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Effects of Multiple Oviposition on Clutch Size in a Leaf-mining Moth, *Paraleucoptera sinuella* (Lepidoptera: Lyonetiidae)

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Abstract. Clutch size in a gregarious leaf-mining moth, *Paraleucoptera sinuella,* was examined in relation to leaf size and multiple oviposition. The field census of clutch distribution on a host plant, *Salix miyabeana,* showed that multiple oviposition occurred frequently, and clutches were distributed aggregatively among leaves. Large leaves were apt to receive multiple clutches. Clutch size was correlated positively with leaf size, but negatively with number of clutches per leaf. This implies that females recognize both leaf size and the presence of conspecific eggs and adjust clutch size accordingly. However, the two variables explained only a small amount of variation in clutch size. Thus, additional mechanisms are needed to explain the large amount of variation in clutch size of the leaf miner.

Key words: clutch size adjustment, leaf miner, leaf size, oviposition behavior, willow.

Introduction

While many leaf-mining insect species lay eggs singly, several species lay eggs in clusters and the larvae form a communal mine within a single leaf (Hespenheide, 1991). In such gregarious leaf miners, clutch size (number of eggs in an egg-mass laid by a single female) is expected to be determined by leaf size to avoid food exhaustion during larval development, because the food of leaf-mining larvae is generally limited to a single leaf. However, mechanisms of clutch size determination in gregarious leaf miners have not been explained except in a few species. Godfray (1986) showed that a leaf-mining fly, *Pegomya nigritarsis* (Zetterstedt), adjusted clutch size to escape from hymenopteran parasitoids while the clutch size was correlated with leaf size. Damman & Cappuccino (1991) also demonstrated that a leaf-mining beetle, *Microrhopala vittata* (Fabricius), laid a clutch, exceeding leaf carrying capacity, to protect eggs from natural enemies. These two studies emphasized that mechanisms for escaping natural enemies are important determinants of clutch size in leaf-mining insects.

Paraleucoptera sinuella (Reutti) (Lepidoptera: Lyonetiidae) is a gregarious leaf-mining moth that lays eggs in clusters on leaves of *Populus* and *Salix* species. In this leaf miner, clutch size was positively correlated with leaf size although the relationship was weak and females laid smaller clutch size than that estimated from leaf carrying capacity (Kagata & Ohgushi, 2002). Thus, clutch size of the leaf miner could not be explained by leaf size alone, and ovipositing females appear to determine clutch size by using several cues. Presence of conspecific eggs would be one of the cues to determine clutch size (Rothschild & Schoonhoven, 1977). Some theoretical studies predicted that clutch size should decrease when several females oviposit at a same site (i.e. multiple oviposition), because multiple oviposition could decrease resource availability and then increase intraspecific competition (Godfray *et al.*, 1991). Multiple oviposition occurred frequently in *P. sinuella.* In the present study, we focus on the effect of multiple oviposition on clutch size, and determine whether clutch size of the leaf miner is explained by leaf size and multiple oviposition.

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Materials and Methods

Clutch size and distribution pattern

Paraleucoptera sinuella is a bivoltine leaf-mining moth (Kuroko, 1982), and adults appear in June and August in Hokkaido. Clutch size and egg distribution pattern of *P. sinuella* were investigated in 1997 and 1998 on *Salix miyabeana* Seemen growing on the Ishikari River flood plain, Ishikari City, Hokkaido (43°11′N, 141°24′E). Female leaf miners lay an egg-clutch on the upper surface of a leaf. Clutch size ranged from one to six on the host plant, *S. miyabeana* (Kagata & Ohgushi, 2002), in which budbreak begins in late April, and new leaves are produced continuously until July (Kagata & Ohgushi, 2001). In early June, the oviposition period of the first generation of the leaf miner, more than 1,000 completely expanded leaves from each of 10 randomly selected trees of *S. miyabeana* were checked and the number of clutches on a leaf and their size were recorded. The length of leaves bearing *P. sinuella* clutches was also measured, and leaf area (Y) was estimated on the basis of leaf length (X) using the regression equation ($Y=0.336X+0.11X^2$, N=50, $r^2=0.98$, $P<0.001$). Phenological change in size of each leaf was not considered because of the following two reasons: (1) data were collected in short period (within a week), and (2) size of each leaf was constant after expansion (Kagata & Ohgushi, 2001).

Statistics

Distribution of clutches among leaves was tested by G test, setting H_0 (null hypothesis) = random distribution, and frequencies in the random distribution were estimated from the Poisson distribution. Frequencies of two or more clutches on a leaf were summed up in the test because of low frequencies: expected frequencies were <5 at classes of three or more clutches. The effect of leaf area on number of clutches per leaf was tested by liner regression. The effects of leaf area and number of clutches per leaf on clutch size were tested by multiple regression.

Results

Multiple oviposition (two or more clutches per leaf) was found frequently on *S. miyabeana*; 46.6% of the observed clutches were deposited with at least one other clutch and 26.1% of oviposited leaves received multiple clutches. The mean number of clutches per leaf was 1.38±0.81 (SD), and the number of clutches was positively correlated with leaf size (N=536, $r^2=0.02$, $P<0.001$, $Y=0.974+0.045X$). The clutches were distributed more aggregatively than expected by chance (G test, df=2, $G=174.3$, $P<0.0001$; Table 1).

Table 1. Observed frequency of number of clutches per leaf and that expected from random distribution on *S. miyabeana*. Frequencies of the random distribution were estimated by Poisson distribution.

Number of clutches per leaf	Frequency	
	Observed	Expected
0	10,531	10,350
1	396	693
2	98	23
3	30	1
4	6	0
5	3	0
6	0	0
7	3	0
Total	11,067	11,067

Table 2. Regression coefficients in the multiple regression.

	Coefficient	SE	t value	P value
Leaf area	0.08	0.01	6.89	<0.001
Number of clutches	−0.11	0.02	−4.53	<0.001
Intercept	1.69	0.11	15.19	<0.001

Clutch size was positively affected by leaf size, but negatively by the number of clutches per leaf, although the amount of variation in clutch size explained by these two variables was small (N=742, $r^2=0.07$, $P<0.001$, Table 2). Leaf area explained 5% and number of clutches explained 2% of the variation in clutch size.

Discussion

Females of some insects species can recognize conspecific eggs previously deposited on a host plant by using marking pheromones or visual stimuli, and then avoid multiple oviposition (Shapiro, 1981). However, females of *P. sinuella* are unlikely to avoid multiple oviposition as avoidance is unknown in most leaf miners (Bultman & Faeth, 1985; Auerbach & Simberloff, 1989; but see McNeil & Quiring, 1983). Aggregative oviposition on a single leaf implies that adult females select oviposition site in response to host plant characteristics: leaf nitrogen (Minkenberg & Ottenheim, 1990), leaf size (Bultman & Faeth, 1986), and the timing of budbreak or leaf abscission (Faeth, 1990; Mopper & Simberloff, 1995). *Paraleucoptera sinuella* females preferred large leaves for oviposition in spite of the presence of conspecific eggs. Oviposition on large leaves may have advantages for larvae because large leaves have low concentrations of defensive compounds (Zucker, 1982) or low likelihood of leaf abscission (Kagata & Ohgushi, 2001).

On the other hand, theoretical studies based on optimal oviposition strategy and ESS predict that clutch size in multiple oviposition should be small because the presence of one or more other clutches on the same oviposition site reduces the amount of food available for each individual on that site (see Godfray *et al.*, 1991). Small clutches in multiple ovipositions have been reported in several insects (Rothschild & Schoonhoven, 1977; Vasconcellos-Neto & Monteiro, 1993) and our data showed that *P. sinuella* clutch size decreased as number of clutches per leaf increased while it increased with increasing leaf size. This implies that females of the leaf miner recognize both leaf size and the presence of conspecific eggs and adjust clutch size accordingly.

However, clutch size adjustment by ovipositing females in response to resource availability was not perfect in the leaf miner since the amount of variation in clutch size was only minimally explained by leaf area and the number of clutches per leaf. The large amount of variation in clutch size which could not be explained by resource availability was also reported in another leaf miner, *Pegomya nigritarsis* (Zettersstedt) (Godfray, 1986) and a gall-fly, *Urophora cardui* (Linnaeus) (Freese & Zwölfer, 1996). These insects may be under severe food limitation during larval period from their feeding style, which expected a tight relationship between clutch size and resource availability. In addition to the factors discussed above, the reproductive age of adult females (Begon & Parker, 1986) and the efficiency of their search for oviposition sites (Charnov & Skinner, 1984) affect clutch size determination. Therefore, additional mechanisms are needed to explain the large amount of variation in clutch size of the leaf miner.

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