

Clutch Size Adjustment of a Leaf-Mining Moth (Lyonetiidae: Lepidoptera) in Response to Resource Availability

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ABSTRACT Clutch size variation of a leaf mining moth *Paraleucoptera sinuella* Reutti was investigated on two host plants, *Populus sieboldii* Miquel and *Salix miyabeana* Seemen. We found that female moths oviposited egg clutches with different size on the two host plants and that clutch size was correlated with leaf area between host species. Therefore, we concluded that females are adjusting clutch size in response to variation in resource availability for offspring. Positive correlation was also found between clutch size and leaf area within host species, however, the relationship was weak. Expected clutch size was determined from estimates of leaf area consumed by a larva until pupation. Females laid significantly smaller clutches than the expected size that could be supported by a single leaf on both host plants. We discussed how the females determined clutch size in response to resource availability.

KEY WORDS *Paraleucoptera sinuella*, leaf miner, clutch size, oviposition behavior, resource availability

SOME HERBIVOROUS INSECTS exhibit gregarious feeding, which provides the following benefits: maintenance of a favorable temperature (Tsubaki 1981, Joos et al. 1988), increased foraging efficiency (Tsubaki 1995), and escape from predators and/or parasitoids (Weis et al. 1983, but see Siemens and Johnson 1992). However, spatial clustering may cause food shortage, which results in larval starvation or increased risk of predation due to searching for an alternative feeding site (Matsumoto 1990, Tsubaki 1995). The size of the feeding group is largely dependent on clutch size (the number of eggs deposited during one oviposition bout of a female). Therefore, ovipositing females should adjust their clutch size in response to resource availability so that their offspring not only enjoy the benefits of gregarious feeding but also avoid the disadvantages due to resource overexploitation.

The evolution of clutch size has been one of the major controversial issues in ecology, and it has been the subject to both theoretical and empirical studies (Godfray 1987, Godfray et al. 1991). Models of clutch size variation in insects have predicted that larger clutches should be laid in a good patch with high quality or abundant resources (Parker and Courtney 1984). Empirical studies have supported this prediction in hymenopteran parasitoids (Charnov and Skinner 1984, Hardy et al. 1992, Vet et al. 1993), lepidopterans (Pilson and Rausher 1988, Vasconcellos-Neto

and Monteiro 1993), and a seed beetle (Fox and Mousseau 1995). These studies indicate that female insects could adjust their clutch size according to food availability for larvae.

Adjustment of clutch size is needed when food resources are limited, and intraspecific competition is likely to occur. The food available to leaf-mining larvae is generally limited to a single leaf, and larvae cannot move to other leaves even if the leaf is heavily exploited. As a result, exploitative competition within a leaf is more likely to occur due to excessive oviposition on a single leaf. Moreover, when larvae encounter other mining larvae in a leaf, one larva often kills the other (i.e., larval interference) without food exhaustion (Auerbach et al. 1995). Such food exploitation and interference competition have been reported in several leaf miners (Murai 1974, Stiling et al. 1984, Auerbach and Alberts 1992). Most leaf miners lay eggs singly, but several species lay eggs gregariously, and larvae form a communal mine (Hespenheide 1991). In the gregarious leaf miners, clutch size is expected to be correlated with leaf size of host plants so that larval competition is avoided. However, we know little about how clutch size of leaf mining insects is determined (Godfray 1986).

In this study, we investigated the causes of clutch size in a gregarious leaf miner *Paraleucoptera sinuella* Reutti in the field. Larvae of *P. sinuella* feed exclusively on several *Salix* and *Populus* species. Leaf size differs considerably between *Salix* and *Populus*. Thus, we investigated whether an ovipositing female is able to modify clutch size in response to leaf size within and between host plant species.

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Materials and Methods

Clutch size of *P. sinuella* was investigated in early June 1997 and 1998 on *Populus sieboldii* Miquel and *Salix miyabeana* Seemen on a flood plain along the Ishikari River, Ishikari City, Hokkaido, in northern Japan. The study site has been disturbed by the river channelization work, and several Salicaceae plants have colonized this area. *P. sieboldii* has a broad and round leaf, and *S. miyabeana* has a narrow and long leaf. *P. sinuella* females lay an egg mass on the upper surface of a leaf. Hatching occurs almost simultaneously within a clutch, and larvae feed gregariously in palisade parenchyma to form a communal mine. The larval period lasts about 20 d, and the larvae never emerge from a leaf until pupation. Pupation occurs in a cocoon outside of a leaf.

The number of eggs per egg mass was counted and the length of leaves with eggs was measured. The leaf area (Y) was estimated from leaf length (X) using the regression equations ($Y = -0.427X + 0.732X^2$, $r^2 = 0.997$ for *P. sieboldii*; $Y = 0.336X + 0.11X^2$, $r^2 = 0.988$ for *S. miyabeana*).

To estimate the leaf area that a single larva required before pupation, we conducted a larval rearing experiment. Leaves with an active mine were collected from *P. sieboldii* and *S. miyabeana* in mid-July, and the number of eggs was counted. Each leaf was placed separately into a plastic case (7 by 2 by 3 cm) to rear larvae in the laboratory. Wet paper was placed on the bottom of each case to maintain high humidity. After all members in a clutch pupated without food exhaustion, consumed leaf area was measured using the NIH Image program (<http://www.tsb.info.nih.gov/nih-image>).

We assumed that expected clutch size was the maximum number of larvae which could be supported by a single leaf. We estimated the expected clutch size for each leaf size from the linear regression equation based on leaf area consumed by larvae ($Y = -0.60 + 0.40X$ for *P. sieboldii*, $Y = -0.70 + 0.46X$ for *S. miyabeana*; $Y =$ expected clutch size, $X =$ leaf area, see details in text). A comparison of the observed and expected clutch size was performed by paired *t*-test for each plant species.

Results

There was a significant difference in clutch size distribution of *P. sinuella* between *P. sieboldii* and *S. miyabeana* (Kolmogorov-Smirnov test: $\chi^2 = 687.6$, $P < 0.0001$, Fig. 1). Clutch size varied from one to 15 eggs on *P. sieboldii* and one to 6 eggs on *S. miyabeana*. The mean clutch size on *P. sieboldii* was twice of that on *S. miyabeana* (mean \pm SE: 4.74 ± 0.07 on *P. sieboldii*, 2.22 ± 0.03 on *S. miyabeana*; Mann-Whitney *U* test: $U = 108458$, $P < 0.0001$, Table 1). *P. sieboldii* leaves with eggs were significantly larger than those of *S. miyabeana*, and were twice of those of *S. miyabeana* (mean \pm SE: 19.3 ± 0.24 cm² for *P. sieboldii*, 9.1 ± 0.10 cm² for *S. miyabeana*; Mann-Whitney *U* test: $U = 66287$, $P < 0.0001$, Table 1).

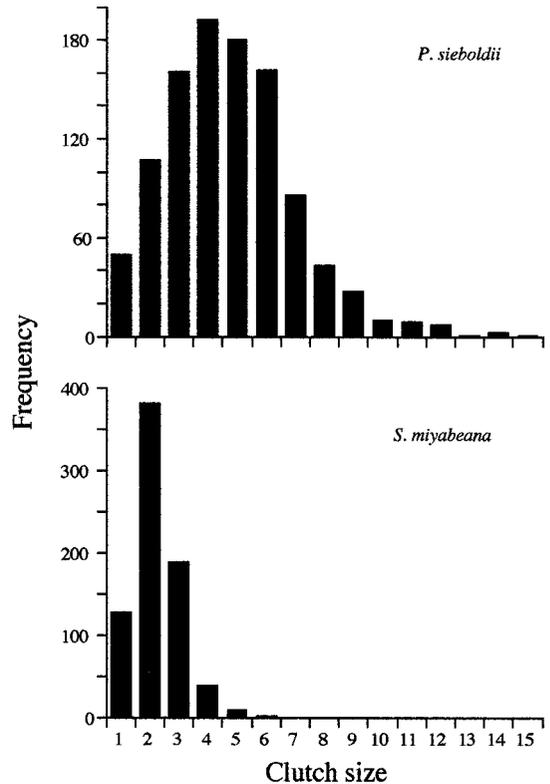


Fig. 1. Frequency distribution of clutch size of *P. sinuella* on *P. sieboldii* (top) and *S. miyabeana* (Bottom).

A significant positive correlation was found between leaf area and clutch size for *P. sieboldii* and *S. miyabeana*, although the amount of variation in clutch size explained by leaf area was small in both plant species (Fig. 2).

There was no significant difference in the leaf area consumed by larvae between plant species (ANCOVA: $MS = 3.77$, $F = 1.83$, $df = 1$, $P > 0.05$). Based on the regression lines ($Y = 1.47 + 2.50X$ for *P. sieboldii* and $Y = 1.51 + 2.16X$ for *S. miyabeana*, Fig. 3), we determined expected clutch size on each plant species. The observed clutch size was significantly smaller than the expected size on both plant species (Table 2). Hence, ovipositing females lay eggs in

Table 1. Clutch size of *P. sinuella* and leaf area of *P. sieboldii* and *S. miyabeana*

	Plant species	
	<i>P. sieboldii</i>	<i>S. miyabeana</i>
Sample size	1,038	742
Clutch size		
Mean (SE)	4.74 (0.07)	2.22 (0.03)
Range	1-15	1-6
Leaf area (cm ²)		
Mean (SE)	19.3 (0.24)	9.1 (0.10)
Range	4.1-55.8	3.2-20.2

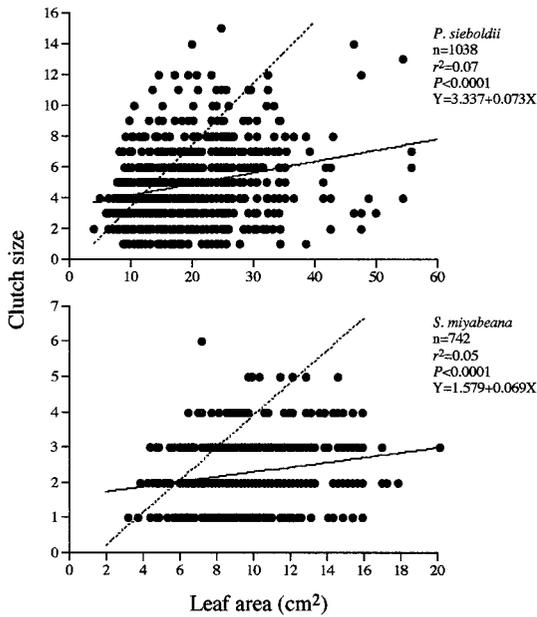


Fig. 2. Relationship between clutch size and leaf area on *P. sieboldii* (top) and *S. miyabeana* (bottom). Regression lines of observed clutch size are shown by solid lines. Expected clutch size is shown by dotted lines ($Y = -0.60 + 0.40X$ for *P. sieboldii*, $Y = -0.70 + 0.46X$ for *S. miyabeana*).

smaller clutches than the maximum number of larvae, which could be supported by a single leaf.

Discussion

Several herbivorous insects vary clutch size in response not only to different host plants (Godfray 1986, Vasconcellos-Neto and Monteiro 1993, Ekblom 1998), but also conspecific host plant variation (Pilson and Rausher 1988, Tsubaki 1995, Fox and Mousseau 1995). The results of the current study clearly illustrate that *P. sinuella* females oviposit significantly different-sized clutches on *P. sieboldii* and *S. miyabeana*. The different clutch size was clearly caused by the interspecific difference in leaf size. Because the required leaf area per larva until pupation was not different on the both host plants, *P. sieboldii* with larger leaves can support more larvae in a single leaf than *S. miyabeana*. Hence, ovipositing females would lay eggs in larger clutches on *P. sieboldii*. We also found that the clutch size was correlated with leaf size within host plant species. These results support a model of clutch size determination: host plants that provide rich resources to offspring of insect females should receive larger clutches than hosts that provide poor resources (Parker and Courtney 1984), which has been supported by studies of other herbivorous insects (Matsumoto et al. 1994, Freese and Zwölfer 1996, Ekblom 1998). In the current study, however, leaf size did not solely explain a large amount of variation in clutch

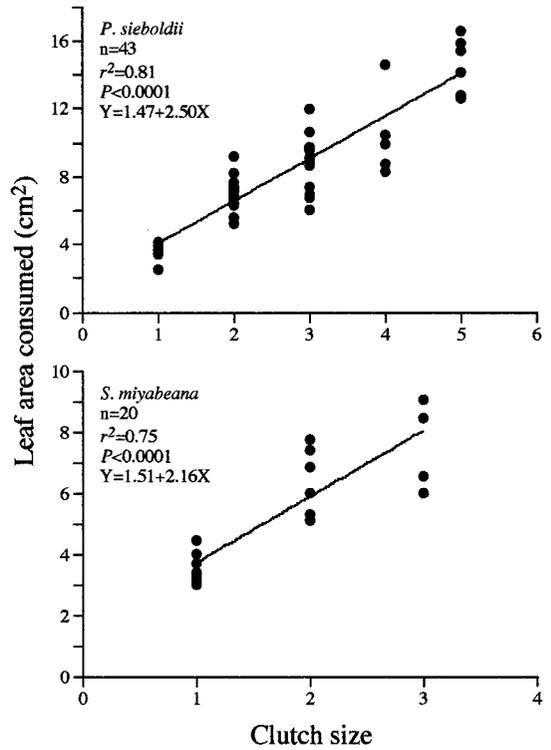


Fig. 3. Consumption of leaf area by larvae against different clutch size on *P. sieboldii* (top) and *S. miyabeana* (bottom).

size, and cause(s) of the large variation remained unclear.

Perception of leaf size by ovipositing females across plant species is particularly important for insects which use several plant species in the same location. *P. sinuella* larva feeds exclusively on *Populus* and *Salix* plants, however leaf size varies greatly among the species. Therefore, an ability to evaluate resource availability among plant species should evolve in the leaf-mining moth. We frequently observed females walking carefully around several leaves of a host plant before laying eggs. This behavior may enable a female to measure leaf size and adjust clutch size accordingly. However, Godfray (1986) suggested the possibility that females of a leaf-mining fly measure leaf size by using chemical composition relevant to leaf size.

Godfray (1986) showed that there was an optimal clutch size in a leaf-mining fly, *Pegomya nigritarsis* Zetterstedt, to escape from hymenopteran parasitoids, and that this optimal clutch size was observed most frequently on a single host species in the field. His study demonstrated that clutch size was largely de-

Table 2. A comparison of observed and expected clutch sizes

Host plant	df	Mean difference	t value	P
<i>P. sieboldii</i>	1,037	-2.38	-22.88	<0.0001
<i>S. miyabeana</i>	741	-1.36	-28.21	<0.0001

terminated by the impact of natural enemies rather than by resource availability, although natural enemies did not explain the difference in clutch size among plant species. The impact of predation and parasitism on clutch size has often been reported in herbivorous insects (Subinprasert and Svensson 1988, Siemens and Johnson 1992). In their model, Parker and Courtney (1984) predicted that females would maximize their fitness, not by laying as many eggs as a patch could support, but rather by laying larger clutches to compensate for the larval death from mortality causes other than intraspecific competition among clutch members. Damman (1991) studied the pyralid moth *Omphalocera munroei* Martin to test this hypothesis and demonstrated that the clutch size exceeded that which could be supported by a single patch and that larvae had to migrate to an alternative patch. Large clutch size was thus explained as compensation for larval loss from predation during the migration.

The current study, however, showed that realized clutch size of *P. sinuella* was smaller than the expected clutch size based on leaf area in the absence of mortality. One hypothesis to explain the small clutch size is that all of the area of a leaf would not be available to the leaf-mining larva. If the larva uses only a part of the leaf, the realized clutch size should be smaller than the expected size. Leaf venation would affect mining behavior, and many leaf miners cannot cross the leaf midvein (Stiling et al. 1987, Hespeneheide 1991). Moreover, quality as larval food would be different on different parts within a single leaf (Schoonhoven et al. 1998). In several leaf miners, it is known that the larvae mine specific positions of a leaf (Nielsen 1978, Sato 1991). The larva of *P. sinuella* can cross the leaf midvein. However, whether all parts of a leaf are equally preferable for the leaf miner remains unclear.

Another hypothesis is that optimal clutch size should be small when multiple oviposition per patch is common, because the oviposition by multiple females increases the likelihood of food shortage during the larval period (Parker and Courtney 1984, Ives 1989). In general, leaf-mining larvae tend to aggregate among leaves, due to multiple oviposition by several females (Stiling et al. 1987, Auerbach and Simberloff 1989). As a consequence, larval competition within a leaf increases (Bultman and Faeth 1986). In *P. sinuella*, approximately half of observed egg masses co-occurred on a leaf with other egg masses (unpublished data). Hence, multiple oviposition may explain why females lay eggs in smaller clutch sizes than the expected size that is supported by availability of a single leaf.

As we expected, clutch size of the leaf miner was principally determined by leaf size of host plants. The clutch size adjustment by adult females in response to leaf size would decrease larval competition for limited food within a leaf. However, leaf size alone did not explain a large amount of variation in clutch size and the small clutch size compared with maximum size that a leaf supports. Further studies are required to determine which factors affect the clutch size determination in the leaf miner.

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