

# Resource Partitioning among Three Willow Leaf Miners: Consequence of Host Plant Phenology

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**Abstract.** Host use pattern of three lepidopteran leaf miners was studied on the host plant, *Salix miyabeana*. Interspecific co-occurrence on a single leaf was exceptional, and the three leaf miner species used leaves in different size. There was a positive correlation between leaf size and leaf area required for larval development of each species. This host use pattern depending on leaf size is probably due to leaf-flushing pattern of the host plant. Since *S. miyabeana* produced temporal variation in leaf size throughout the growing season, size of leaves available to the leaf miners varied temporally depending on different ovipositing timing of the leaf miners. Thus, it is concluded that different timing of oviposition results in resource partitioning among the leaf miners.

**Key words:** Leaf miner, larval distribution, plant phenology, leaf size.

## Introduction

Distribution of leaf-mining insects is strongly determined by host plant variation. For example, oviposition site selection by adult females is largely influenced by variation in leaf structure (Ezcurra *et al.*, 1987; Reavey & Gaston, 1991), leaf age (Faeth *et al.*, 1981; Auerbach & Simberloff, 1984), leaf size (Bultman & Faeth, 1986a; Faeth, 1991), leaf chemistry (Stiling *et al.*, 1982; Minkenbergl & Ottenheim, 1990), and herbivory (Faeth, 1986; Simberloff & Stiling, 1987). Because larval emergence from leaves until pupation does not occur in most leaf miner species, the spatial distribution is mostly determined by oviposition site selection by adult females. When larval performance is largely determined by characteristics of leaves on which females lay eggs, oviposition behaviour would be favored by natural selection so as to maximize offspring performance.

Many studies have demonstrated that conspecific larvae of leaf miners tend to co-occur on a single leaf (Potter, 1985; Auerbach & Simberloff, 1989; Faeth, 1990), despite that the aggregation of oviposition would cause interference and/or exploitative competition during larval period (Murai, 1974; Quiring & McNeil, 1984; Stiling *et al.*, 1984). Moreover, Bultman & Faeth (1985) showed that heterospecific leaf miners co-occurred on a leaf more frequently than

expected by chance. On the other hand, a few studies have found resource partitioning in terms of leaf size and/or mine positions within a leaf among leaf miner species (Nielsen, 1978; Bultman & Faeth, 1986b; Sato, 1991). These studies suggest that temporal and/or spatial differences in resource utilization would contribute to food resource partitioning in the leaf miners.

In this study, we investigated temporal and spatial distribution patterns of three lepidopteran leaf miners, *Caloptilia stigmatella* (Fabricius) (Gracillariidae), *Phyllonorycter pastorella* (Zeller) (Gracillariidae), and *Paraleucoptera sinuella* (Reutti) (Lyonetiidae) on the host plant, *Salix miyabeana* (Seemen). We examined how the three leaf miners partition available leaves of the host plant.

## Materials and Methods

### *Study site*

All censuses were conducted in 2000 at a flood plain along the Ishikari River, Ishikari City, Hokkaido, in northern Japan. In and around the study site, several willow species have widely occurred (see Ishihara *et al.*, 1999).

### *Plant phenology and leaf miner appearance*

Eight trees of *S. miyabeana* in similar size (4–5 m in height) were randomly selected in mid-May for monitoring of plant phenology and occurrence of leaf miners. Plant phenology was examined at 6 to 7 day intervals from late May to late July. We selected one

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apical shoot per each tree, and recorded shoot length, number of flushing and abscission leaves, and length of individual leaves. Each leaf area was estimated from the equation,  $Y=0.336X+0.11X^2$  ( $Y$ =leaf area,  $X$ =leaf length). During the same period, we also randomly selected eight branches (including >200 shoots) per tree for the eight trees, and censused leaf miners at 3 to 4 day intervals on these branches. We counted newly emerged mines and marked them to avoid double counting. We made species identification of leaf miners by specific mine shape and oviposition location (Table 1).

#### Spatial distribution of leaf miners and leaf size

To determine spatial distribution pattern of leaf miners, we randomly selected 12 trees of *S. miyabeana* in late June and took 150 to 200 leaves on each tree. Leaf length and leaf miner species in each leaf were recorded. Species association of the leaf miners was evaluated by Iwao's  $\omega$  index, which is derived from the interspecies mean crowding (Iwao, 1977). This index indicates spatial association between two species, varying from  $-1$  to  $1$ . When  $\omega=0$ , two species are distributed independently. When  $\omega=1$ , the distributions of two species overlap completely. When  $\omega=-1$ , the distributions of two species are completely exclusive.

Moreover, we measured leaf length of >200 leaves with each leaf miner species, to determine whether the three leaf miner species, *C. stigmatella*, *P. pastorella*, and *P. sinuella*, used different leaves in size.

#### Leaf consumption by a leaf-mining larva

We measured total leaf area that one larva of the leaf miners consumed during the mining period. A larva of *C. stigmatella* emerges from a leaf in late larval period, and makes a few leaf-rolls until pupation. *P. pastorella* and *P. sinuella* never emerge from a leaf until pupation. We collected mined leaves with *C. stigmatella* in late June, and *P. pastorella* and *P. sinuella* in mid July. When alive larvae were found in the leaf, the larvae were reared in laboratory until larval emergence from a leaf for *C. stigmatella* or pupation for *P. pastorella* and *P. sinuella*. Leaf area consumed by a larva of each leaf miner species was measured by using the public domain soft NIH image program.

## Results

#### Plant phenology

Budbreak of *S. miyabeana* began in late April.

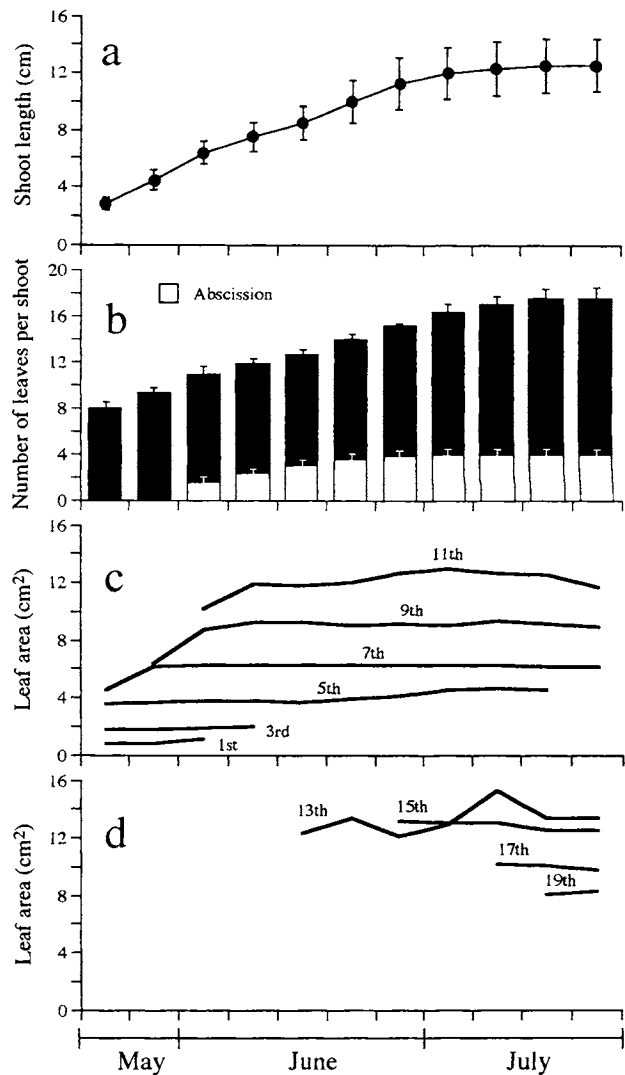


Fig. 1. Seasonal changes in (a) shoot length, (b) number of expanded and abscised leaves, (c-d) leaf area at different position from the base of a current shoot of *S. miyabeana*. Means are presented. Vertical bars show SE. Leaf area (c, d) are illustrated during the period from 50% flushing to 50% abscission of each leaf position.

Shoot growth continued up to mid July, and number of leaves increased with shoot elongation (Fig. 1a and b). Leaf abscission was firstly observed in early June, and occurred in leaves which emerged early in the season. Leaf abscission was no longer observed in July. Size of newly flushed leaves increased as the season advanced, and leaves reached at the maximum size in late June. Leaf expansion completed within one week after its appearance, and leaf size was constant thereafter (Fig. 1c and d).

#### Appearance of leaf miners

Seven species of leaf miners, including five lepidop-

Table 1. Density, mine type and oviposition location of leaf-mining insects on *S. miyabeana*. S=serpentine type, B=blotch type, L=lower leaf surface, U=upper leaf surface.

Family Species	No. of larvae/100 shoots (SD)	Mine type	Oviposition location
Gracillariidae			
<i>Caloptilia stigmatella</i> (Fabricius)*	4.19(2.34)	S-B	L
<i>Phyllonorycter pastorella</i> (Zeller)*	4.60(1.78)	B	L
<i>Phyllonorycter salicicolella</i> (Sircom)	0.18(0.35)	B	L
Lyonetiidae			
<i>Paraleucoptella sinuella</i> (Reutti)*	16.62(36.98)	B	U
Phyllocnistidae			
<i>Phyllocnistis saligna</i> (Zeller)	0.78(1.17)	S	L
Unknown sp. 1	0.11(0.21)	B	U
Unknown sp. 2	0.04(0.13)	B	L

\* species in the study

teran and two unknown species, were identified on *S. miyabeana* (Table 1). Fig. 2 shows temporal pattern of numbers of the three major species, *C. stigmatella*, *P. pastorella*, and *P. sinuella*. The earliest species *C. stigmatella* appeared in late May. Two weeks later, *C. stigmatella* larvae reached at leaf-rolling stage. Next, *P. pastorella* occurred in early June, followed by *P. sinuella* in mid June. Because approximately two weeks are required for egg hatch in the leaf miners (unpublished data), the oviposition period would be in mid May for *C. stigmatella*, late May for *P. pastorella*, and early June for *P. sinuella*. The second generation of *C. stigmatella* and that of *P. pastorella* were observed in July. Although *P. sinuella* is also bivoltine in Hokkaido (unpublished data), we did not observe the second generation because our monitoring did not extend the emergence of the second generation in August.

#### Distribution of leaf miners and leaf size

Leaf miners were found in 5.1% of 2130 sampled leaves of *S. miyabeana*. There was only one leaf in which two species coexisted: *P. pastorella* and *P. sinuella*. Neither co-occurrence of *C. stigmatella* and *P. pastorella* nor *C. stigmatella* and *P. sinuella* were observed. These three species of leaf miners did not overlap each other, indicating interspecific segregation on Iwao's  $\omega$  index (Table 2). Comparison between observed and expected frequency of co-occurrence showed that the three leaf miners were distributed independently among leaves from one another ( $\chi^2$  test,  $P > 0.05$ , Table 2).

Leaf size of *S. miyabeana* varied from 0.5 to 23.6 cm<sup>2</sup> (mean leaf area  $\pm$  SE =  $6.14 \pm 0.08$ ) in late June. The three leaf miners used different leaves in size (ANOVA with Scheffé test,  $df = 3$ ,  $F = 107.7$ ,  $P < 0.05$ , Fig. 3 and Table 3). These leaves were used by each

leaf miners of the first generation, because the second generation of each species has not appeared in June (Fig. 2). *C. stigmatella* larvae mined the smallest leaves ( $5.77 \pm 0.18$ ) among three species, and moved to larger leaves for making leaf-rolls. *P. pastorella* used leaves ( $7.07 \pm 0.19$ ) larger than those for *C. stigmatella*. However, there was no significant difference in leaf size between *C. stigmatella* leaf-rolling stage and *P. pastorella*. *P. sinuella* larvae used the largest leaves ( $9.72 \pm 0.18$ ) among three species. In these three miners, extremely small leaves were not used as an oviposition site in spite of a large proportion of small leaves.

#### Leaf consumption by a leaf-mining larva

Larvae of *C. stigmatella* and *P. pastorella* started mining from the lower side of a leaf, and fed on mesophyll penetrating to upper side. In contrast, *P. sinuella* started mining from upper side, and fed exclusively on upper mesophyll. Consumed leaf area by a leaf-mining larva was significantly different among species (ANOVA with Scheffé test,  $df = 2$ ,  $F = 445.4$ ,  $P < 0.0001$ , Table 3). Leaf consumption by *C. stigmatella* larvae during mining period was the least. *P. sinuella* larvae required the largest leaf area for development among three species, and *P. pastorella* was the intermediate between *C. stigmatella* and *P. sinuella*.

## Discussion

Herbivorous insects often have a positive species association because of preference to the same characteristics of the host plant (Kidd *et al.*, 1985; Bultman & Faeth, 1985; Fritz *et al.*, 1987a, b). For example, Fritz *et al.* (1987b) demonstrated that four sawfly species considerably co-occurred on long shoots of willows, which provide good resource with high qual-

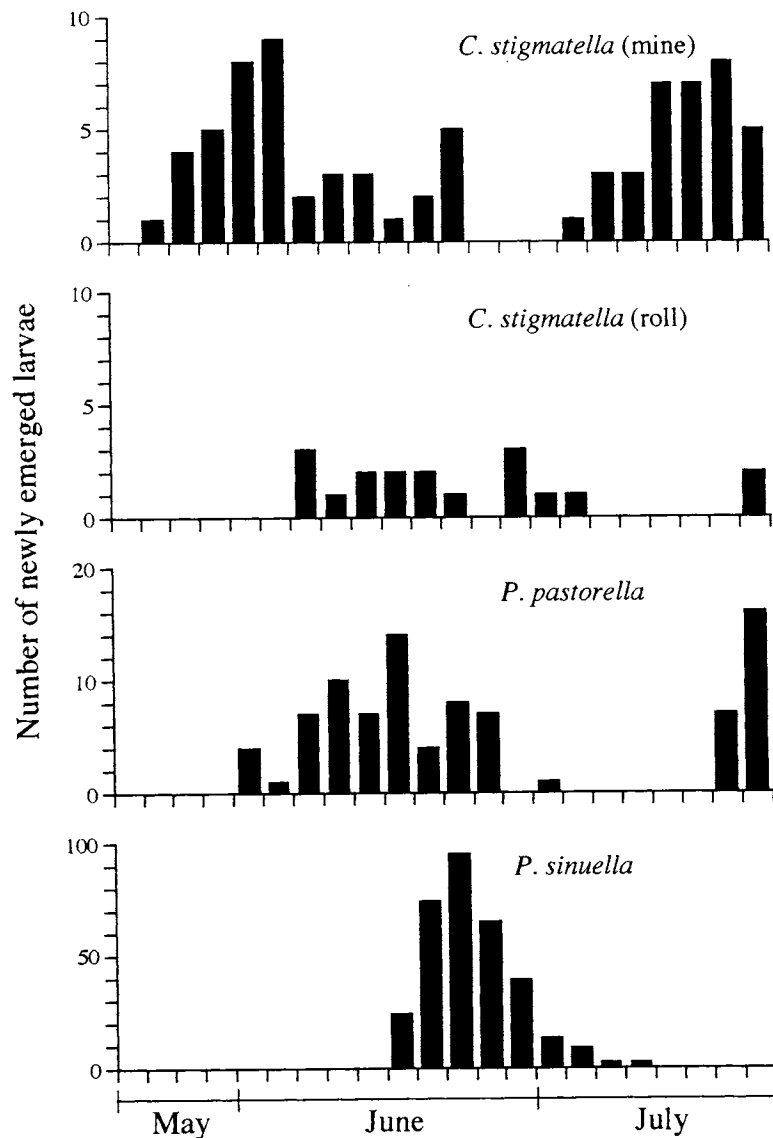


Fig. 2. Seasonal changes in number of larvae of three leaf miners on *S. miyabeana*.

Table 2. Species association of leaf miners on *S. miyabeana*. No significant difference ( $\chi^2$  test,  $P > 0.05$ ) between observed and expected frequency of co-occurrence is shown as ns. Expected frequency is calculated from probability of co-occurrence by chance. Sample size = 2130.

Species combination (A and B)	Leaves with:				Expected	$\omega$ value
	None	A	B	Both		
<i>C. stigmatella</i> and <i>P. pastorella</i>	2089	13	28	0	0 <sup>ns</sup>	-1
<i>P. pastorella</i> and <i>P. sinuella</i>	2034	27	68	1	1 <sup>ns</sup>	-0.24
<i>P. sinuella</i> and <i>C. stigmatella</i>	2048	69	13	0	0 <sup>ns</sup>	-1

ity to increase larval survival of sawflies (Price *et al.*, 1987; Craig *et al.*, 1989; Price & Ohgushi, 1995). Therefore, these sawflies have a positive species association because females preferred long shoots for oviposition regardless of the presence of other species. In contrast, our study illustrated that three leaf miner species were distributed exclusively from each other

among leaves, and co-occurrence on a single leaf was exceptional. However, comparison between observed and expected frequencies of co-occurrence showed that the distribution was not significantly different from independence. This discrepancy would be due to small sample size. Hence, further analysis with larger sample size may clearly determine the distribution

pattern of the three leaf miners. In either case, our result does not agree with other leaf miners on oak trees which show a positive interspecific correlation (Bultman & Faeth, 1985; Sato, 1991).

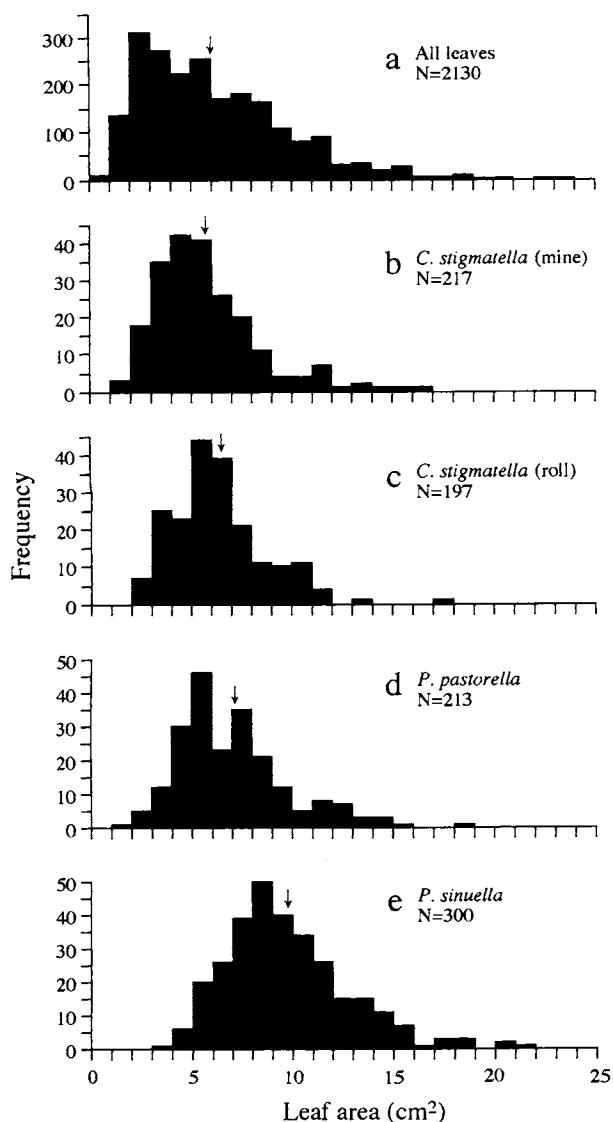


Fig. 3. Frequency of leaf area of *S. miyabeana*: (a) all leaves, and leaves used by (b) *C. stigmatella* leaf-mining stage, (c) *C. stigmatella* leaf-rolling stage, (d) *P. pastorella*, (e) *P. sinuella*. Arrows show mean leaf area. N shows sample size.

The distribution of the three leaf miners would be caused by different utilization pattern depending on leaf size. *S. miyabeana* had a large variation in leaf size, and the leaves used by three leaf miner species differed significantly in size. This size-specific host use pattern was related to consumed leaf area for larval development: the more leaf miner requires leaf area, the larger leaf is used. Such resource partitioning in terms of leaf size was also reported in leaf miners on oak trees (Bultman & Faeth, 1986a). On the other hand, Nielsen (1978) and Sato (1991) demonstrated that resource partitioning was achieved by differences in mine position within a leaf. These resource partitioning may decrease the likelihood of interspecific competition, and may allow potential competitors to coexist. However, whether interspecific competition among leaf miners promotes the resource partitioning in these leaf miners remains still unclear.

The resource partitioning in terms of leaf size in this study would be explained by leaf-flushing pattern of the host plant. *S. miyabeana* continuously expands leaves throughout growing season. Since budbreak began in late April, most leaves in May were still small and likely to abscise. Thereafter, size of newly emerged leaves increased as season advanced. Hence, size of leaves available to leaf miners varies depending on their oviposition timing. Several studies showed that synchronization between oviposition of leaf miners and budbreak of host plants is important in determining larval distribution (Auerbach & Simberloff, 1988; Auerbach & Alberts, 1992), because the leaf miners preferentially lay eggs on young leaves (Auerbach & Simberloff, 1984; Auerbach, 1991). However, the three leaf miners in this study did not synchronize their oviposition timing with budbreak of *S. miyabeana*, because respective leaf miner species appeared in different timing. Phenological synchronization may be especially important for leaf miners which are univoltine and use host plants with flushing leaves simultaneously. In contrast, synchronization may not be critical for the three leaf miners because they are bivoltine and use host plants with flushing leaves grad-

Table 3. Mined or rolled leaf area and consumption area used by three leaf miners. Means (SE) are presented. Differential letters in column show significant difference (ANOVA with Scheffé test,  $P < 0.05$ ).

Species	Leaf size (cm <sup>2</sup> )		Consumption area (cm <sup>2</sup> )	
	number	Mean (SE)	number	Mean (SE)
<i>C. stigmatella</i>				
Leaf-mining stage	217	5.77(0.18) <sup>a</sup>	38	0.70(0.02) <sup>a</sup>
Leaf-rolling stage	197	6.34(0.17) <sup>b</sup>	—	—
<i>P. pastorella</i>	213	7.07(0.19) <sup>b</sup>	29	1.01(0.03) <sup>b</sup>
<i>P. sinuella</i>	300	9.72(0.18) <sup>c</sup>	20	3.21(0.14) <sup>c</sup>

ually. For early emerging species on the willow, laying eggs on small leaves is highly risky, because small leaves are easy to abscise. The leaf abscission is one of the most important mortality factors for leaf-mining insects (Bultman & Faeth, 1986b; Preszler & Price, 1993). Larvae of the first emerging species, *C. stigmatella*, shifts from the natal small leaf to a large leaf for making a leaf-roll in the late larval period. The larvae would avoid a risk of leaf abscission in a small leaf by shortening mining period due to the change in feeding behaviour. In contrast, later emerging species can lay eggs on larger leaves. Therefore, *P. sinuella* whose larvae need large leaf area appeared late, when large leaves are available to females for oviposition. Since *P. pastorella* appeared at intermediate timing between emergence of *C. stigmatella* and *P. sinuella*, it uses intermediate leaf size. The different utilization of leaves would be realized by preference to large leaves of each leaf miner species as well as by differential oviposition timing. The three leaf miners did not use small leaves as an oviposition site in spite of a large proportion of small leaves (see Fig. 3). This indicates that females of each leaf miner species selected newly emerged large leaves for oviposition. Consequently, the three leaf miner species use different leaves in size according to different timing of oviposition, resulting in resource partitioning among the leaf miners.

Iwao's  $\omega$  index showed that the three leaf miners are distributed exclusively from each other. Thus, behavioral avoidance to the interspecific co-occurrence may cause the distribution of the three leaf miners. Ovipositing females of several insects recognize previously deposited conspecific eggs on a host plant by using marking pheromones or visual stimulus, and avoid multiple oviposition (McNeil & Quiring, 1983; Vasconcellos-Neto & Monteiro, 1993). However, it is unknown whether leaf miners avoid multiple oviposition for heterospecific eggs.

The present study clearly demonstrated that there is resource partitioning in terms of leaf size among three leaf miners sharing the same host plant. This host use pattern is more likely to be caused by temporal changes in size of available leaves for oviposition, and by different timing of oviposition. Consequently, sequential leaf flushing pattern of *S. miyabeana* plays an important role in interspecific segregation among leaves in the leaf miners.

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