SHORT COMMUNICATION

Oviposition stimuli for host plant recognition and clutch size determination in a leaf-mining moth

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Key words. Clutch size, leaf miner, leaf size, oviposition preference, plant odour.

Introduction

In herbivorous insects that lay eggs in clusters, females must determine where to oviposit (host plant recognition) and how many eggs to deposit (clutch size determination). In general, host plant recognition has been ascribed to chemical stimuli from plant secondary compounds; flavonoids, carboxylic acids, and phenolic glucosides have been identified as recognition cues for ovipositing females (Nishida, 1995; Roininen et al., 1999). Clutch size determination, however, has been linked to physical characters associated with resource abundance (Pilson & Rausher, 1988; Tsubaki, 1995). As a result of this dichotomy, host plant recognition and clutch size determination have long been studied separately and the relationship between their specific stimuli remains unclear.

In the work reported here, therefore, the differences between host plant recognition and clutch size determination stimuli were investigated using the leaf miner Para-leucoptera sinuella Reutti (Lepidoptera: Lyconetidae), by analysing field clutch size on different plant species and by a laboratory oviposition experiment. The separate effects of chemical and physical stimuli were clarified for the two oviposition processes of host plant recognition and clutch size determination.

Materials and methods

Clutch size in the field

Para-leucoptera sinuella larvae are specialist herbivores of Populus and Salix species, however on some Salix species the larvae die soon after initiating a leaf-mining, although egg-laying often occurs on these plants (Kagata & Ohgushi, 2001).

Clutch size was investigated in early June 1997 and 1998 in the Ishikari River flood plain, Ishikari City, Hokkaido, northern Japan (43°11’N, 141°24’E) on four sympatrically growing plant species. Of these, Populus sieboldii Miquel and Salix miyabeana Seemen were host species of the leaf miner and S. haliteni Floderus and S. sachalinensis Fr. Schm. were non-host species. The number of eggs per clutch was counted and the length of leaves with eggs was measured on 10–14 randomly selected trees of each species over the 2 years. Leaf area was calculated on the basis of leaf length using the regression equations: P. sieboldii:  Y = −0.427X + 0.732X^2, r^2 = 0.997; S. miyabeana:  Y = 0.336X + 0.11X^2, r^2 = 0.988; S. haliteni:  Y = 0.665X + 0.276X^2, r^2 = 0.955; S. sachalinensis:  Y = 0.778X + 0.07X^2, r^2 = 0.976.

Oviposition test

This experiment was designed to separate the effects of leaf volatiles on oviposition behaviour from those of physical leaf traits such as leaf size, shape, and surface texture. Adult moths were obtained from larvae collected from Populus alba in early July and reared in plastic cases (70 x 120 x 30 mm) lined on the bottom with wet paper to keep humidity high. Oviposition was tested in an environmental chamber at 23 °C, L:D 16:8 h. When adults eclosed, a male–female pair was isolated and used immediately for the experiment. Transparent plastic cups, 90 mm in diameter and 40 mm in height, were used to examine oviposition preference and clutch size. Each cup was subdivided 20 mm above the base by a paper mesh and leaf materials were placed in the lower chamber. The pair of adult moths was placed in the upper chamber but could not pass through the mesh and therefore had no direct contact with the leaf materials. Instead, females deposited eggs on the lid and walls of the cup, with an area of ≈120 cm^2. The leaf materials were either wet paper alone or wet paper with one or two leaves of one of the four plant species. Each experiment lasted until the female died, and the number of eggs

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was counted. Twenty replicates were conducted for each of the four plant species and for the control.

Females sometimes added eggs to clutches that had already been deposited, because differential stages of embryogenesis within an egg mass were formed. These additional clutches were separated successfully from the primal clutch and were treated independently in the analysis.

Results and discussion

Clutch size in the field

Clutch size differed significantly among plant species in the field (Kruskal-Wallis test, d.f. = 3, \( H = 813.0, \ P < 0.001; \) Fig. 1a). The largest clutches were on *P. sieboldii* while *S. miyabeana* had the smallest clutches, half the size of those on *P. sieboldii*. The size of leaves bearing eggs also differed significantly among plant species (Kruskal-Wallis test, d.f. = 3, \( H = 539.3, \ P < 0.001; \) Table 1). *Populus sieboldii* with large egg clutches had large leaves, while *S. miyabeana* and *S. sachalinensis* with small clutches had small leaves (Fig. 2). *Salix huleni*, having large leaves, received the second largest clutches among the four plant species. Clutch size was correlated significantly and positively with leaf size when the plant species were pooled (\( n = 46, \ r^2 = 0.52, \ P < 0.001; \) Fig. 2). Significant, though weak, positive correlations were also detected when each plant species was analysed separately, except for *S. huleni* (Table 2).

Oviposition test

The lifespan of female moths was 6.9 ± 1.2 (mean ± SD) days under the experimental conditions, and did not differ among treatments. (ANOVA, d.f. = 4, \( F = 0.85, \ P > 0.05. \) Mating and oviposition were observed in all treatments.

The number of eggs differed significantly among treatments (Kruskal-Wallis test, d.f. = 4, \( H = 9.60, \ P < 0.05; \) Fig. 1b). Females with *P. sieboldii* leaves present laid 2.5 times as many eggs as when leaves were absent (i.e. control). The second highest egg production occurred with *S. miyabeana*, although there was no significant difference between *S. miyabeana* and the control. Egg numbers with *S. huleni* and *S. sachalinensis* leaves were approximately equal to that in the control.

There was no significant difference in clutch size among plant species in the experiment (Kruskal-Wallis test, d.f. = 4, \( H = 3.36, \ P > 0.05; \) Fig. 1c). Most clutches had fewer than 10 eggs, although the largest, in a cup with *P. sieboldii* leaves, contained 29 eggs.

Oviposition preference

Many studies have shown that herbivorous insects use plant odours to recognise their host plants (see References in Visser, 1986). This study also showed that leaf volatiles are important cues for recognition of host plants by *P. sinuella*. Females were more stimulated by the volatiles from host plants than by the volatiles from non-host plants. The difference in egg numbers deposited between host and non-host plants cannot be explained by female lifespan because this did not differ among treatments. Because contact-chemicals on the leaf surface also play an important role for host recognition in some insects (Nishida, 1995), female *P. sinuella* may use both volatiles and contact-chemicals of host plants as oviposition cues. In addition,

![Fig. 1](image-url) **Fig. 1.** (a) Mean clutch size of *Paraueucoptera sinuella* in the field. Different letters show significant differences (Tukey-type multiple comparisons, \( P < 0.05 \)). (b) Mean number of eggs per female in the oviposition test. (c) Mean clutch size in the oviposition test. Vertical bars show SE. *Indicates significant difference when compared with control (non-parametric Dunnet test, \( P < 0.05 \).)

*P. sinuella.* Females were more stimulated by the volatiles from host plants than by the volatiles from non-host plants. The difference in egg numbers deposited between host and non-host plants cannot be explained by female lifespan because this did not differ among treatments. Because contact-chemicals on the leaf surface also play an important role for host recognition in some insects (Nishida, 1995), female *P. sinuella* may use both volatiles and contact-chemicals of host plants as oviposition cues. In addition,

**Table 1.** Mean leaf area ±SE of four plant species. Different letters in the column show significant differences (Tukey-type multiple comparisons, \( P < 0.05 \)). Data for *P. sieboldii* and *S. miyabeana* are from Kagata and Ohgushi (2002).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Mean leaf area (cm²)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. sieboldii</em></td>
<td>19.3 ± 0.24 a</td>
<td>4.1–55.8</td>
</tr>
<tr>
<td><em>S. miyabeana</em></td>
<td>9.1 ± 0.10 b</td>
<td>3.2–20.2</td>
</tr>
<tr>
<td><em>S. huleni</em></td>
<td>17.6 ± 0.27 a</td>
<td>5.6–36.3</td>
</tr>
<tr>
<td><em>S. sachalinensis</em></td>
<td>9.0 ± 0.15 b</td>
<td>3.2–20.7</td>
</tr>
</tbody>
</table>
deterrent chemicals may also be important for discrimination between host and non-host plants (Huang & Renwick, 1993). If the non-host plants, S. hultenii and S. sachalinensis, have deterrent volatiles, females would be expected to avoid oviposition on these plants, but this is not the case. No deterrent effects of leaf volatiles on oviposition behaviour were detected.

The oviposition experiment showed that the most preferred plant for oviposition was P. sieboldii, and that S. miyabeana was the second preferred plant although it did not receive significantly more eggs than the control. The oviposition choice experiment using fresh leaves showed the same preference hierarchy, which corresponds with larval performance (Kagata & Oghushi, 2001). It is expected that natural selection will favour a good correlation between oviposition preference and larval performance, and this has been documented in several herbivorous insects (Nylin, 1988; Craig et al., 1989; Barker & Maczka, 1996; but see Thompson, 1988). It is not clear, however, why female P. sinuella lay eggs on non-host plants in the field, because under experimental conditions females could distinguish host plants from non-host plants.

Clutch size determination

Grant and Langev (1995) found that stimulating extracts of host and non-host plants had no effects on the clutch size of the spruce budworm Choristoneura fumiferana. In contrast, they found that the clutch size of the jack pine budworm C. pinus increased in response to a stimulant extract and decreased in response to a deterrent extract of jack pine hosts. This indicates that clutch size is determined by chemical stimuli without physical stimuli in some insect species. In the present study, the effect of leaf volatiles on determination of clutch size was separated successfully from that of physical stimuli. The results showed that clutch size of P. sinuella was not affected by volatile chemicals emitted from leaves of either host or non-host plants, although there remains the possibility that chemical substances on the leaf surface affect oviposition behaviour.

In contrast to volatile chemicals, a positive correlation was found between clutch size and leaf area in P. sieboldii, S. miyabeana, and S. sachalinensis, and when all plant species were pooled. Therefore, the difference in clutch size among plant species could be explained by the difference in resource abundance (i.e. leaf size) for offspring, depending on plant species. This agrees with several studies showing that clutch size was adjusted by ovipositing females in response to differences in resource abundance among host plants (Godfray, 1986; Vasconcellos-Neto & Monteiro, 1993; Ekbom, 1998). Furthermore, the present study showed that the clutch size did not differ depending on preference hierarchy of plant species, and that the positive correlation was detected between leaf size and clutch size even in the non-host plant S. sachalinensis. These results indicate that females adjust clutch size in response to leaf size, independent of chemical cues used in host-plant recognition.

Previous studies on clutch size determination have sought the relationship between resource availability and clutch size to test optimal models of clutch size, which assume that resource limitation is the most important selective force. Less attention has been paid to mechanisms by which clutch size is determined in the oviposition process. When testing clutch size adjustment across different plant species, field observations would not provide a clue to determine whether clutch size is adjusted according to plant species or resource abundance, in spite of the positive correlation between clutch size and resource abundance. The present study illustrated that clutch size in the leaf miner was determined by leaf size, not affected by plant species, to which ovipositing females have preference hierarchy. This indicates that the distinction of the two oviposition processes is a more appropriate and powerful approach to understanding clutch size adjustment in herbivorous insects.

Acknowledgements

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Table 2. Regression summary of clutch size on leaf area in four plant species. ***P < 0.001, NS = P > 0.05. Data for P. sieboldii and S. miyabeana are from Kagata and Oghushi (2002).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Sample size</th>
<th>Regression coefficient</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. sieboldii</td>
<td>1038</td>
<td>0.07</td>
<td>0.07***</td>
</tr>
<tr>
<td>S. miyabeana</td>
<td>742</td>
<td>0.07</td>
<td>0.05***</td>
</tr>
<tr>
<td>S. hultenii</td>
<td>671</td>
<td>-0.01</td>
<td>&lt;0.01 NS</td>
</tr>
<tr>
<td>S. sachalinensis</td>
<td>411</td>
<td>0.11</td>
<td>0.09***</td>
</tr>
</tbody>
</table>
References


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