Weak parasitoid-mediated apparent competition between two *Phyllonorycter* (Lepidoptera: Gracillariidae) leafminer species on a deciduous oak *Quercus dentata*

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Running title: Apparent competition in *Phyllonorycter*

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Abstract

Parasitoid assemblages and the rates of parasitism on tissue-feeding larvae of two *Phyllonorycter* leafminer species, *P. persimilis* and *P. leucocorona*, were studied from the autumn generation in 2002 to the summer generation in 2005 to understand whether parasitoids mediate interactions of the two host species. Fourteen species of parasitoids emerged from *P. persimilis*, and 11species emerged from *P. leucocorona*. The parasitism rate was high; i.e., 24.1 -92.6 % in *P. persimilis* and 58.9 -81.7 % in *P. leucocorona*. Thus, parasitism was a major mortality factor in the present *Phyllonorycter* species. The parasitoid composition was distinctly different between the two host species, although most parasitoids were able to parasitize both leafminer species. The analysis based on the quantitative parasitoid overlap revealed that the present parasitoids could mediate interactions between the present leafminer species, but their effects would be weak. This is attributable to that most parasitoids preferentially parasitize either of the leafminer species.

Key words: parasitism, tissue-feeding larvae, wasps.

INTRODUCTION

Parasitoids are major enemies of many insects and are capable to mediate interspecific interactions of hosts. For example, an increase in abundance of one host species may increase the parasitic rate on another species by increasing the local abundance of shared parasitoids, a situation termed as "apparent competition" (Holt 1977; Holt & Kotler 1987; Holt & Lawton 1994). Bonsall and Hassell (1997) revealed with a laboratory system consisting of one parasitoid and two host species that one of the host species is eliminated owing to the effect of apparent competition. In addition, Morris *et al*. (2004) revealed that apparent competition occurs between leafminers in a tropical ecosystem, and Rott and Godfray (2000) suggested that parasitoids have a potential to mediate interspecific interactions of leafminers in a temperate ecosystem. However, it is still uncertain whether parasitoids are important agents mediating apparent competition or not. For example, host specialization, a characteristic that has often been reported for parasitoid species (Waage & Hassell 1982; Miller & Ehler 1990), would lower their capability to mediate apparent competition.

In this study, we assessed the potential of parasitoids as agents mediating indirect interactions of two leafmining species, *Phyllonorycter leucocorona* (Kumata) and *P. persimilis* Fujihara, Sato et Kumata, on a deciduous oak *Quercus dentata* Thunberg in northern Japan. These two *Phyllonorycter* species are predominant leafminers on this oak and are attacked by a number of parasitoid species (Sato 1986, 1990, 1995; Shibata *et al*. 2001; Ishida *et al*. 2003, 2004; Kitamura *et al*. 2007; Nakamura *et al*. 2008).

METHODS

Study site and leafminers

The present study was carried out in a *Q. dentata* forest on Ishikari Coast (43° 12’ N, 141° 19’ E) in Hokkaido, northern Japan. In this area, nearly pure forests of *Q. dentata* develop along the seashore (Ishida *et al*. 2004). Trees in this area, especially near the forest edge at the seashore, are dwarfed, perhaps because of winds from the sea.

Leafminers on this species of oak in the study area are mainly Lepidoptera; two species of *Phyllonorycter* (Gracillariidae), one species of *Caloptilia* (Gracillariidae), three species of *Stigmella* (Nepticulidae), and two species of *Tischeria* (Tischeriidae) (Sato 1990; Shibata *et al*. 2001; Ishida *et al*. 2003, 2004; Nakamura *et al*. 2008). In addition, two species of the family Tenthredinidae (Hymenoptera) are also known (Sato 1991; Shibata *et al*. 2001). In this study, parasitism was examined for the two dominant leafminer species, *Phyllonorycter persimilis* and *P. leucocorona*.

The larval stage of *Phyllonorycter* species is divided into two in terms of feeding type: sap-feeding and tissue-feeding stages. The two *Phyllonorycter* species are hardly discriminated from each other at the sap-feeding stage, but they are easily distinguished at the tissue-feeding stage; fully-expanded mines of *P. leucocorona* are about one-fourth of those of *P. persimilis* in size, and mature larvae of *P. leucocorona* make a pupal chamber of frass (Sato 1986).

These two *Phyllonorycter* species produce two (summer and autumn) generations in a year (Sato 1990). Summer generation mines begin to appear from mid June, and larvae within the mines grow to the tissue-feeding stage in early July. They pupate at the end of July, and adults emerge from the mines in August. Autumn generation mines occur from late August. In mid September sap-feeding larvae grow to the tissue-feeding stage, and pupate by early November. They overwinter as pupae, and emerge from mines in early June of the next year.

Sampling

Some hundred leaves with *Phyllonorycter* mines of the tissue-feeding stage were collected from 6-9 trees at the study site for six generations, from the autumn generation in 2002 to the summer generation in 2005. In 2003, *P. leucocorona* mines of the summer generation were not collected because of our circumstances. Sampling of the summer generation was made in late July or early August, and that of the autumn generation was made in early October.

For the summer generation, collected leaves were brought back to the laboratory, and individual mines were chopped off and reared in plastic cases under outdoor conditions. For the autumn generation, collected leaves were left outdoors until next spring, and then mines were reared as above. *Phyllonorycter* adults and parasitoid wasps having emerged from the mines were identified and counted.

Host density

To assess the densities of tissue-feeding larvae of *P. persimilis* and *P. leucocorona* in this site, 50 “sun” leaves were randomly collected from the canopy area (2-5 m above the ground) of 25 trees growing in this site in early October (Nakamura *et al*. 2008). Collected leaves were examined for the number of tissue-feeding mines and area. Mines were discriminated into the summer and autumn generations according to the color and conditions. Leaf area was measured using an image processor (Image J program developed by the US National Institutes of Health) after leaf outline was scanned using an image scanner (CanoScan LiDE 60, Canon Co., Tokyo, Japan). The density of mines was given as the number of mines per unit leaf area (100 cm^2).

Data analysis

Similarity of parasitoid assemblages between samples of two *Phyllonorycter* species at different generations and years is evaluated by the Horn's (1966) measurement of

$$R_0 = \frac{\sum_i (n_{ij} + n_{ij'}) \log (n_{ij} + n_{ij'}) - \sum_i n_{ij} \log n_{ij} - \sum_i n_{ij'} \log n_{ij'}}{(N_j + N_{j'}) \log (N_j + N_{j'}) - N_j \log N_j - N_{j'} \log N_{j'}}$$

overlap:

where n_{ij} (or $n_{ij'}$) = number of individuals of parasitoid species i in sample j (or j'), and N_j (or $N_{j'}$) = total number of individuals in sample j (or j'). The resulting similarity matrix was reduced to a dendrogram by UPGMA (unweighted pair-group method using arithmetic average; Sneath & Sokal 1973).

To assess the potential for indirect interactions between two host species, quantitative parasitoid overlap between the two host species was calculated with the following equation (Müller *et al*. 1999; Rott & Godfray 2000),

$$d_{ij}[t] = \sum_k \left[\frac{\alpha_{ik}[t]}{\sum_l \alpha_{il}[t]} \frac{\alpha_{jk}[t-1]}{\sum_k \alpha_{mk}[t-1]} \right]$$

where $\alpha_{ik}[t]$ is the strength of the link between host *i* and parasitoid *k* in generation *t*. $\alpha_{ik}[t]$ was calculated from the rate of parasitism by parasitoid *k* on host *i* and the density of host *i* in generation *t*. The quantity $d_{ij}[t]$ summarizes interactions between two hosts via all possible shared parasitoids and hence the outer summation is taken over all parasitoids. The first quantity within the square bracket represents the relative importance of species *k* as a parasitoid of host *i* in the generation *t* and the second quantity is the fraction of parasitoid species *k* that developed on host species *j* in the previous generation.

RESULTS

Parasitoid complexes

Fourteen species of wasps belonging to four families (Ichneumonidae, Pteromalidae, Encyrtidae and Eulophidae) emerged from 1250 *Phyllonorycter persimilis* mines, and 11 species belonging to three families (Ichneumonidae, Encyrtidae and Eulophidae) emerged from 617 *P. leucocorona* mines (Table 1). The rate of parasitism considerably varied from generation to generation in *P. persimilis* (24.1 - 92.6 %), but less varied in *P. leucocorona* (58.9 - 81.7 %).

The composition of parasitoid species was clearly different between the two *Phyllonorycter* species (Fig. 1), although most parasitoids attacked both host species. Major parasitoids were *Sympiesis sericeicornis*, *Ageniaspis* sp. and *Achrysocharoides* sp. A in *P. persimilis*, whereas *Achrysocharoides* sp. B and *Cirrospilus diallus* in *P.*

leucocorona (Table 1). On the other hand, the composition of parasitoid species was similar between the summer and autumn generations in each species (Fig. 1).

Potential for indirect interactions

The quantitative parasitoid overlap between the two leafminer species, d_{ij}, was given in Table 2. This measure represents the importance of species *j* in a generation as a source of parasitoids attacking species *i* in the following generation. The measure d_{ii} represents the importance of species *i* in a generation as a source of parasitoids attacking conspecifics in the following generation. In *P. persimilis*, d_{ij} was larger than d_{ii} in the autumn generation of 2004 and the summer generation of 2005, indicating the potential for apparent competition. In these two cases, however, d_{ij} was only slightly larger than d_{ii}, whereas d_{ii} was much larger than d_{ij} in the other one case. In *P. leucocorona*, on the other hand, d_{ii} was always much larger than d_{ij} in two cases and slightly larger in one case. Thus, apparent competition could occur between these leafminers, but it would be weak.

DISCUSSION

In this paper, we report parasitoid composition in two *Phyllonorycter* leafminer species on *Quercus dentata* leaves, *P. persimilis* and *P. leucocorona*, at the tissue-feeding stage. At an earlier developmental stage (the sap-feeding stage), these two leafminer species cannot be discriminated, and therefore it is difficult to examine the parasitoid composition separately for the two leafminer species. Thus, the present data did not cover all parasitoids that attack the present *Phyllonorycter* species, but it is certain that parasitism is a major mortality factor of these leafminer species; i.e. about two third of tissue-feeding larvae were killed by parasitoids.

The analysis based on the quantitative parasitoid overlap between the present two leafminer species revealed that the present parasitoid complexes have a potential to mediate the interactions of host leafminers. Such potential has also been reported for a number of polyphagous predators and parasitoids (Hochberg & Hawkins 1992; Holt & Lawton 1994; Müller *et al*. 1999; Rott & Godfray 2000; Chaneton & Bonsall 2000; Lewis *et al*. 2002; Morris *et al*. 2004). In the present leafminers, however, apparent competition seems to be weak at least at the tissue-feeding stage. This is because most parasitoids, especially the dominant and common ones, preferentially parasitize either of the host species.

It is not known why these parasitoids prefer either of the host species, but there are some hypotheses. First, the two *Phyllonorycter* species may differ in resistance to parasitoids. According to the theoretical study of Jokela *et al*. (2000), however, resistance may not evolve at all in hosts like the present *Phyllonorycter* species that are attacked by various types of parasitoids. Another hypothesis is concerned with the difference in the position of mines between the two host species. Sato (1986, 1991) observed that *P. persimilis* makes mines at the mid position of leaves whereas *P. leucocorona* does at the margin or base of leaves. Therefore, differential host use could arise, if parasitoids preferentially search hosts at either of mid or marginal positions of leaves. In addition, the difference of body size between the host species (*P. persimilis* is larger than *P. leucocorona*; Sato 1986) may affect the host selection of parasitoids. For example, large-bodied parasitoids may avoid exploiting smaller species. It is also possible that parasitoids have differentiated in host use to avoid interspecific competition between them. In fact, competition would be severe among the present parasitoid species, since the parasitic rate is high.

It is also interesting how so many parasitoid species coexist on the present host species. Theory suggests that their coexistence is possible if competitive ability and some life history characteristics (e.g., host finding ability) are under trade-offs (Bonsall *et al*. 2002). This condition is likely if parasitoids differ in the host stage they attack; i.e., parasitoids that attack small early-stage hosts may confront with a difficulty in finding hosts but may have competitive advantages owing to priority effects. This theory may explain the present diversity of parasitoids, since the present parasitoid species on each host vary in the host stage they preferentially attack (Sato 1990, 1995).

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Table 1 Rates (%) of parasitism by each parasitoid species on tissue-feeding larvae of *Phyllonorycter persimilis* and *P. leucocorona* from the autumn generation in 2002 to the summer generation in 2005. The total number of mines collected and the host density was also given. S=summer generation; A=autumn generation.

	Phyllonorycter persimilis						*Phyllonorycter leucocorona*					
	2002	2003		2004		2005	2002	2003		2004		2005
Family/Subfamily/Species	A	S	A	S	A	S	A	S	A	S	A	S
Ichneumonidae												
Unidentified species	6.7	0.2	2.5	-	5.2	2.6	-		2.8	-	4.4	-
Pteromalidae												
Pteromalus sp.	-	0.2	-	-	-	-	-		-	-	-	-
Encyrtidae												
Ageniaspis sp.	9.9	12.9	25.9	17.7	1.7	21.3	8.3		9.9	1.9	1.8	2.2
Eulophidae												
Eulophinae												
Elachertus fenestratus (Walker)	0.3	2.4	2.5	-	-	-	3.3		-	-	-	-
Cirrospilus diallus Walker	0.3	2.5	2.5	3.2	1.7	2.6	3.3		9.9	5.6	10.0	7.7
Cirrospilus lyncus Walker	-	0.7	2.5	1.6	-	-	-		7.0	1.9	2.3	3.3
Sympiesis sericeicornis (Nees)	52.5	62.3	14.8	22.6	10.3	15.5	1.7		4.2	9.3	1.8	1.1

Pnigalio sp.	0.3	2.0	-	1.6	-	-	-		-	-	-	-
Entedontinae												
Pleurotroppopsis japonica (Kamijo)	-	0.2	-	-	-	-	-		-	-	-	-
Chrysocharis laomedon (Walker)	-	0.4	-	-	-	-	-		-	-	0.3	-
Chrysocharis ujiyei Kamijo	-	-	-	-	1.7	-	-		11.3	-	7.3	-
Achrysocharoides sp. A	0.9	8.2	29.6	17.7	-	7.1	-		-	1.9	-	1.1
Achrysocharoides sp. B	0.3	0.2	2.5	1.6	3.4	1.3	48.3		36.6	50.0	31.1	58.2
Chrysonotomyia sp.	-	-	-	-	-	-	-		-	-	-	1.1
Closterocerus trifasciatus Westwood	-	0.5	-	-	-	-	-		-	-	-	-
Total parasitism (%)	71.1	92.6	82.7	66.1	24.1	50.3	65.0		81.7	70.4	58.9	74.7
Total number of mines collected	343	510	81	62	58	155	60		71	54	341	91
Host density (per 100 cm^2 of leaf)	0.44	0.32	0.09	0.02	0.04	0.05	0.12	0.27	0.02	0.10	0.03	0.07

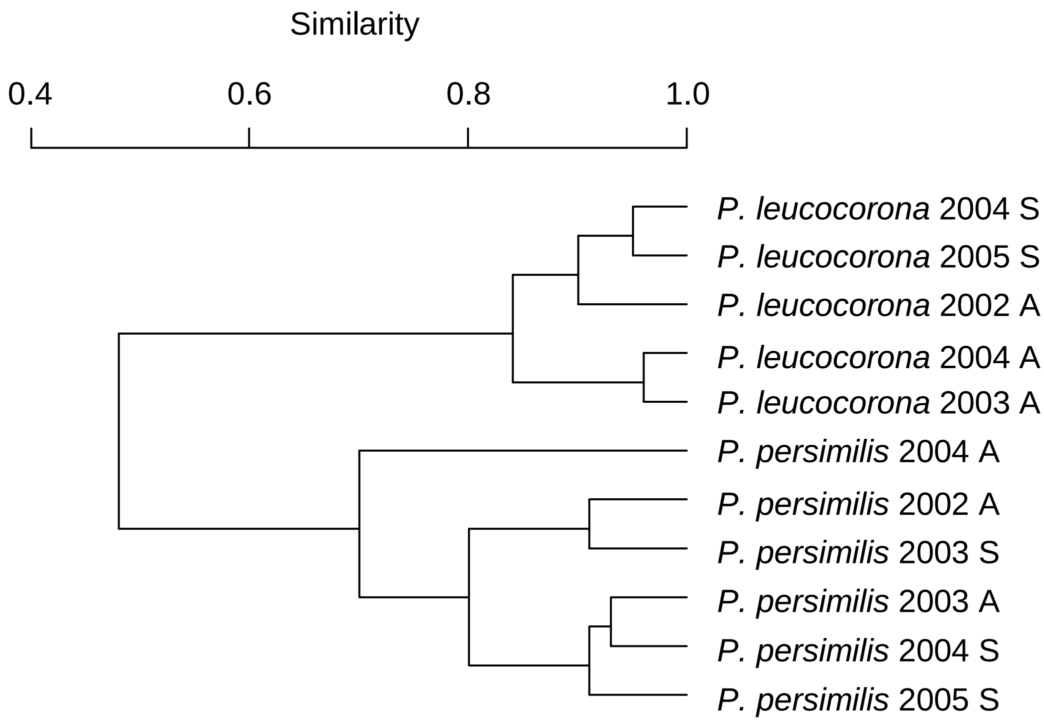
Table 2 Quantitative effects of parasitoids at generation *t*-1 on parasitism at generation *t*. S: summer generation, A: autumn generation.

species *i*	2004 S		2004 A		2005 S	
	d_{ii}	d_{ij}	d_{ii}	d_{ij}	d_{ii}	d_{ij}
P. persimilis	0.888	0.088	0.227	0.488	0.305	0.554
P. leucocorona	0.590	0.410	0.730	0.066	0.925	0.045

Figure legend

Figure 1 UPGMA analysis on the composition of parasitoid species. S=summer generation, A=autumn generation.

Fig. 1


Similarity
0.4
0.6
0.8
1.0
P. leucocorona 2004 S
P. leucocorona 2005 S
P. leucocorona 2002 A
P. leucocorona 2004 A
P. leucocorona 2003 A
P. persimilis 2004 A
P. persimilis 2002 A
P. persimilis 2003 S
P. persimilis 2003 A
P. persimilis 2004 S
P. persimilis 2005 S