INTRODUCTION

The serpentine leafminer, *Liriomyza trifolii* (Burgess), is one of the most serious pests of various floricultural and vegetable crops, because this species has developed resistance to chemical insecticides (Keil and Parrella, 1990; Sher et al., 2000). Thus, several biological control agents have been used for *L. trifolii* control in greenhouses (Parrella et al., 1982; Harris et al., 1990; Williams and Walters, 1994). In particular, the ectoparasitic wasp, *Diglyphus isaea* (Walker), and the endoparasitic wasp, *Dacnusa sibirica* Telenga, are used as commercial biological control agents against *L. trifolii* in many countries (Ravensberg, 1992; van Lenteren, 1995). The two parasitoid species are different from each other in many ecological characteristics. *D. sibirica* is an endoparasitic, proovigenic, and koinobiont wasp. On the other hand, *D. isaea* is an ectoparasitic, synovigenic, and idiobiont wasp. Furthermore, *D. sibirica* attacks all instars of host larvae, while *D. isaea* prefers later stage larvae for oviposition (Minkenberg and van Lenteren, 1986).

The European strains of *D. isaea* and *D. sibirica* are frequently used simultaneously as biological control agents in greenhouses. If these parasitic wasps are used simultaneously, then we can expect that multiple parasitism occurs between them. Such multiple parasitism may decrease the activity of parasitoid species for biological control. However, the influence of multiple parasitism between the two parasitoid species has not been studied. Furthermore, we assume that these two parasitic wasps interact with several native parasitic wasps both in greenhouses and in the field (Saito et al., 1996; Ozawa et al., 1999). For example, *Hemiptarsenus varicornis* (Girault) is one of the most dominant parasitoids observed in Japan against *L. trifolii* in commercial tomato greenhouses. This wasp species is easily found in greenhouses where the European strains of *D. isaea* and *D. sibirica* are released (Saito et al., 1996). As in *D. isaea*, *H. varicornis* is an ectoparasitic, synovigenic, idiobiont, and later-stage-larvae preferring wasp.

In many cases, when a solitary parasitic wasp larva meets another wasp larva on a host, they fight each other until the death of one (e.g., Godfray,
Many researchers have noted such competitive relationships between solitary parasitic wasps (e.g., Leveque et al., 1993; Gauthier et al., 1999). Interestingly, interspecific competition is highly asymmetric in almost all parasitoid-parasitoid combinations reported (e.g., Leveque et al., 1993; Gauthier et al., 1999). Especially, almost all ectoparasitic wasps are idiobiont parasitoids. Then, when an ectoparasitoid attacks the host parasitized by an endoparasitoid, it kills the both host larva and endoparasitoid larva simultaneously. On the other hand, many endoparasitic wasps are koinobiont parasitoids. So, an endoparasitoid does not attack the host parasitized by an ectoparasitoid. As a rule, when multiple parasitism between the ectoparasitoid and the endoparasitoid occurs, the ectoparasitic wasp always wins.

Many superior species do not have a competition avoidance mechanism, while almost all inferior species do (e.g., Leveque et al., 1993; Gauthier et al., 1999). In our situation, the endoparasitic, and competitive-inferior species, D. sibirica shows competition avoidance of the sympatric ectoparasitic, and the competitive-superior species, D. isaea, but two ectoparasitic, and competitive-superior wasps, D. isaea and H. varicornis show no competition avoidance of D. sibirica. That is, the two ectoparasitic wasps did not show any oviposition and host-feeding preference between parasitized and non-parasitized hosts by D. sibirica (Mitsunaga and Yano, unpublished). We assume the driving force of such asymmetric competition avoidance mechanisms is a difference in the probability of survival for competition by parasitoid species. Until now, the probability of survival for competition for superior species has not been examined.

The ectoparasitic wasps beat the endoparasitic wasp in multiple parasitism, but the ectoparasitoid may pay the cost. We can assume the possible cost for multiple parasitism as follows: (1) The ectoparasitic wasp may increase the handling time in attacking parasitized hosts by endoparasitoids. In general, handling time is one of the most important components for lifetime fitness of parasitoids (e.g., Hassell, 1978). (2) The host parasitized by endoparasitoids may be a low-quality host for the ectoparasitic wasp. For example, the development may become slow and survival rate of ectoparasitoid larvae may decrease on the parasitized hosts by endoparasitoids. The size of newly emerged ectoparasitoid may be reduced when they are reared on the parasitized hosts by endoparasitoids. The size of parasitic wasps strongly relates to fecundity (e.g., King, 1987; Godfray, 1994). In this paper, we conducted experiments to examine the possible costs of superior species in multiple parasitism. Moreover, we discuss the relationship between the influence of multiple parasitism and successful biological control.

MATERIALS AND METHODS

Insect rearing. All insects were maintained and tested in an environmental chamber controlled at 25±1°C, 50±10% r.h., and a photoperiod of L14: D10. L. trifolii was collected in Hamamatsu, Shizuoka Prefecture, Japan, in 1991. This strain has been kept under the laboratory conditions for more than 100 generations between collection and the beginning of the experiments. The laboratory culture was reared on cotyledons of kidney bean, Phaseolus vulgaris L. All parasitoid species were reared on L. trifolii larvae in the laboratory. The strains of D. isaea and D. sibirica were purchased from Tomen Corporation (Tokyo, Japan). These parasitic wasps were kept in the laboratory for more than 10 generations between the purchase and the beginning of the experiments. The strain of H. varicornis was collected in Iwata, Shizuoka Prefecture, Japan in 1995. This strain was kept in the laboratory setting for more than 50 generations between the collection and the beginning of the experiments.

Preparation of wasps and hosts for experiments. D. isaea and H. varicornis were reared on third-instar leafminer larvae. After emergence, female wasps were placed with three males and a 50% water solution of honey in a plastic cylinder for insemination (15 cm diam.×12 cm long). One day later, they were used for the experiments.

Two groups of host leafminer larvae were prepared. One group was composed of healthy third-instar L. trifolii larvae (H type host). The other group was composed of third-instar L. trifolii larvae parasitized by D. sibirica three days before the experiments (P type host). To prepare P type hosts, we simultaneously placed one leaf infested with ca. 40 first-instar leafminer larvae, 10 inseminated females of D. sibirica, and a small cotton soaked with honey inside a plastic cylinder (15 cm diame-
One day later, wasps were removed. The parasitization rate was about 95% (total parasitized host 119/total host 126). *D. sibirica* females prefer the younger-stage host larvae (e.g., Mitsunaga and Yano, unpublished) and the parasitization decreases when later-stage host larvae are supplied. The larvae of *D. sibirica* do not develop until leafminer hosts pupate (Croft and Copland, 1994). Thus we assumed that the host deterioration did not occur between preparation and the experiment. For experiments, we cut leaves squares of 3 cm×3 cm with a single host on each leaf.

**Experiment 1: Handling time.** The differences in handling time were examined between the *H* type hosts and *P* type hosts. The wasp behavior was observed with binoculars connected to a video recorder and monitor system. An inseminated *D. isaea* or *H. varicornis* female was placed with a *H* type host or a *P* type host in a glass cylinder (4 cm diam.×2 cm long). Then, the time from the start of drumming on the host to the end of the oviposition of wasp was measured as the handling time (c.f. Finidori-Logli et al., 1996). The handling time was recorded with a video recorder. To avoid time wasted by incapable wasps, each trial was performed for 30 min with about 80 percent of all wasps ovipositing within this time interval on both host types. Twenty replications per host type and per wasp species were performed. The handling time was interpreted by using a univariate ANOVA to evaluate the effect of host type.

**Experiment 2: Mortality, sex ratio, developmental period, and body size of newly emergent wasps.** Several life history traits of wasps emerging on *H* type hosts were compared to those of wasps emerging on *P* type hosts. An inseminated *D. isaea* or *H. varicornis* female was placed with a *H* type host or a *P* type host in a glass cylinder (4 cm diam.×2 cm long). After parasitization, the female wasp was removed and the developmental period until emergence, the sex and the length of hind tibia of newly emerged adults were recorded. Thirty replications per host type and per wasp species were performed and G-tests conducted for comparison of mortality and sex ratio (Sokal and Rohlf, 1981). Sex ratio was defined as the proportion of males. The developmental period after the log-transformation was interpreted using a two-factor ANOVA to evaluate the effect of host type and sex of newly emerged wasps. The body size of newly emerged wasps was compared by a two-factor ANCOVA to evaluate the effect of host type and sex of newly emergence with the developmental period as a covariate. The body size was estimated by the length of the hind tibia.

**RESULTS AND DISCUSSION**

**Handling time**

Females of *D. isaea* and *H. varicornis* made no distinction between *H* type hosts and *P* type hosts in handling time (the result of univariate ANOVA: *D. isaea*; MS=27,180.300, *F*<sub>1,28</sub>=0.222, *p*=0.641, *H. varicornis*; MS=154,464.215, *F*<sub>1,28</sub>=1.729, *p*=0.199) (Fig. 1). In the two-way choice experiment, *D. isaea* and *H. varicornis* females showed no host preference regardless of parasitization by *D. sibirica* (Mitsunaga and Yano, unpublished). Thus, we conclude that the oviposition behaviour of two competitively superior species, *D. isaea* and *H. varicornis* was not affected by parasitization by the competitively inferior species, *D. sibirica*.

When an ectoparasitoid female attacks a host, it kills the host in almost all cases (e.g., Godfray, 1994). If the hosts attacked by ectoparasitic wasps have already been parasitized by endoparasitic wasps, the larvae of endoparasitic wasps may be simultaneously killed with the hosts (e.g. Rosenheim et al., 1995). Thus, we can easily assume that competition avoidance of endoparasitoids need not have evolved for ectoparasitoids in almost all cases.

![Graph](https://via.placeholder.com/150)

**Fig. 1.** The handling time of *Diglyphus isaea* and *Hemiptarsenus varicornis* for *P* type hosts and *H* type hosts.
Mortality, sex ratio, developmental period, and body size of newly emergent wasps

The mortality and sex ratio of *D. isaea* and *H. varicornis* did not differ between the H and P groups (Table 1). The developmental period of the two ectoparasitic wasps was not affected by the presence of *D. sibirica* in the hosts (Table 2 and Fig. 2). Furthermore, the host type did not affect the length of the hind tibia of the two ectoparasitic wasps (Table 3 and Fig. 3). Thus, we conclude that several characteristics related to adaptive strategy of the two ectoparasitic wasps are independent of the presence of *D. sibirica* in the hosts.

In many studies on interspecific interaction between parasitoids, several kinds of competition avoidance mechanisms by the inferior species have been reported (e.g., McBrien and Mackauer, 1989; van Alebeek et al., 1993; Leveque et al., 1993; Gauthier et al., 1999). On the other hand, competition avoidance by superior species has not been observed. Such an asymmetric response to interspecific competition has been explained by a difference in survival rate between winner and loser (e.g., van Alebeek et al., 1993; Gauthier et al., 1999). However, almost no studies have measured

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**Table 1.** The number emerged and sex ratio of two ectoparasitoids, *Diglyphus isaea* and *Hemiptarsenus varicornis* from two types of hosts. Sex ratio is defined as the proportion of males. G-tests were conducted for comparison (Sokal and Rohlf, 1981). No comparisons showed significant differences.

<table>
<thead>
<tr>
<th></th>
<th>P type</th>
<th>H type</th>
<th>G-value</th>
<th>p-value</th>
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<tr>
<td><em>Diglyphus isaea</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Emerged</td>
<td>27</td>
<td>25</td>
<td>0.582</td>
<td>0.445</td>
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<tr>
<td>Not Emerged</td>
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<td>5</td>
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<td></td>
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<tr>
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<td>10/25</td>
<td>0.350</td>
<td>0.554</td>
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<tr>
<td><em>Hemiptarsenus varicornis</em></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Emerged</td>
<td>22</td>
<td>26</td>
<td>1.693</td>
<td>0.193</td>
</tr>
<tr>
<td>Not Emerged</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex ratio</td>
<td>9/22</td>
<td>10/26</td>
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<td>0.863</td>
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</table>

**Table 2.** Results of the ANOVA for effects of host type and sex on the developmental periods of two ectoparasitoids, *Diglyphus isaea* and *Hemiptarsenus varicornis*. Logarithmic transformation was conducted for the comparison (Sokal and Rohlf, 1981).

<table>
<thead>
<tr>
<th></th>
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<th>Mean square</th>
<th>F-value</th>
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<tr>
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<tr>
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<td>1.121</td>
<td>0.2951</td>
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<tr>
<td>Sex</td>
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<td>1.956</td>
<td>0.1684</td>
</tr>
<tr>
<td>Host type×Sex</td>
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<td>0.002</td>
<td>0.155</td>
<td>0.6954</td>
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<tr>
<td>Residuals</td>
<td>48</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hemiptarsenus varicornis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Host type</td>
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<td>0.004</td>
<td>0.246</td>
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</tr>
<tr>
<td>Sex</td>
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<td>0.040</td>
<td>2.564</td>
<td>0.1165</td>
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<tr>
<td>Host type×Sex</td>
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<td>Residuals</td>
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**Fig. 2.** Mean developmental period for both sexes of *Diglyphus isaea* and *Hemiptarsenus varicornis* in each host type. Bars indicate the standard errors.

**Fig. 3.** Mean length of the hind tibia of emerged males and females of *Diglyphus isaea* and *Hemiptarsenus varicornis* in each host type. Bars indicate the standard errors.
other costs of competition, such as decrease in adult body size, increase in developmental period, and change in sex ratio, of the winner. Thus, this study is one of the first reports on the cost to the winner of interspecific interaction between parasitoids.

In Japan, *D. isaea* and *D. sibirica* are frequently used simultaneously as biological control agents in greenhouses. In our experiments, multiple parasitism did not influence the activity of *D. isaea*. On the other hand, the activity of *D. sibirica* strongly decreased in the presence of *D. isaea* (Mitsunaga and Yano, unpublished). Thus, the effect of simultaneous usage of *D. isaea* and *D. sibirica* on successful biological control may be almost the same as that of single usage of *D. isaea* when the environmental conditions are suitable for *D. isaea*.

At present, many species of parasitic wasps are produced commercially and imported to other countries as biological control agents in many countries. Such imported wasps will interact with native parasitic wasps in many environments. The influences to interspecific competition by imported natural enemies on native natural enemies are one of the most serious environmental problems in utilization of natural enemies (Elliott et al., 1996; Hirose, 1999). In the case of *L. trifolii* parasitoids, all dominant native species are ectoparasitoids in Japan (Konishi, personal communication). As mentioned above, when an ectoparasitic wasp fights an endoparasitic wasp, the ectoparasitic wasp always wins. Furthermore, ectoparasitic wasps show no observable costs for multiple parasitism. Thus, *D. sibirica*, an imported endoparasitoid, is considered to be a safe biological control agent for use in Japan.

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