

Interspecific competition between the ichneumonid *Campoletis chlorideae* and the braconid *Microplitis mediator* in their host *Helicoverpa armigera*

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Abstract

We investigated interspecific competition between *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) and *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) in their host, the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) under laboratory conditions. Cotton bollworm larvae were allowed to be parasitized by both wasp species simultaneously or sequentially at different time intervals. When host larvae were parasitized simultaneously by both parasitoids, the majority of the cocoons produced were of *M. mediator*. When host larvae were parasitized initially by *M. mediator* followed by *C. chlorideae* at 12 or 24 h, parasitoids emerging from the multiparasitized hosts were mainly *M. mediator*. In contrast, when host larvae were parasitized initially by *C. chlorideae*, followed by *M. mediator* 12 or 24 h later, parasitoids emerging from the multiparasitized hosts were mainly *C. chlorideae*. Dissections of host larvae at various time intervals after parasitization by the two parasitoids showed that first instars of *M. mediator* could physically attack the larvae of *C. chlorideae*, but not the eggs of *C. chlorideae*. When a host was parasitized by both wasp species sequentially, more host larvae died and the number of wasp offspring was significantly reduced compared to a host parasitized by only one wasp. Conversely, in simultaneous multiparasitism, the host mortality and wasp offspring production were not significantly different from those parasitized by single wasp species.

Introduction

Multiparasitism is a natural phenomenon that occurs when a host is simultaneously parasitized by two or more species of parasitoids. Because a multiparasitized host can only support the complete development of the progeny from one species (Godfray, 1994), interspecific conflicts set in between the two endoparasitoid species. In an asymmetric competition, the superior competitor displaces the inferior (Reitz & Trumble, 2002). Because parasitoids are main biological control agents, their interspecific competitions deserve careful studies for various reasons. For example, interspecific competition can influence the size, structure, and stability of insect communities (Jalali et al., 1988; Leveque et al., 1993; Pijls et al., 1995), and may also lead to a reduction of the overall parasitism index and efficiency of

pest population regulation (Leveque et al., 1993; Wen & Brower, 1995).

A number of researchers have investigated interspecific competition in insect parasitoids (Kfir & van Hamburg, 1988; Pijls et al., 1995; Wen & Brower, 1995; Infante et al., 2001; Marktl et al., 2002; Sallam et al., 2002; Utsunomiya & Iwabuchi, 2002; Wang & Messing, 2003; Shi et al., 2004). Various mechanisms of the competition, such as physical attack and physiological suppression, have been previously addressed (Fisher, 1971; Vinson & Iwantsch, 1980; Mackauer, 1990). Physical attack by using mandibles has been observed in some solitary parasitoid larvae (Vinson, 1972; McBrien & Mackauer, 1990), whereas physiological suppression by secreting substances toxic to the competitors (Strand & Vinson, 1984; Hagver, 1988), shutting out oxygen (Fisher, 1963), or locking out the supply of nutrients from the host has also been reported. The method of such attacks usually depends on the age of the parasitoid (Wang, 2001).

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Two solitary endoparasitoids, *Campoletis chloridae* Uchida (Hymenoptera: Ichneumonidae) and *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae), play a major role in the natural control of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), which is a major agricultural pest throughout the world (Fitt, 1989; Wu & Guo, 2005). *Campoletis chloridae* is an important early larval endoparasitoid of many noctuid species, and has been widely reported as a potential biological control agent for *H. armigera* in China, Korea, and India (Zheng & Lu, 1981; Dai, 1990; Nandihlli & Lee, 1995a,b; Kumar et al., 2000; Wang, 2001; You et al., 2002; Liu et al., 2004; Pandey et al., 2004). It produces 8–10 generations per year in China and is mainly distributed in the Yellow River Valley and Yangtze River Valley as a predominant parasitoid of *H. armigera* (Zheng & Lu, 1981; You et al., 2002). *Campoletis chloridae* oviposits in early instars of *H. armigera*, but prefers second and third instars (Dai, 1990; Wang, 2001). The average rates of parasitism by *C. chloridae* on early instars of *H. armigera* were 23.7% during 1986–1989 (Dai, 1990). *Microplitis mediator*, on the other hand, is a solitary braconid parasitoid of some noctuids and geometrids (Arther & Mason, 1986; Wang & Hun, 1992). It is also an important natural enemy of *H. armigera* in the cotton fields of Yellow River Valley, China. This parasitoid prefers first and second instars of *H. armigera*. The average annual rates of parasitism by *M. mediator* on first and second instars of *H. armigera* were 12.2, 35.5, 21.1, and 22.6% in 1979, 1980, 1981, and 1982, respectively (Wang et al., 1984). However, the rate of parasitism on second generation larvae of *H. armigera* was as high as 43.3% in 1980 in Hebei province, China (Wang et al., 1984).

Because both *C. chloridae* and *M. mediator* parasitize *H. armigera* larvae at early stages in the Yellow River Valley, the two parasitoids compete for limited resources available for their development in the same host. However, very little is known about the strategies that each parasitoid species uses in order to be successful in the same host *H. armigera*. This study was designed to investigate the consequences of the competitive interactions between the two parasitoids and the possible mechanisms involved, with the aim of providing basic information necessary for their introduction and evaluation as biological agents against the cotton bollworm.

Materials and methods

Insect culture

Helicoverpa armigera were reared on artificial diets and incubated at 26 ± 1 °C, 80% r.h., and L16:D8 as previously described (Wang & Dong, 2001). The two colonies of the parasitoids, *C. chloridae* and *M. mediator*, were initiated

with cocoons collected from the fields of Zhengzhou and Shijiazhuang in China, respectively, and maintained on *H. armigera* larvae reared in the laboratory. Mated female wasps were allowed to sting the host larvae (late second or early third instars) once or twice, and the parasitized larvae were reared continuously in an incubator under the same conditions as described above until the formation of cocoons. Twenty cocoons were collected and kept in a glass tube (2 cm in diameter \times 10 cm in height), plugged with cotton wool, until adult emergence. Twenty adults of each parasitoid species were selected in a sex ratio of 1:1 and were kept in a cage (10 cm in diameter \times 20 cm in height) with 20% (wt/vol) honey solution as a food source.

Development of *Campoletis chloridae* and *Microplitis mediator* within *Helicoverpa armigera* larvae

To investigate the possible effects of relative hatch time on the success of larval competition of the two parasitoid species, 60 third-instar host larvae were parasitized by *C. chloridae* and another 60 parasitized by *M. mediator*. The host larvae were then dissected at various times between 24 and 50 h after parasitization. Each parasitized host larva was placed in a drop of Ringer's solution on a slide glass and dissected under a stereomicroscope (Olympus SZ, Tokyo, Japan) at 7–40 \times .

Multiparasitism of *Helicoverpa armigera* by *Campoletis chloridae* and *Microplitis mediator*

Helicoverpa armigera larvae were transferred into a cage containing either *C. chloridae* or *M. mediator* parasitoids and allowed to be stung only once by the wasps. The female wasps used for parasitization were 5–10 days old. The host larvae were in the late second or early third instar, and no more than 1 cm in length. Using this technique, 80–100 parasitized larvae were obtained per hour. The stung larvae were dissected immediately and the number of parasitized larvae was recorded. The parasitism ratio (Pr) was calculated by the formula $Pr = \text{number of larvae parasitized} / \text{number of larvae stung}$.

Twenty-four host larvae stung by either *C. chloridae* or *M. mediator* were placed individually in 24-well cell culture plates supplied with artificial diets to prevent them from biting one another. At 12- or 24-h intervals after initial parasitization by either *C. chloridae* or *M. mediator*, the parasitized larvae were exposed again to *M. mediator* or *C. chloridae* for further parasitization. Multiparasitized larvae were reared as previously described and the number of cocoons formed was recorded for each parasitoid species. Our experiments included six different combinations: the Cc-0-Mm, Cc-12-Mm, and Cc-24-Mm representing the host larvae first parasitized by *C. chloridae* (Cc) followed by *M. mediator* (Mm) with an interval of <10 min (designated

as simultaneous parasitizations), 12, and 24 h, respectively, whereas the Mm-0-Cc, Mm-12-Cc, and Mm-24-Cc combinations representing the host larvae first parasitized by Mm followed by Cc with an interval of <10 min, 12, and 24 h, respectively.

Survival of *Campoletis chloridae* and *Microplitis mediator* in multiparasitized *Helicoverpa armigera* larvae

Multiparasitized host larvae were obtained using the procedure described above. Dissections of host larvae were carried out 72 h after the first parasitization occurred, to evaluate the competition outcomes at earlier stages. Each treatment contained 25–40 host larvae. To further understand the mechanisms involved in the interspecific competition of these two wasp species, multiparasitized host larvae in the Cc-0-Mm, Cc-12-Mm, and Cc-24-Mm treatments were dissected at four time intervals (38–40, 48–51, 63–65, and 70–72 h) after the host larvae were first stung by the first wasp species. Each group contained 31–44 host larvae, and a total of 179 were dissected. The number of host larvae containing eggs or larvae of either one species alone or two species together was recorded.

Effect of multiparasitism on the weight of *Helicoverpa armigera* larvae

In order to test whether multiparasitism affected the growth of the host larvae, 20 multiparasitized larvae (Cc-0-Mm) were weighed individually at 1, 3, 5, and 7 days postparasitism (p.p.). Larvae of similar age parasitized by the first parasitoid species only were used as the control. Each treatment and control was replicated three times.

Data analysis

To explore the possible factors influencing competition outcomes between the two parasitoids, we used logistic analyses, assuming a binomial distribution of error variances (Hardy & Field, 1998; Stokkebo & Hardy, 2000). The competition outcome was defined as a binary response variable (1, Mm cocoons produced; 0, Cc cocoons produced), whereas the order of the two parasitizations and the time interval between the parasitizations were defined as the explanatory variable. The percentages of emergence of wasps were subjected to a t-test. All data were analyzed using SPSS 13.0 for Windows (SPSS Inc.).

Results

Developmental times of *Campoletis chloridae* and *Microplitis mediator* embryos in *Helicoverpa armigera* larvae

The hatch times of *M. mediator* and *C. chloridae* were about 33–34 and 44–46 h, respectively, after parasitism. Thus, the embryos of *M. mediator* developed approximately



Figure 1 Outcomes of competition between *Microplitis mediator* (Mm) and *Campoletis chloridae* (Cc) and estimated probabilities of the production of Mm cocoons. The contest data are binary (1, production of Mm cocoons; 0, production of Cc cocoons). Negative differences indicate that Mm parasitization was later than Cc. The fitted curve shows the probability (P) of host producing Mm cocoons from logistic regression; $P = 1 / [1 + 1/\exp(0.1X + 0.740)]$, with X = difference in parasitization.

12 h faster than those of *C. chloridae* under the given conditions.

Competition outcomes between *Campoletis chloridae* and *Microplitis mediator* in multiparasitized *Helicoverpa armigera*

Among 720 parasitized host larvae, only 273 produced wasp cocoons, 172 from *M. mediator* and 101 from *C. chloridae*. The competition data were then explored by logistic analysis with the binary response variable defined as 1, Mm cocoons produced and 0, Cc cocoons produced. The fitted explanatory variables were absolute time between the two parasitizations, which represents the sequence and interval of the two parasitizations. This absolute time was the only variable that influenced the outcome of the contest ($G = 73.98$, d.f. = 1, and $P < 0.001$; Figure 1).

Parasitization of *Helicoverpa armigera* by *Campoletis chloridae* after initial parasitism by *Microplitis mediator*

Campoletis chloridae can oviposit in *H. armigera* larvae previously parasitized by *M. mediator*. The multiparasitism ratios were similar between the Mm-0-Cc and Mm-12-Cc, but both were higher than those of the Mm-24-Cc (Table 1). When *M. mediator* and *C. chloridae* parasitized at approximately the same time (Mm-0-Cc) or *M. mediator* parasitized first (Mm-12-Cc; Mm-24-Cc), the majority of cocoons produced were those of *M. mediator* (78.7, 78.7, and 76.5%, respectively). *Microplitis mediator* showed a competitive advantage in both simultaneous parasitization or when it parasitized first (Figure 2). The rate of successful parasitism based on parasitoid cocoon formation between Mm-0-Cc and Mm was similar, but higher than those of

Table 1 Parasitism ratio (%) of *Helicoverpa armigera* larvae parasitized by *Microplitis mediator* (Mm), *Campoletis chloridae* (Cc), or both. The numbers between Mm and Cc are the intervals (h) between the two parasitizations. In each treatment, 96 larvae were used for parasitization

Treatments	<i>C. chloridae</i>	<i>M. mediator</i>	<i>C. chloridae</i> + <i>M. mediator</i>
Mm-0-Cc	84 (87.5)	90 (93.8)	79 (82.3)
Mm-12-Cc	81 (84.4)	87 (90.6)	73 (76.0)
Mm-24-Cc	69 (71.9)	88 (91.7)	63 (65.6)
Mm		88 (91.7)	
Cc-0-Mm	90 (93.8)	82 (85.4)	77 (80.2)
Cc-12-Mm	89 (92.7)	87 (90.6)	80 (83.3)
Cc-24-Mm	91 (94.8)	69 (71.9)	64 (66.7)
Cc	92 (95.8)		

the Mm-12-Cc and Mm-24-Cc (Figure 3) treatments. More hosts died before the parasitoids formed their cocoons in the Mm-12-Cc and Mm-24-Cc treatments than in Mm-0-Cc and Mm alone (Figure 3). Based on our dissections, *M. mediator* was in the lead during interspecific competition at 72 h p.p. Surviving *C. chloridae* and *M. mediator* larvae could be observed in less than 10% of the hosts (Table 2).

Parasitization of *Helicoverpa armigera* by *Microplitis mediator* after initial parasitism by *Campoletis chloridae*

Microplitis mediator can also oviposit in *H. armigera* larvae previously parasitized by *C. chloridae*. The multiparasitism ratios were similar between the Cc-0-Mm and Cc-12-Mm treatments, but both were higher than those of Cc-24-Mm

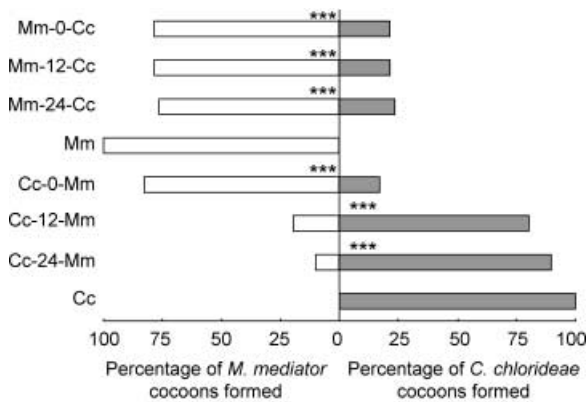


Figure 2 Percentages of emergence of *Microplitis mediator* (Mm) and *Campoletis chloridae* (Cc) when the host was parasitized by both wasp species. Asterisks indicate a significant difference within each time interval (t-test: *** $P < 0.001$). The numbers between Mm and Cc are the intervals (h) between the two parasitizations.

(Table 1). When *C. chloridae* parasitized first (Cc-12-Mm and Cc-24-Mm), the majority of cocoons produced were those of *C. chloridae* (80.5 and 89.9%, respectively). However, the cocoons were mainly *M. mediator* in the Cc-0-Mm treatment (82.7%; Figure 2). When a host larva was parasitized by *C. chloridae* only (i.e., Cc), more parasitoid cocoons were formed than in Cc-12-Mm and Cc-24-Mm, but the number was similar to that of the Cc-0-Mm (Figure 3) treatment. The host mortality prior to the formation of the parasitoid cocoons in Cc-0-Mm and Cc was lower than those of Cc-12-Mm and Cc-24-Mm. Few host larvae could develop to pupae in all treatments (Figure 3). In fact, interspecific competitions could be easily observed at 72 h after parasitization by *C. chloridae*. Thus, *C. chloridae* had an advantage in Cc-12-Mm and Cc-24-Mm, whereas *M. mediator* had an advantage in the Cc-0-Mm. Living larvae of both *C. chloridae* and *M. mediator* could be found in 12–16% of host larvae in various treatments, including Cc-0-Mm, Cc-12-Mm, and Cc-24-Mm (Table 2).

The mechanism involved in the interspecific competition

First instars of *M. mediator* have a pair of huge mandibles (Figure 4A), which start to degenerate from the second instars. However, these mandibles are not found in first instars of *C. chloridae* (Figure 4B). In order to know whether the first instars of *M. mediator* attack the egg and larvae of *C. chloridae*, we dissected parasitized *H. armigera* of the Cc-0-Mm treatment around 5 h after *M. mediator* and *C. chloridae* hatched. At 48–51 h p.p. of *C. chloridae* (approximately 5 h after the hatch of *C. chloridae*), the first instars of *M. mediator* were normal, but 80% of *C. chloridae* larvae had died in the multiparasitized host larvae, with obvious bite marks (Figure 4C). Furthermore, surviving larvae of both parasitoid species were found in only 6.7% of the host larvae (Table 3). At 38–40 h p.p. of *C. chloridae* (approximately 5 h after the hatch of *M. mediator*), however, no attack marks of *M. mediator* larvae on *C. chloridae* eggs were found. In Cc-12-Mm, both species of parasitoid hatched approximately at the same time. Dissections were carried out at 49–51 h after parasitization by *C. chloridae*. In the host larvae containing both wasp species, 83.3% were found with *C. chloridae* living and *M. mediator* dead larvae, and 8.3% with living larvae of both wasp species. In the Cc-24-Mm treatment, eggs of *C. chloridae* were already hatched at 48–51 h p.p. of *C. chloridae*, and the eggs of *M. mediator* showed no bite marks by *C. chloridae* larvae. Conversely, dissections conducted 63–66 h after parasitization by *C. chloridae* indicated that *C. chloridae* larvae survived, but *M. mediator* larvae died in 50% of the multiparasitized hosts (Table 3).

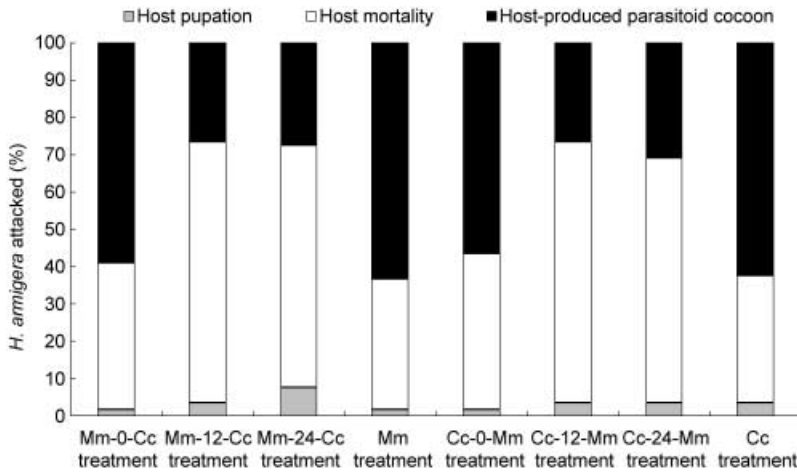


Figure 3 Fate (percentage) of *Helicoverpa armigera* larvae attacked by *Campoletis chloridae* (Cc) and/or *Microplitis mediator* (Mm) in eight parasitizing treatments. The numbers between Mm and Cc are the intervals (h) between the two parasitizations. In each treatment, 120 larvae were used.

Effect of multiparasitism on host weights

When host larvae were parasitized, their larval weight decreased significantly in contrast to the unparasitized larvae. However, the weight of host larvae parasitized by *M. mediator* alone was not significantly different from those parasitized by both parasitoid species, but was lower than that of the host larvae parasitized by *C. chloridae* alone (Figure 5).

Discussion

The current study provides convincing evidence that the braconid *M. mediator* has a competitive advantage over the ichneumonid *C. chloridae* in simultaneous multiparasitization. When the host larvae were parasitized by both species of parasitoid at the same time, most of *M. mediator* could survive and develop into cocoons, whereas only few *C. chloridae* could (Figures 1 and 2). The hatching time of *M. mediator* was about 12 h shorter than that of *C. chloridae*. The faster development of *M. mediator* could explain the advantage of this species during interspecific

competition. Our results are consistent with those of De Moraes et al. (1999) showing that *Microplitis croceipes*, with a shorter hatching time, dominates competition against another parasitoid, *Cardiochiles nigriceps*, when the interval between primary and secondary parasitization did not exceed 8 h.

Physical attack by *M. mediator* is another factor contributing to the competitive advantage of *M. mediator* over *C. chloridae*. The first instars of *M. mediator* can attack *C. chloridae* with sickle-like mandibles and eliminate them (Figure 4). This notion is well-supported by our dissection results. In the Cc-0-Mm and Mm-0-Cc treatments, *M. mediator* coexisted with the first instars of *C. chloridae*. The former physically attacked the latter and killed them. Kfir & van Hamburg (1988) reported that when competition occurred between *Telenomus ullyetti* and *Trichogrammatoidea lutea* within *H. armigera* eggs, regardless of which species parasitized first, the aggressive mandibulate larva of the former eliminated the larvae of the latter. Similarly, Shi et al. (2004) reported that the braconid *Cotesia plutellae*, with sickle-like mandibles, obviously had an advantage over the ichneumonid

Table 2 Dissection results at 72 h after the first wasp parasitization when the host was parasitized by both wasps, *Microplitis mediator* (Mm) and *Campoletis chloridae* (Cc). The numbers between Mm and Cc are the intervals (h) between the two parasitizations

Treatments	Number of host larva dissected	Number of hosts with Cc larva	Number of hosts with Mm larva	Number (%) of hosts with both Cc and Mm larva	Number (%) of hosts parasitized by Cc or Mm larvae
Mm-0-Cc	31	7	18	3 (9.7)	28 (90.3)
Mm-12-Cc	25	0	20	2 (8.0)	22 (88.0)
Mm-24-Cc	40	0	34	4 (10.0)	38 (95.0)
Mm	25	0	21	0	21 (84.0)
Cc-0-Mm	39	6	25	5 (12.8)	36 (92.3)
Cc-12-Mm	25	20	0	4 (16.0)	24 (96.0)
Cc-24-Mm	25	21	0	3 (12.0)	24 (96.0)
Cc	25	19	0	0	19 (76.0)



Figure 4 *Campoletis chloridae* and *Microplitis mediator* larvae. (A) First instar of *M. mediator* (the arrow indicates the large mandibles of *M. mediator*); (B) first instar of *C. chloridae*; (C) second instars of *M. mediator* (left) and the biting mark of *C. chloridae* (right) as indicated by the arrow.

Diadegma semiclausum in competition within their host *Plutella xylostella*.

In the experiments of sequential multiparasitism, the species that parasitized first succeeded in completing its larval development. Because hatching time of *C. chloridae* was about 12 h longer than *M. mediator*, both parasitoids should have hatched at almost the same time in the Cc-12-Mm treatment. *Microplitis mediator* should have been dominant; however, in our results, most of *C. chloridae*, which oviposited first, survived to cocoon formation. This suggests that offspring of the first parasitoid uses physiological suppression toward the later one. Many studies indicate that parasitoids attacking hosts early are better competitors than those attacking hosts at a later developmental stage (Force & Messenger, 1965; Bokonon-Ganta et al., 1996, 2005; Wang et al., 2003). Host conditions that are altered by the first parasitizing wasps may partly explain their competitive advantage. Previous parasitism reduces the

quality of a host for subsequent parasitoid female wasps (van Alphen & Visser, 1990; Zaviezo & Mills, 2001). Endoparasitoids usually release some substances into hosts to regulate the development and physiological conditions of the host in order to optimize their own development. Both species of parasitoid inject some regulatory substances/compounds, such as polydnavirus and venom, into the host at oviposition (Li et al., 2001; Yin et al., 2003; Zhang & Wang, 2003), but *M. mediator* also release teratocytes into the host hemocoel when their eggs hatch (Qin et al., 2000). Microvilli covering the surface of the teratocytes are believed to enhance the ability of these cells to absorb nutrients from, or secrete proteins or other materials into, the host's hemolymph (Nakamatsu et al., 2002). The specific physiological suppression factors in *M. mediator* may also contribute to the fact that the weight of hosts parasitized by *M. mediator* was lower than the host parasitized by *C. chloridae* (Figure 5).

Table 3 Dissection results at 38–66 h after parasitization of the first wasp, *Campoletis chloridae* (Cc). Mm, *Microplitis mediator*. The numbers between Mm and Cc are the intervals (h) between the two parasitizations

Treatment	Number of host larvae dissected	Dissection time after the first parasitization (h)	Number of host larvae with different conditions							
			Mm larvae surviving, Cc dead	Cc larvae surviving, Mm dead	Mm larvae surviving, Cc no hatching	Cc larvae surviving, Mm no hatching	Both larvae surviving	Only with Mm larvae	Only with Cc larvae	Unparasitized
Cc-0-Mm	31	38–40	0	0	17	0	0	7	6	1
Cc-0-Mm	37	48–51	12	0	2	0	1	14	7	1
Cc-12-Mm	44	49–51	0	10	0	1	1	12	15	5
Cc-24-Mm	31	48–51	0	0	0	9	0	2	16	4
Cc-24-Mm	34	63–66	1	3	0	1	1	4	22	2

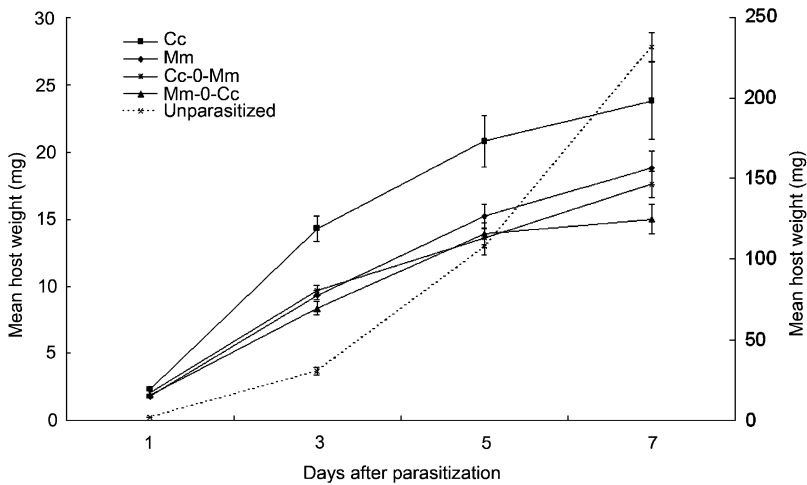


Figure 5 Weight of host larvae parasitized by *Microplitis mediator* (Mm), *Campolitis chloridae* (Cc), or by both (mean \pm SE). The values of the solid lines refer to the left Y-axis, whereas the values of dotted line refer to the right Y-axis. The numbers between the Mm and Cc are the intervals (h) between the two parasitizations.

An intriguing phenomenon is that eggs of both wasp species could hatch during all multiparasitization treatments, and most competitions ended at the wasp larval stage. This may have occurred, because the egg provides sufficient nutrition and the eggshell provides a certain degree of protection for the development of the wasp embryo. Therefore, the egg stage is less susceptible to both physiological suppression and physical attack. However, wasp larvae exposed to host hemocoel succumbed to physical attack and were also susceptible to possible physiological suppression factors elicited by interspecific competitors.

Relatively fewer females of both wasp species appeared to accept hosts parasitized 24 or more hours earlier in which their offspring would have a very low probability of surviving. However, they did accept 12-h parasitized hosts (and also 0-h parasitized) as frequently as unparasitized hosts, even though their offspring also had significantly reduced survival in such hosts (Table 1). The discrepancy between female choice and offspring survival may be due to a lack of discrimination ability during the first 24 h, and especially the first 12 h after parasitism.

Higher mortality of hosts and lower parasitoid production occurred in sequential multiparasitization (Figure 3). Double parasitism further adversely affected host physiology through the injection or secretion by both parasitoids or by their offspring. Furthermore, the braconids' physical attacks may easily damage host tissues because of the limited space (Harvey et al., 1993). All this may result in the increase of host death and consequently a reduction in parasitoid survival rates. Competition for nutrition and space are probably yet other factors contributing to reduction in wasp survival. However, it seems that this could not completely explain the difference in offspring production and host mortality between simultaneous and sequential

parasitization. It is possible that the rapid development of *M. mediator* dominates the competition in simultaneous multiparasitism by both physiological suppression and physical attack. For example, the first instars, with their powerful mandibles, killed *C. chloridae* larvae as soon as the latter hatched. The obvious dominant status and the quick killing of the opponent larvae will not only ensure a better host condition for the development of *M. mediator*, but also reduce the adverse effects toward the host. Therefore, low host mortality and high parasitoid offspring production occurred during simultaneous multiparasitism.

Competition between parasitoids may play a key role in community dynamics of tritrophic plant–herbivore–parasitoids systems and has important implications for the management of herbivorous insect pests (Bogran et al., 2002; De Moraes & Mescher, 2005). In this study, multiparasitism of *C. chloridae* and *M. mediator* in *H. armigera* larvae did not further decrease host survival rate, but offspring production was significantly decreased in sequential multiparasitization. This competition suppresses population increase of parasitoids and could reduce their efficiency in the next generation. It should be pointed out, however, that competition between the two parasitoids in the field will not be as intense as that in the laboratory. Many factors in the field may lead to the occurrence of multiparasitization that does not completely overlap in time and space, such as landscape structure (Thies & Tscharrntke, 1999), host plant preference of the pest (Talekar & Yang, 1993), host-searching behavior and efficiency of the two parasitoid species (Wang & Keller, 2002), the parasitoids' host stage preference (Wang et al., 1984; Dai, 1990; Wang, 2001), the parasitoids differential adaptation to a temperature range (Zheng & Lu, 1981; Wang et al., 1984; You et al., 2002), and life-history trade-offs (Lei & Hanski, 1998; Amarasekare,

2000; Bonsall et al., 2002). Different parasitoid species may coexist in the same locality, and enhance the control efficiency against the pest through their cooccurrence in time and space (Talekar & Yang, 1993). Nevertheless, our results showed that interspecific competition between the ichneumonid *C. chlorideae* and the braconid *M. mediator* exists in younger larvae of *H. armigera*. The intensity of such a competition in the field and its effect on efficiency of biological control of *H. armigera* need to be further investigated.

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References

- van Alphen JJM & Visser ME (1990) Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology* 35: 59–79.
- Amarasekare P (2000) Spatial dynamics in a host-multiparasitoid community. *Journal of Animal Ecology* 69: 201–213.
- Arther AP & Mason PG (1986) Life history and immature stages of the parasitoid *Microplitis mediator* (Hymenoptera: Braconidae), reared on the bertha armyworm *Mamestra configurata* (Lepidoptera: Noctuidae). *The Canadian Entomologist* 118: 487–491.
- Bogran CE, Heinz KM & Ciomperlik MA (2002) Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. *Ecology* 83: 653–668.
- Bokonon-Ganta AH, Ramadan MM, Wang XG & Messing RH (2005) Biological performance and potential of *Fopius ceratitivorus* (Hymenoptera: Braconidae), an egg-pupal parasitoid of tephritid fruit flies, newly imported to Hawaii. *Biological Control* 33: 238–247.
- Bokonon-Ganta AH, van Alphen JJM & Neuenschwander P (1996) Competition between *Gyranusoideas tebygi* and *Anagyrus mangicola*, parasitoids of mango mealybug, *Rastrococcus invadens*: interspecific host discrimination and larval competition. *Entomologia Experimentalis et Applicata* 79: 179–185.
- Bonsall MB, Hassell MP & Asefa G (2002) Ecological trade-offs, resource partitioning, and coexistence in a host-parasitoid assemblage. *Ecology* 83: 925–934.
- Dai XF (1990) Biology of *Camponotus chlorideae* (Hymenoptera: Ichneumonidae) and its field control effect on cotton bollworm. *Chinese Journal of Biological Control* 6: 153–156.
- De Moraes CM, Cortesero AM, Stapel JO & Lewis WJ (1999) Intrinsic and extrinsic competitive interactions between two larval parasitoids of *Heliothis virescens*. *Ecological Entomology* 24: 403–410.
- De Moraes CM & Mescher MC (2005) Intrinsic competition between larval parasitoids with different degrees of host specificity. *Ecological Entomology* 30: 564–570.
- Fisher RC (1963) Oxygen requirements and the physiological suppression of supernumerary insect parasitoids. *Journal of Experimental Biology* 40: 531–540.
- Fisher RC (1971) Aspects of the physiology of endoparasitic Hymenoptera. *Biological Reviews* 46: 243–278.
- Fitt GP (1989) The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology* 34: 17–52.
- Force DC & Messenger PS (1965) Laboratory studies on competition among three parasites of the spotted alfalfa aphid *Therioaphis maculata* (Buckton). *Ecology* 46: 853–859.
- Godfray H CJ (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, NJ, USA.
- Hagver EB (1988) Multiparasitism of the green pea aphid, *Myzus persicae*: competition in the egg stage between *Aphidius matricariae* and *Ephedrus cerasicola*. *Entomologia Experimentalis et Applicata* 47: 275–282.
- Hardy ICW & Field SA (1998) Logistic analysis of animal contests. *Animal Behaviour* 56: 787–792.
- Harvey JA, Harvey IF & Thompson DJ (1993) The effect of superparasitism on development of the solitary parasitoid wasp, *Venturia canescens* (Hymenoptera: Ichneumonidae). *Ecological Entomology* 18: 203–208.
- Infante F, Mumford J, Baker P, Barrera J & Fowler S (2001) Interspecific competition between *Cephalonomia stephanoderis* and *Prorops nasuta* (Hym., Bethyridae), parasitoids of the coffee berry borer, *Hypothenemus hampei* (Col., Scolytidae). *Journal of Applied Entomology* 125: 63–70.
- Jalali SK, Singh SP, Ballal CR & Kumar P (1988) Competitive interaction between *Cotesia kazak* and *Hyposoter didymator*, exotic parasitoids of *Heliothis armigera*. *Entomologia Experimentalis et Applicata* 46: 221–225.
- Kfir R & van Hamburg H (1988) Interspecific competition between *Telenomus ullyetti* (Hymenoptera: Scelionidae) and *Trichogrammatoidea lutea* (Hymenoptera: Trichogrammatidae) parasitizing eggs of the cotton bollworm *Heliothis armigera* in the laboratory. *Environmental Entomology* 17: 664–670.
- Kumar N, Kumar A & Tripathi CPM (2000) Sex ratio of *Camponotus chlorideae* Uchida in response to *Helicoverpa armigera* (Hübner) density. *Insect Science and Its Application* 22: 73–76.
- Lei GC & Hanski I (1998) Spatial dynamics of two competing specialist parasitoids in a host metapopulation. *Journal of Animal Ecology* 67: 422–433.
- Leveque L, Monge JP, Rojas-Rousse D, van Alebeek F & Huigard H (1993) Analysis of multiparasitism by *Eupelmus vuilleti* (Craw) (Eupelmidae) and *Dinarmus basalis* (Rond) (Pteromalidae) in the presence of one of their common hosts, *Bruchidius atrolineatus* (Pic) (Coleoptera: Bruchidae). *Oecologia* 94: 272–274.
- Li X, Qin QL, Ding C & Liu HH (2001) Characterization of *Microplitis mediator* polydnavirus. *Virologica Sinica* 16: 373–376.

- Liu WX, Wan FH & Yuan ST (2004) Mass-rearing and bionomics of *Campoletis chloridae*. Chinese Journal of Biological Control 20: 17–20.
- Mackauer M (1990) Host discrimination and larval competitions in solitary endoparasitoids. Critical Issues in Biological Control (ed. by M Mackauer, LE Ehler & J Roland), pp. 41–62. Intercept Ltd., Andover, Hants, UK.
- Marktl RC, Stauffer C & Schopf A (2002) Interspecific competition between the braconid endoparasitoids *Glyptapanteles porthetriae* and *Glyptapanteles liparidis* in *Lymantria dispar* larvae. Entomologia Experimentalis et Applicata 105: 97–109.
- McBrien H & Mackauer M (1990) Heterospecific larval competition and host discrimination in two species of aphid parasitoids: *Aphidius ervi* and *Aphidius smithi*. Entomologia Experimentalis et Applicata 56: 145–153.
- Nakamatsu Y, Fuji S & Tanaka T (2002) Larvae of an endoparasitoid, *Cotesia kariyai* (Hymenoptera: Braconidae), feed on the host fat body directly in the second stadium with the help of teratocytes. Journal of Insect Physiology 48: 1041–1052.
- Nandihli BS & Lee JH (1995a) Effect of host food plants on the biology of the host, *Helicoverpa assulta* (Guenée), and its parasitoid, *Campoletis chloridae* Uchida. Advances in Agricultural Research in India 3: 22–32.
- Nandihli BS & Lee JH (1995b) Seasonal occurrence of *Campoletis chloridae* Uchida and its control efficacy on the oriental tobacco budworm, *Helicoverpa assulta* (Guenée), in tobacco fields in Suwon. Korean Journal of Applied Entomology 34: 147–153.
- Pandey P, Kumae N & Tripathi CPM (2004) Impact of males on the progeny sex ratio of *Campoletis chloridae* (Hym., Ichneumonidae), a parasitoid of *Helicoverpa armigera* (Hübner) (Lep., Noctuidae). Journal of Applied Entomology 128: 254–257.
- Pijls JWAM, Hofker KD, van Staalduinen MJ & van Alphen JJM (1995) Interspecific host discrimination and competition in *Apoanagyrus (Epidinocarsis) lopezi* and *A.(E.) diversicornis*, parasitoids of the cassava mealybug *Phenacoccus manihoti*. Ecological Entomology 20: 326–332.
- Qin QL, Gong H & Ding C (2000) Two collagenases are secreted by teratocytes from *Microplitis mediator* (Hymenoptera: Braconidae) cultured in vitro. Journal of Invertebrate Pathology 76: 79–80.
- Reitz SR & Trumble JT (2002) Competitive displacement among insects and arachnids. Annual Review of Entomology 47: 435–465.
- Sallam MN, Overholt WA & Kairu E (2002) Intraspecific and interspecific competition between *Cotesia flavipes* and *Cotesia sesamiae* (Hymenoptera: Braconidae), gregarious larval endoparasitoids of lepidopteran stemborers. Biocontrol Science and Technology 12: 493–506.
- Shi ZH, Li QB & Li X (2004) Interspecific competition between *Diadegma semiclausum* Hellen (Hym., Ichneumonidae) and *Cotesia plutellae* (Kurdjumov) (Hym., Braconidae) in parasitizing *Plutella xylostella* (L.) (Lep., Plutellidae). Journal of Applied Entomology 128: 437–444.
- Stokkebo S & Hardy ICW (2000) The importance of being gravid: egg load and contest outcome in a parasitoid wasp. Animal Behaviour 59: 1111–1118.
- Strand MR & Vinson SB (1984) Facultative hyperparasitism by the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Annals of the Entomological Society of America 77: 679–686.
- Talekar NS & Yang JC (1993) Influence of crucifer cropping system on the parasitism of *Plutella xylostella* (Lep., Yponomeutidae) by *Cotesia plutellae* (Hym., Braconidae) and *Diadegma semiclausum* (Hym., Ichneumonidae). Entomophaga 38: 541–550.
- Thies C & Tschardt T (1999) Landscape structure and biological control in agroecosystems. Science 285: 893–894.
- Utsunomiya A & Iwabuchi K (2002) Interspecific competition between the polyembryonic wasp *Copidosoma floridanum* and the gregarious endoparasitoid *Glyptapanteles pallipes*. Entomologia Experimentalis et Applicata 104: 353–362.
- Vinson SB (1972) Competition and host discrimination between two species of tobacco budworm parasitoids. Annals of the Entomological Society of America 65: 229–236.
- Vinson SB & Iwantsch GF (1980) Host regulation by insect parasitoids. The Quarterly Review of Biology 55: 143–165.
- Wang CZ (2001) Effects of host size on oviposition and development of the endoparasitoid, *Campoletis chloridae* Uchida. Chinese Journal of Biological Control 17: 107–111.
- Wang CZ & Dong JF (2001) Interspecific hybridization of *Helicoverpa armigera* and *H. assulta* (Lepidoptera: Noctuidae). Chinese Science Bulletin 46: 489–491.
- Wang DA & Hun Z (1992) Diapause and overwintering characteristics of *Microplitis mediator*. Chinese Journal of Biological Control 6: 211–218.
- Wang XG & Keller MA (2002) A comparison of the host-searching efficiency of two larval parasitoids of *Plutella xylostella*. Ecological Entomology 27: 105–114.
- Wang XG & Messing RH (2003) Intra- and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of tephritid fruit flies. Biological Control 27: 251–259.
- Wang XG, Messing RH & Bautista RC (2003) Competitive superiority of early acting species: a case study of opine fruit fly parasitoids. Biocontrol Science and Technology 16: 391–402.
- Wang DA, Nan LZ, Sun X & Li XZ (1984) Study on a bionomics of *Microplitis* species, larval parasitic wasp of *Helicoverpa armigera*. Natural Enemies of Insects 6: 211–218.
- Wen BR & Brower JH (1995) Competition between *Anisopteromalus calandrae* and *Choetospila elegans* (Hymenoptera: Pteromalidae) at different parasitoid densities on immature rice weevils (Coleoptera: Curculionidae) in wheat. Biological Control 5: 151–157.
- Wu KM & Guo YY (2005) The evolution of cotton pest management practices in China. Annual Review of Entomology 40: 31–52.
- Yin LH, Zhang C, Qin JD & Wang CZ (2003) Polydnavirus of *Campoletis chloridae*: Characterization and temporal effect on host *Helicoverpa armigera* cellular immune response. Archives of Insect Biochemistry and Physiology 52: 104–113.
- You LS, Lei RH, Jiang JX, Bo LY & Xiao ZS (2002) Bionomic of *Campoletis chloridae* (Hymenoptera: Ichneumonidae) as a

- parasitoid of the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Entomologia Sinica* 9: 29–37.
- Zaviezo T & Mills N (2001) The response of *Hyssopus pallidus* to hosts previously parasitized by *Ascogaster quadridentata*: heterospecific discrimination and host quality. *Ecological Entomology* 26: 91–99.
- Zhang C & Wang CZ (2003) cDNA cloning and molecular characterization of a cysteine-rich gene from *Campoletis chloridae* polydnavirus. *DNA Sequence – The Journal of Sequencing and Mapping* 14: 413–419.
- Zheng YS & Lu ZK (1981) Studies on the biology of *Campoletis chloridae*. *Natural Enemies of Insects* 3: 10–13.