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Potential Host Range of the Larval Endoparasitoid *Cotesia vestalis* (=*plutellae*) (Hymenoptera: Braconidae)

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ABSTRACT: Many parasitoid wasps are highly specialized in nature, attacking only one or a few species of hosts. Host range is often determined by a range of biological and ecological characteristics of the host including diet, growth potential, immunity, and phylogeny. The solitary koinobiont endoparasitoid wasp, *Cotesia vestalis*, mainly parasitizes diamondback moth (DBM) larvae in the field, although it has been reported that to possess a relatively wide lepidopteran host range. To better understand the biology of *C vestalis* as a potential biological control of hosts other than the DBM, it is necessary to determine suitability for potential hosts. In this study, the potential host range of the wasp and its developmental capacity in each host larva were examined under laboratory conditions using 27 lepidopteran species from 10 families. The wasp was able to parasitize 15 of the 27 species successfully. Some host species were not able to exclude *C vestalis* via their internal physiological defenses. When parasitization was unsuccessful, most hosts killed the parasitoid at the egg stage or early first-instar stage using encapsulation, but some host species disturbed the development of the parasitoid at various stages. No phylogenetic relationships were found among suitable and unsuitable hosts, revealing that host range in some endoparasitoids is not constrained by relatedness among hosts based on immunity.

KEYWORDS: Diamondback moth, parasitoid wasp, physiological host range, encapsulation rate

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Introduction

Parasitoid wasps are insects whose eggs and larvae live in, or on the bodies of other arthropods (the "host"), whereas the adults are free-living.[1] Moreover, parasitoids obligatorily kill their hosts during the process of parasitism. Ecological and physiological interactions among hosts and their parasitoids are generally very intimate. Consequently, evolution has often reduced host range to one or just a few host species in nature for many parasitoid species.[1–3] This is particularly true among endoparasitoids whose eggs and/or larvae are found in the host hemocoel and must confront host immune defenses which are quite potent in some host species.[4] Moreover, host range in endoparasitoids is also smaller than in ectoparasitoids because immune defenses in many insects are phylogenetically conserved.

The host range of parasitoids in the field is also influenced by a wide array factors that often work synergistically. For instance, in addition to constraints imposed by immunity, the host food plant differentially affects the parasitoid in terms of its direct effects on attraction through the release of volatiles that are recognized as cues by the adult female parasitoid[5] or through indirect effects on development and survival.[6,7] Therefore, the realized range of parasitoids in the field is narrow, despite a broad fundamental or potential host range in the lab.[8–10] For example, *Campoletis sonorensis*, a Nearctic larval endoparasitoid of several species of moths in the Noctuidae, is capable of attacking and successfully developing in the larvae of several completely novel Palearctic noctuids.[11] Similarly, *Hyposter didymator*, a relative of *C sonorensis* native to the Paleractic, develops well in the caterpillars of some Nearctic noctuids.[11] In both parasitoids, the novel hosts were closely related to the natural hosts (eg, Noctuidae), suggesting that the immune systems were also similar because of phylogenetic conservatism. However, in the field, many endoparasitoids are known to attack only a small percentage of hosts that they can develop under lab conditions, revealing the importance of plant-based or ecological-based constraints on host range. Alternatively, a small number of endoparasitoids are capable

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of attacking a very broad range of hosts in many different families. For example, the solitary braconid *Meteorus pulchricornis* attacks the caterpillars of a very wide range of lepidopteran hosts (eg, up to 12 families) that include species of both micro- and macro-Lepidoptera[12] with immensely different growth potentials. Moreover, understanding the factors that delineate host range under both lab and field conditions is helpful in assessing a parasitoid's potential as a beneficial organism in biological control programs.

Cotesia vestalis (Hymenoptera, Braconidae) is a solitary larval endoparasitoid of the diamondback moth (DBM), a major worldwide pest of brassicaceous crops (eg, cabbages and mustards) with a strong propensity to develop insecticidal resistance. This wasp originates in warmer parts of the Palearctic but has been introduced to other regions for the control of DBM.[13–17] In Japan, *C vestalis* is one of the most important natural enemies of the DBM. The wasp preferentially parasitizes L2 and L3 instars of the DBM[18–20] and takes approximately 15 days to complete its egg-to-adult development 25°C.[14,19] Although it has been reported that *C vestalis* has a relatively wide host range (22 species in 12 families),[21] it is predominantly viewed as a parasitoid of the DBM[15,20,22,23] and is therefore considered a specialist.[24–26] Natural hosts of *C vestalis* in Japan include the DBM,[16,18,27] *Autographa gamma*,[28] *Autographa nigrisigna*,[23,28] and *Leuroperna sera*.[29] Other host species listed by Papp[21] have as of yet not been reported for this parasitoid in Japan. Consequently, an important question regarding the host range of *C vestalis* in Japan is whether host range varies across spatial and/or temporal gradients or, alternatively, if populations native to Japan exhibit a narrower realized host ranges based on physiological constraints. If *C vestalis* parasitizes a range of other lepidopteran species that feed on many kinds of weeds grown around cruciferous crop fields, then the parasitoid can survive near such fields even when cruciferous crops are out of season. Evaluating the potential host range by the difference of the host immune response might provide important additional information for the effective development of *C vestalis* as a biological control agent for integrated pest management programs involving pests other than the DBM.[30]

In this study, we aimed to clarify whether the potential host range of a Japanese strain of *C vestalis* is broader than the DBM by studying developmental interactions between this parasitoid and various other lepidopteran species under laboratory conditions. Specifically, we investigated the behavioral response of the wasp to various hosts and the suitability of these hosts for parasitoid development after oviposition.

Materials and Methods

Host insects

This study was conducted in 2002 to 2004 using 26 lepidopteran species from 10 families (Table 1). Most of the potential host species were collected in Tokyo and Saitama. However, *Ephestia kuehniella*, *Crocidolomia binotalis*, *Helicoverpa armigera*, and *Mythimna* (=*Pseudaletia) separata* were obtained from Ryukyu Sankei Co., Ltd and Sankei Chemical Co., Ltd or from the Laboratory of Tropical Crop Protection and Tokyo University of Agriculture.

Insect rearing

Larvae of each host species were reared under 16-hour photophase and 8-hour scotophase (16L:8D) at 25°C, except maintaining at 20°C for each colony to avoid the high humidity and fungus in rearing case. Diets for each host species were as follows: seedlings of radish for DBM; Insecta LF-S (Nihon Nosan Co., Japan) of commercial artificial diet for *Homona magnanima*, *Spodoptera litura*, *Spodoptera exigua*, *Peridroma saucia*, *H armigera*, *M separata*, *Mamestra brassicae*, *A nigrisigna*, *Macdunnoughia confusa*, *Trichoplusia ni*, *Trichoplusia intermixta*, *Xanthorhoe saturata*, and *Hyphantria cunea*; artificial diet[31] for *C binotalis*; radish sprout powder in exchange for cabbage powder as an alternative composition for *C binotalis* and *Hellula undalis*; and a mixture of wheat and bran (4:1) for *E kuehniella*. Other host insects were fed on the leaves of plant species on which they are often found in the field. Seventeen *C vestalis* cocoons that emerged from DBM larvae were collected from cabbages cultivated in Nerima, Tokyo, in July 2000, and their progeny were successively reared using DBM larvae as the host.

Experimental protocols

Single naive females of *C vestalis*, 1 week after adult emergence and with no experience of oviposition, were confined with 10 unparasitized larvae of respective potential hosts in plastic Petri dishes (9 cm in diameter). First and second instars were used for each host species because they corresponded approximately to the size of second to third instar of DBM to observe the wasp behavior when attacked to each host. For example, L2 (second instar) or L3 (third instar) of pyralids fit L1 (first instar) of noctuid larvae. After insertion and removal of the ovipositor, which confirmed an oviposition event, the female wasp was removed from its dish, and the parasitized host larvae were thereafter fed on the diet suitable for each species under 16L:8D at 25°C.

Successful cocoon formation rate

Cocoon production was measured as an indicator of successful emergence from a host. In one experiment, 10 larvae per host species were confined with a female wasp for 3 hours in a Petri dish (12 cm in diameter). This design was repeated at least more than 10 times for each host species, except in *Brachmia triannulella*, where 10 larvae were the minimum. The replication time was different for each species (Table 1). After 3 hours, experimental hosts were transferred to plastic dishes or

Table 1. Cocoon formation rate (successful parasitism) of *Cotesia vestalis* and food plants of the tested lepidopteran species.

FAMILY	HOST SPECIES TESTED[a]	FAMILY OF FOOD PLANT FOR HOST LARVAE TESTED	TOTAL NO. OF HOST INSECT USED	NO. OF REPLICATION	TOTAL NO. OF PARASITOID COCOONS	SUCCESSFUL PARASITISM (COCOON FORMATION RATE)
Plutellidae	1. *Plutella xylostella*	Brassicaceae	118	12	40	33.9
Gelechiidae	2. *Evuooe structus*	Rosaceae	112	11	0	0
Tortricidae	3. *Homona magnanima*	Theaceae, Rutaceae, Mimosaceae	40	4	0	0
Pyralidae	4. *Herpetogramma luctuosalis*	Vitaceae	82	8	5	6.1
	5. *Hellula undalis*	Brassicaceae, Capparaceae	230	23	19	8.3
	6. *Crocidolomia binotalis*	Brassicaceae	181	18	28	15.5
	7. *Ephestia kuehniella*	flour	466	47	38	8.2
	8. *Pyrausta panopealis*	Oleaceae	48	48	2	4.2
	9. *Palpita nigropunctalis*	Lamiaceae	320	32	0	0
	10. *Hymenia recurvalis*	Chenopodiaceae, Cucurbitaceae, Amaranthaceae	198	20	0	0
Noctuidae	11. *Peridroma saucia*	Brassicaceae, Oxalidaceae, Asteraceae, Polygonaceae	102	10	31	30.4
	12. *Helicoverpa armigera*	Brassicaceae, Solanaceae, Rosaceae, Malvaceae, etc	886	89	73	8.2
	13. *Mythimna separata*	Poaceae	2546	255	404	15.9
	14. *Macdunnoughia confusa*	Brassicaceae, Asteraceae, Polygonaceae	50	5	4	8
	15. *Autographa nigrisigna*	Brassicaceae, Fabaceae, Apiaceae	170	17	36	21.2
	16. *Trichoplusia ni*	Asteraceae, Cucurbitaceae	210	21	12	5.7
	17. *Trichoplusia intermixta*	Asteraceae, Apiaceae	130	13	2	1.5
	18. *Spodoptera litura*	Fabaceae, Convolvulaceae	1151	12	0	0
	19. *Spodoptera exigua*	Brassicaceae, Liliaceae, Solanaceae, Chenopodiaceae	2010	201	0	0

(Continued)

Table 1. *(Continued)*

FAMILY	HOST SPECIES TESTED[a]	FAMILY OF FOOD PLANT FOR HOST LARVAE TESTED	TOTAL NO. OF HOST INSECT USED	NO. OF REPLICATION	TOTAL NO. OF PARASITOID COCOONS	SUCCESSFUL PARASITISM (COCOON FORMATION RATE)
	20. *Mamestra brassicae*	Brassicaceae, Chenopodiaceae	2070	207	0	0
	21. *Aedia leucomelas*	Convolvulaceae	439	44	0	0
Arctiidae	22. *Hyphantria cunea*	Salicaceae, Juglandaceae, Rosaceae, etc	179	18	29	16.2
Bombycidae	23. *Bombyx mori*	Moraceae	710	71	0	0
Geometridae	24. *Xanthorhoe saturata*	Brassicaceae, Apiaceae, etc	150	15	0	0
	25. *Abraxas miranda*	Celastraceae	160	16	0	0
Lycaenidae	26. *Zizeeria maha*	Oxalidaceae	1367	137	19	1.4
Pieridae	27. *Pieris rapae curcivora*	Brassicaceae	731	73	0	0

Oviposited eggs were confirmed in 10 dissections of 15 host species except small number of samples 1 day after oviposition in a preliminary experiment.
[a]A serial number assigned to each lepidopteran host tested is used in the subsequent tables.

containers (30 cm in length × 22.5 cm in width × 6 cm in height) to observe for egression and cocoon formation of the parasitoid (ie, successful parasitism). Host larvae that died before parasitoid emergence were not included in the data analyses.

Furthermore, to determine the realized host range of *C vestalis* in the field, host larvae of 10 species (*Herpetogramma luctuosalis* [n = 37 individuals collected], *H undalis* [87], *C binotalis* [80], *Pyrausta panopealis* [21], *Palpita nigropunctalis* [28], *S litura* [83], *M brassicae* [572], *Aedia leucomelas* [217], *Zizeeria maha* [48], and *Pieris rapae curcivora* [519]) were collected from fields in Tokyo and Saitama prefectures, and all larvae were reared in the laboratory to verify whether they were parasitized or not.

Number of wasp stings per host

Observation of stinging behavior is useful to elucidate the host range because it represents one measure of host acceptance. More suitable hosts may also be more attractive to parasitoids. The number of stings made by *C vestalis* in larvae of each host species was counted for 30 minutes during a foraging bout.

Degree of host suitability

To evaluate the degree of host suitability, differences in the growth and development of the parasitoid eggs and larvae in each host species were examined.

Host caterpillars that had been kept with a female wasp for 3 hours, as mentioned above, were dissected in saline on each of 0, 1, 2, 3, 5, 7, and 10 days after oviposition with at least 10 replications per each *C vestalis*/host species combination. Unparasitized hosts were excluded from the analyses. Although *C vestalis* parasitoid eggs typically hatched ~36 hours after oviposition in *Plutella xylostella* larvae at 25°C, delayed development of the parasitoid in different hosts suggested that the duration of embryogenesis and larval growth may vary across the different host species. To measure growth and development, the volume of parasitoid larvae at 3, 5, and 7 days after oviposition in different host species was calculated by spheroidal equation ($4/3\pi*(L/2)*(W/2)^2$) (L: total length of larval body, W: width in swollen part of abdomen). However, the calculation was slightly overestimated because the actual larval body is not a simple spheroid shape. When 2 or more parasitoid eggs and/or larvae were found in one host larva, the older developmental stage of the parasitoid was recorded.

Furthermore, to determine whether a host's defensive response to *C vestalis* eggs or larva had occurred, the encapsulation rate of parasitoid eggs and larvae in each host species was observed on each day after parasitization.

Results

Successful larval parasitoid egression and cocoon formation

Female wasps of *C vestalis* stung all 27 host species examined (Table 1). Oviposited eggs were confirmed in all the 27 host species with 10 dissections at least 1 day after oviposition to check whether wasps oviposited in a preliminary experiment (personal observation). The successful adult emergence was observed from all cocoons that were formed because the cocoon formation rate of *C vestalis* was defined as "successful parasitization" (Table 1).

Table 2. Number of stings by *Cotesia vestalis* on each larva of the tested lepidopteran species.

SPECIES	NO. OF TRIALS	NO. OF STINGS (MEAN ± SD)	RANGE
1. *Plutella xylostella*	14	15.6 ± 6.9	6-27
4. *Herpetogramma luctuosalis*	10	12.3 ± 7.3	4-25
5. *Hellula undalis*	12	2.4 ± 2.9**	0-10
6. *Crocidolomia binotalis*	7	0.4 ± 0.5**	0-10
7. *Ephestia kuehniella*	15	1.8 ± 2.7**	0-9
12. *Helicoverpa armigera*	15	7.1 ± 6.7*	0-21
13. *Mythimna separata*	18	9.9 ± 5.2	2-17
14. *Macdunnoughia confusa*	6	4.0 ± 3.2*	0-8
15. *Autographa nigrisigna*	12	11.5 ± 6.2	4-23
16. *Trichoplusia ni*	14	3.2 ± 3.7**	0-11
18. *Spodoptera litura*	11	2.6 ± 2.0**	0-6
19. *Spodoptera exigua*	22	4.3 ± 2.8**	0-11
20. *Mamestra brassicae*	14	6.6 ± 5.2*	1-17
21. *Aedia leucomelas*	23	4.7 ± 5.7**	0-23
22. *Hyphantria cunea*	20	4.4 ± 5.4**	0-17
23. *Bombyx mori*	20	6.8 ± 9.0**	0-30
24. *Xanthorhoe saturata*	6	1.8 ± 3.6**	0-9
26. *Zizeeria maha*	9	3.9 ± 4.0**	1-13
27. *Pieris rapae curcivora*	21	3.0 ± 4.7**	0-16

Statistical significance (*$P < .05$; **$P < .01$) was examined by the Dunnett test after 1-way analysis of variance after Box-Cox transformation of data for multiple comparison between *P xylostella* and each host.

In case of *P xylostella*, cocoon formation rate was low due to a large number of host individuals that died for unknown reasons during the experiments.

Cocoons production by *C vestalis* larvae at a low rate (<than 10% successful parasitism) was recorded in 9 host species (*H luctuosalis, H undalis, E kuehniella, P panopealis, H armigera, M confusa, T ni, T intermixta*, and *Z maha*) when reared in the laboratory. No *C vestalis* emerged from the larvae of any of these host species when collected in the field, revealing that they are rarely, if ever used as hosts. There were 7 host species in which with >15% successful parasitism occurred, including *P xylostella*, which has long been considered to be the main or preferred host in the field.

The number of stings observed in 3 host species (*H luctuosalis, M separata*, and *A nigrisigna*) was more than those observed in other host species and was not significantly different when compared with the sting number of its preferred host, *P xylostella*. Especially, in *M separata* and *A nigrisigna* host species, a high frequency of stinging behavior was observed to be a high cocoon formation rate. However, even when the sting frequency was lower, parasitism success was often high, for example, when *C binotalis* served as host (Tables 1 and 2).

Comparison of the growth and development of *C vestalis* in the different host species after oviposition revealed that it was delayed in other host species and was arrested as L1 in some unsuitable hosts, although in *P xylostella* host the parasitoid larva was 2 to 3 days for L1 (first larval instar) and 5 days for L2 (second larval instar) of developmental duration. In *H undalis*, *C binotalis*, *H armigera*, *M separata*, and *A nigrisigna* (Table 3) which sorted in same group (Table 4), even though parasitoid eggs usually hatched 2 days, molting from L1 to L2 tended to be delayed and even 7 days after oviposition most larvae were still L1s. Five host species—*E kuehniella*, *P nigropunctalis*, *S litura*, *S exigua*, and *P rapae curcivora*—had the parasitoid larva stayed in the egg and first larval stage until 7 to 10 days after oviposition, affirming the sort in 2 groups with the stepwise regression analysis (Table 4). In 12 host species except *C binotalis* and *Bombyx mori*, a smaller first instar of parasitoid was observed in size when compared with parasitoid larvae of the same age developing in *P xylostella* caterpillars (Table 7). Larvae of *C vestalis* in *H armigera* and *M separata* grew at approximately the same rate (Table 3), but in *H armigera*, the fewer larvae egressed and successfully formed cocoons due to encapsulation of the parasitoid as L2 (Table 1, Table 5). *Autographa nigrisigna* was a suitable host for *C*

Table 3. Difference in development and growth of *Cotesia vestalis* after oviposition in each host species.

HOST SPECIES	TOTAL NO. OF HOSTS DISSECTED	NUMBER OF EACH HOST WITH *C VESTALIS* EGG OR LARVA ON EACH DAY AFTER OVIPOSITION																							
		1d				2d				3d				5d				7d				10d			
		NO.	E	L1	L2	NO.	E	L1	L2	NO.	E	L1	L2	NO.	E	L1	L2	NO.	E	L1	L2	NO.	E	L1	L2
1. *Plutella xylostella*	88	20	20	0	0	18	0	18	0	11	0	11	0	28	0	0	28	11	0	2	9	—	—	—	—
5. *Hellula undalis*	79	13	13	0	0	22	16	6	0	12	0	12	0	11	1	10	0	19	7	8	4	2	0	2	0
6. *Crocidolomia binotalis*	64	10	10	0	0	12	0	12	0	13	0	13	0	19	12	7	0	10	0	4	6	—	—	—	—
7. *Ephestia kuehniella*	69	10	10	0	0	12	12	0	0	14	14	0	0	13	0	13	0	13	0	13	0	7	3	4	0
9. *Palpita nigropunctalis*	121	25	25	0	0	18	16	2	0	23	19	4	0	31	26	5	0	17	14	3	0	7	6	1	0
12. *Helicoverpa armigera*	103	10	10	0	0	28	2	26	0	17	0	17	0	16	0	16	0	18	0	14	4	14	0	7	7
13. *Mythimna separata*	118	42	42	0	0	15	1	14	0	24	0	24	0	18	0	18	0	8	0	6	2	11	0	0	11
15. *Autographa nigrisigna*	89	13	13	0	0	16	0	16	0	9	0	9	0	45	0	29	16	6	0	4	2	—	—	—	—
16. *Trichoplusia ni*	61	11	11	0	0	4	0	4	0	16	1	15	0	15	0	15	0	14	0	14	0	1	0	0	1
18. *Spodoptera litura*	49	12	12	0	0	16	11	5	0	5	3	2	0	14	13	1	0	2	2	0	0	—	—	—	—
19. *Spodoptera exigua*	109	20	20	0	0	20	17	3	0	45	33	12	0	10	5	5	0	12	10	2	0	2	0	2	0
20. *Mamestra brassicae*	145	18	18	0	0	32	6	26	0	20	5	15	0	27	2	25	0	34	2	32	0	14	0	14	0
21. *Aedia leucomelas*	179	20	20	0	0	54	3	51	0	43	1	42	0	38	1	37	0	24	0	24	0	—	—	—	—
22. *Hyphantria cunea*	113	15	15	0	0	37	0	37	0	15	1	14	0	5	0	5	0	35	0	35	0	6	0	5	1
23. *Bombyx mori*	76	10	10	0	0	11	1	10	0	20	0	20	0	8	0	8	0	17	0	17	0	10	0	10	0
27. *Pieris rapae curcivora*	50	5	5	0	0	39	25	14	0	—	—	—	—	—	—	—	—	6	2	4	0	—	—	—	—

Abbreviations: E, egg; L1, first instar; L2, second instar of *C vestalis*.
The number in dark halftone shows the total number of dissected host in each day after parasitization.

Table 4. Statistical analysis by stepwise method using ordinary logistic regression analysis.

FACTORS	LIKELIHOOD RATIO, χ^2	*P*
{Pn&Sl-Se}	4.31252874	.0378
{Prc-Ek}	1.17301435	.2788
{Hu&Ms&Cb&Mb&Tn&Bm&Al&Hc-Ha&An&Px}	219.311292	<.0001
{Hu-Ms&Cb&Mb&Tn&Bm&Al&Hc}	18.4545711	<.0001
{Ms&Cb&Mb-Tn&Bm&Al&Hc}	3.14561347	.0761
{Ms&Cb-Mb}	10.6405048	.0011
{Ms-Cb}	4.65003722	.0311
{Tn-Bm&Al&Hc}	0.05603925	.8129
{Ha&An-Px}	64.4869583	<.0001
{Ha-An}	23.1083874	<.0001
DAP	266.793775	<.0001
{Pn&Sl&Se&Prc&Ek-Hu&Ms&Cb&Mb&Tn&Bm&Al&Hc&Ha&An&Px}*DAP	103.614232	<.0001
{Pn&Sl&Se-Prc&Ek}*DAP	7.29313867	.0069
{Pn&Sl-Se}*DAP	2.29755678	.1296
{Prc-Ek}*DAP	3.28442009	.0699
{Hu&Ms&Cb&Mb&Tn&Bm&Al&Hc-Ha&An&Px}*DAP	118.455449	<.0001
{Hu-Ms&Cb&Mb&Tn&Bm&Al&Hc}*DAP	3.02268377	.0821
{Ms&Cb-Mb}*DAP	20.6784609	<.0001
{Ms-Cb}*DAP	7.19778414	.0073*
{Tn-Bm&Al&Hc}*DAP	7.8088384	.0052*
{Ha&An-Px}*DAP	73.2408399	<.0001
{Ha-An}* DAP	36.8324945	<.0001

Abbreviations: An, *Autographa nigrisigna*; AL, *Aedia leucomelas*; Bm, *Bombyx mori*; DAP; days after parasitization; Ha, Helicoverpa armigera; Hc, *Hyphantria cunea*; Hu, *Hellula undalis*; Mb, *Mamestra brassicae*; Ms, *Mythimna separata*; Pn, *Palpita nigropunctalis*; Prc, *Pieris rapae curcivora*; Se, *Spodoptera exigua*; SL, *Spodoptera litura*; Tn, *Trichoplusia ni*.

vestalis, and there was a low rate of encapsulation in the early larval stages of the parasitoid (Table 5). In *H undalis*, *C vestalis* eggs were encapsulated in more than half of the dissected host larvae on most days more than 7 days after oviposition, although no encapsulation was observed in 2 hosts 10 days after parasitization (Table 5), causing a low cocoon formation rate (Table 1). In *T ni* and *H cunea*, second instar parasitoid larvae were also observed 10 days after parasitism (Table 5), coincident with result of cocoon formation rate (Table 1).

The numbers in a parenthesis indicates the number of insects dissected. Different alphabets indicate the significant difference by the Tukey test after 1-way analysis of variance after Box-Cox transformation.

Stepwise regression analysis based on the least Akaike information criterion value[32] shows that 4 host species—*P nigropunctalis*, *S exigua*, *M brassicae*, and *P rapae curcivora*—were grouped in 1 cluster, 5 host species—*M separata*, *A nigrisigna*, *T ni*, *A leucomelas*, and *H cunea*—were grouped in other cluster including *H armigera, S litura*, and *B mori*, meaning that 2 groups show the different developmental degrees against the host defense reaction (Table 6).

In 7 host species (*E kuehniella*, *P nigropunctalis*, *S litura*, *S exigua*, *A leucomelas*, *B mori*, and *P rapae curcivora*), development of the parasitoid was arrested at L1. However, in *E kuehniella*, 2 L2 *C vestalis* with smaller body volumes than those in other host species were found (Table 7). In *P nigropunctalis, S litura, S exigua*, and *P rapae curcivora*, most of the parasitoid eggs did not hatch, but a small number of first parasitoid instars were found and most were rapidly encapsulated (Tables 3 and 5), indicating that these host species are unsuitable for development of *C vestalis*. Both *S litura* and *S exigua* exhibited a high rate of encapsulation (Table 5) and arrested parasitoid development during

Table 5. Encapsulation rate of *Cotesia vestalis* eggs or larvae in each host larvae.

HOST SPECIES	TOTAL NO. OF DISSECTED LARVAE	ENCAPSULATION RATE (%) OF PARASITOID EGGS OR LARVAE IN PARASITIZED HOSTS EACH DAY AFTER PARASITIZATION							
		0	1	2	3	5	7	10	14 d
1. *Plutella xylostella*	94	0 (12)	0 (20)	0 (12)	0 (11)	0 (28)	0 (11)	—	—
5. *Hellula undalis*	58	0 (10)	23.1 (13)	58.8 (17)	40.0 (5)	100 (1)	63.6 (11)	0 (2)	—
9. *Palpita nigropunctalis*	134	0 (12)	56.0 (25)	100 (18)	88.5 (26)	89.7 (29)	94.1 (17)	100 (7)	—
12. *Helicoverpa armigera*	130	0 (25)	0 (10)	0 (28)	0 (17)	25.0 (16)	16.7 (18)	0 (16)	—
13. *Mythimna separata*	152	0 (33)	0 (42)	6.7 (15)	0 (24)	0 (18)	0 (8)	0 (12)	—
15. *Autographa nigrisigna*	93	0 (5)	0 (13)	0 (16)	11.1 (9)	0 (45)	0 (5)	—	—
16. *Trichoplusia ni*	68	0 (7)	0 (11)	0 (4)	6.3 (16)	0 (15)	0 (14)	0 (1)	—
18. *Spodoptera litura*	58	0 (10)	8.3 (12)	50.0 (16)	40.0 (5)	38.5 (13)	50.0 (2)	—	—
19. *Spodoptera exigua*	133	0 (14)	15.0 (20)	70.0 (20)	86.6 (45)	95.0 (20)	91.7 (12)	100 (2)	—
20. *Mamestra brassicae*	136	0 (16)	0 (18)	22.2 (9)	71.4 (21)	87.5 (24)	76.7 (30)	72.7 (11)	57.1 (7)
21. *Aedia leucomelas*	206	0 (35)	0 (20)	18.9 (53)	5.6 (36)	36.8 (38)	33.3 (24)	—	—
22. *Hyphantria cunea*	108	0 (11)	0 (15)	26.1 (23)	0 (15)	0 (5)	0 (33)	0 (6)	—
23. *Bombyx mori*	72	—	0 (10)	44.4 (9)	50.0 (20)	0 (8)	0 (15)	0 (10)	—
27. *Pieris rapae curcivora*	100	—	40.0 (5)	60.5 (38)	100 (24)	100 (18)	100 (3)	—	—

Number in parenthesis shows the number of dissected hosts on each day. Hosts with no parasitoid egg or larva after attack were discarded from data.

the L1 (Table 3). Similarly, in *B mori* and *P rapae curcivora*, all parasitoid larvae were encapsulated as L1 within 2 to 3 days of parasitism.

Discussion

Our results show that there were profound differences in the suitability and quality among the different hosts for the development of *C vestalis*. For hosts in the micromoth family Pyralidae, *C vestalis* survived poorly in *H luctuosalis, H undalis, E kuehniella*, and *P panopealis*, and even those that were able to reach L2 experienced developmental delay. Furthermore, several hosts in the macromoth family Noctuidae (*H armigera*, *M confusa*, *T ni*, and *T intermixta*) were also of low suitability for *C vestalis*, also with low egression and cocoon formation rates and developmental delay as L2. In both of the above families, the physiological state in the hemocoel of hosts was clearly marginal at best for the development of *C vestalis* larva. In other noctuids (*P nigropunctalis*, *Hymenia recurvalis*, 2 *Spodoptera* species, *M brassicae*, and *A leucomelas*), the parasitoid was unable to develop past L1, even though they showed no signs of being encapsulated, ie, melanization. This reveals that young larvae may have been unable to use the fat body tissue of the host as a food source. Larvae of *C vestalis* and other species in the Microgastrinae use polydnaviruses (PDVs) and venoms that are injected into the host during the oviposition sequence to regulate host growth and abrogate the host immune system.[33] Polydnaviruses are also found in parasitoid species from a few other subfamilies of the Braconidae and Ichenumonidae and have been shown to be important factors in parasitoid development and survival.[34–38] Polydnaviruses regulate the physiological state of host by-products that are translated in the host cells, such as fat bodies and hemocytes, soon after parasitization.[39–41]

Furthermore, in braconid endoparasitoids, such as *C vestalis*, other regulatory factors, including as teratocytes and secretions from the parasitoid larva(e), also influence host growth and immunity and thus enhance parasitoid survival. It is well known that teratocytes assist the growth and development of parasitoid larvae by controlling the physiological state of the host during the parasitoid larval stage.[42–47] In hosts "conditioned" with PDV and venom, teratocytes also provide a trophic function and thus enhance the nutrition of late larval stages of the parasitoid.[33] The fact that L2 *C vestalis* failed to develop in some hosts could be due to a death of circulating teratocytes and the inability of PDV to regulate host development effectively. It is known that incomplete host regulation by PDV and/or teratocytes appears to strongly affect the physiological host range.[48] Host physiological defenses are strongly phylogenetically conserved, and PDVs have co-evolved intimately with parasitoids to regulate the immunity and development of a narrow range of closely related hosts.

When *C vestalis* superparasitized hosts, as was the case in *H undalis*, some parasitoid larvae were able to avoid the host defense reaction, despite conspecific larvae being encapsulated in host hemocoel. This suggests that superparasitism can be adaptive if multiple ovipositions "overwhelm" host internal

Table 6. Statistical analysis by stepwise regression based on least Akaike information criterion value.

FACTORS	LIKELIHOOD RATIO, χ^2	*P*
{Ms&An&Tn&Hc&AL&Ha&Bm&SL&Hu-Mb&Se&Pn&Prc}	464.357056	<.0001
{Ms&An&Tn&Hc-AL&Ha&Bm&SL&Hu}	51.868549	<.0001
{Ms&An&Tn-Hc}	4.1975045	.0405
{AL&Ha&Bm-SL&Hu}	25.6776314	<.0001
{AL&Ha-Bm}	11.9295923	.0006
{AL-Ha}	15.5082795	<.0001
{Mb&Se-Pn&Prc}	17.703903	<.0001
{Mb-Se}	34.1441169	<.0001
{Pn-Prc}	4.45368918	.0348
DAP	37.6377259	<.0001
{Ms&An&Tn&Hc&AL&Ha&Bm&SL&Hu-Mb&Se&Pn&Prc} *DAP	61.0497225	<.0001
{Ms&An&Tn&Hc-AL&Ha&Bm&SL&Hu}*DAP	6.50624679	.0107
{Ms&An&Tn-Hc}*DAP	0.49321979	.4825
{AL&Ha-Bm}*DAP	54.5198877	<.0001
{AL-Ha}*DAP	10.5095869	.0012
{Mb&Se-Pn&Prc}*DAP	4.5658298	.0326
{Mb-Se}*DAP	22.8875413	<.0001
{Pn-Prc}*DAP	6.76090709	.0093

Abbreviations: An, *Autographa nigrisigna*; AL, *Aedia leucomelas*; Bm, *Bombyx mori*; DAP; days after parasitization; Ha, Helicoverpa armigera; Hc, *Hyphantria cunea*; Hu, *Hellula undalis*; Mb, *Mamestra brassicae*; Ms, *Mythimna separata*; Pn, *Palpita nigropunctalis*; Prc, *Pieris rapae curcivora*; Se, *Spodoptera exigua*; SL, *Spodoptera litura*; Tn, *Trichoplusia ni*.

defense responses. In the solitary *Microplitis rufiventris*-*Spodoptera littoralis* association, superparasitization of the final instars of the host caterpillars (an atypical condition) increased the number of live wasp larvae that emerged[49] even in low-quality (late instar) hosts.[50] Consequently, physiological host range in the Microgastrinae is significantly influenced by the ability of the parasitoid to regulate the host's physiological condition through the expression of factors such as PDV, venom, and teratocytes. Superparasitism by *C vestalis* enables the parasitoid to survive at low rates in low-quality hosts such as *P panopealis*, *T ni*, *T intermixta*, and *Z maha*.

Examination of the suitability of different host species for *C vestalis* is important for determining the parasitoid's host range. *Cotesia vestalis* successfully parasitized 15 host species from 5 families including its preferred hosts in the Plutellidae. No phylogenetic relationships between host species and successful parasitism were found in our study. However, we did not determine whether successful parasitization of the different host species is correlated with the preferred plant diets of these hosts in nature. If so, overlap in plant dietary regimes may generate similarities in physiological conditions among closely related host species, rendering them suitable or not. *Cotesia vestalis* failed to develop in 2 species of *Spodoptera* that are known dietary generalists but which clearly have evolved internal metabolic defenses that are similar.

When the DBM and other host species were placed together with *C vestalis*, the wasp often preferred to parasitize host species other than the DBM (personal observations). This raises the possibility that the wasp may prefer to oviposit in host species other than the DBM when these species are locally sympatric in the field. When other host species grow near a DBM population, it is also possible that *C vestalis* parasitizes these other species as well, although this needs further verification. Various weed species (eg, plants in the family Asteraceae or Fabaceae or Poaceae) grow sympatrically in or around the fields where cultivated brassicaceous plants grow. This may enable multiple lepidopteran host species to exist sympatrically in the same field, each exploiting different plants growing in heterogeneous stands. Multiple host species that live sympatrically and feed on the same plant species may develop a similar defense system against the parasitoid.

Brodeur and Vet[51] suggested that host acceptance and suitability is affected not only by the host immunologic compatibility but also by traits influencing its foraging behavior. Vos and Vet[52] reported geographic variation in host acceptance between American and European parasitoid strains of the gregarious

Table 7. Change of *Cotesia vestalis* larvae in volume with age after attack.

HOST SPECIES	TOTAL NO. OF LARVAE FOUNDED	VOLUME OF PARASITE LARVAE ([MEAN ± SD] $\mu M^3 \times 10^3$)				
		DAYS AFTER PARASITIZATION (STAGE OF PARASITOID LARVA)				
		3 d	5 d		7 d	
		(FIRST INSTAR)	(FIRST INSTAR)	(SECOND INSTAR)	(FIRST INSTAR)	(SECOND INSTAR)
1. *Plutella xylostella*	82	9.5 ± 4.6 (17)abc	553.5 ± 742.6 (38)a	—	114.2 ± 203.5 (9)ab	1513.4 ± 770.5 (18)a
5. *Hellula undalis*	34	5.4 ± 2.2 (12)bcd	7.9 ± 6.8 (10)cdef	—	48.2 ± 50.3 (8)ab	482.2 ± 199.2 (4)ab
6. *Crocidolomia binotalis*	39	16.6 ± 9.1 (12)a	116.2 ± 157.9 (13)ab	524.0 ± 213.9 (6)a	89.6 ± 59.1 (4)a	1108.0 ± 622.7 (6)ab
7. *Ephestia kuehniella*	48	5.7 ± 1.4 (14)abcd	22.3 ± 21.3 (11)bc	105.7 ± 72.0 (2)b	21.3 ± 27.3 (19)ab	489.0 ± 170.3 (2)ab
9. *Palpita nigropunctalis*	9	3.1 ± 0.8 (4)def	2.6 ± 1.1 (2)defg	—	6.0 ± 3.4 (3)abcd	—
12. *Helicoverpa armigera*	46	5.3 ± 1.7 (15)bcd	8.9 ± 8.6 (13)cdf	—	31.3 ± 40.8 (13)ab	1108.4 ± 758.0 (5)ab
13. *Mythimna separata*	73	5.3 ± 1.2 (24)bcd	18.0 ± 21.4 (17)cd	—	35.7 ± 37.0 (23)ab	543.1 ± 268.9 (9)b
15. *Autographa nigrisigna*	51	4.1 ± 0.7 (8)cde	—	—	42.7 ± 39.8 (25)ab	752.2 ± 551.5 (18)b
16. *Trichoplusia ni*	42	4.0 ± 0.8 (14)d	5.8 ± 3.8 (14)cdef	—	13.0 ± 10.7 (14)ab	—
18. *Spodoptera litura*	6	2.1 ± 1.2 (6)f	—	—	—	—
19. *Spodoptera exigua*	14	2.3 ± 0.8 (10)f	2.0 ± 0.4 (2)efg	—	2.7 ± 0.5 (2)bcde	—
20. *Mamestra brassicae*	40	2.5 ± 0.6 (13)ef	2.1 ± 0.9 (15)g	—	1.6 ± 0.7 (12)e	—
21. *Aedia leucomelas*	68	3.9 ± 1.0 (32)d	3.4 ± 1.2 (27)e	—	2.3 ± 0.8 (10)de	—
22. *Hyphantria cunea*	27	3.3 ± 1.1 (3)bcdef	5.6 ± 1.6 (5)cdef	—	3.9 ± 2.4 (19)cd	—
23. *Bombyx mori*	32	163.2 ± 173.7 (20)ab	140.5 ± 208.5 (6)bcd	—	78.5 ± 174.7 (6)abc	—

Significance for 'a to e' - Different alphabetical letter in the same column means significantly difference (one way ANOVA, Tukey-Kramer test, $p<0.05$).

endoparasitoid *Cotesia glomerata*. *Cotesia glomerata* and the 2 pierid hosts are native to Eurasia but *P rapae* was accidentally introduced into North America in the 19th century, and *C glomerata* was shortly thereafter introduced to control *P rapae*. Importantly, *P (Pieris) brassicae* is absent from North America. The authors found that although the European *C glomerata* strain uses both *P rapae* and *P brassicae* as hosts, the American strain rejected *P brassicae* significantly more often than did the European strain, indicating that was losing the ability to recognize *P brassicae* as a result of frequency-dependent selection. Actually, host range may be more influenced by host density, natural enemy pressure, and competitors than by physiological constraints. However, although *C vestalis* attacked many host species under laboratory conditions (Table 1), no cocoon formation occurred within many of these same host species that were collected in the field. These data suggest that host range seems to be decided through a process, whereby progeny of parasitoids under natural selection by host physiological factors is able to develop successfully in some hosts that also increases their ecological specificity within the environment (a form of local adaptation). *Cotesia vestalis* has long been known to preferentially parasitize *P xylostella* in the field. This is often the dominant species in cruciferous crop fields, potentially adding to the selection for succeeding generations to preferentially parasitize this moth as suggesting with the field reports of Okada.[23]

Conclusions

We have reported that *C vestalis* has a broad physiological host range, which enhances the possibility that this wasp may be retained in or around the fields of cabbage crops that are out of season because the wasp may parasitize the different potential hosts on the other different plants grown sympatrically in or around the same field. If this is the case, it may enable the wasp to control populations of DBM in the early stages of cultivation, rather than later in the growing season when populations have grown.

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Author Contribution

SH: collected the data and wrote the original draft., JAH: contributed to the discussion and check the English., YN, HN: collect the samples, contribute to the discussion., JM: coordinated the whole contents TM: contributed to the statistical analysis., TT: reorganaized the data and rewrote the whole sentence.

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