Potential Host Range of the Larval Endoparasitoid Cotesia vestalis (=plutelliae) (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

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 of 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 parasitoid5 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, Campoplexis 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.12 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
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 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 (15–17). 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 at 25°C. (14,19). Although it has been reported that *C. vestalis* has a relatively wide host range (22 species in 12 families), 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, *C. binotalis*; *Trichoplusia ni*, *Spodoptera exigua*, *Peridroma saucia*, *H. armigera*, *M. separata*, *Mamestra brassicae*, *A. nigrisigna*, *Macunoughia confusa*, *Trichoplusia ni*, *Trichoplusia intermixta*, *Xanthorhoe saturata*, and *Hypantria cunea*, artificial diet (27) for *C. binotalis*; radish sprout powder in exchange for cabbage powder as an alternative composition for *C. binotalis* and *Helissa 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.

### 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*, *Macunoughia confusa*, *Trichoplusia ni*, *Trichoplusia intermixta*, *Xanthorhoe saturata*, and *Hypantria cunea*, artificial diet (27) for *C. binotalis*; radish sprout powder in exchange for cabbage powder as an alternative composition for *C. binotalis* and *Helissa 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 | 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. *Eupoee 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. *Ephesia kuemnella* | flour                                        | 466                           | 47                | 38                              | 8.2                                          |
|            | 8. *Pyrausta panopealis* | Oleaceae                                     | 48                            | 48                | 2                               | 4.2                                          |
|            | 9. *Palpita nigropunctalis* | Lamiaceae                                   | 320                           | 32                | 0                               | 0                                            |
|            | 10. *Hyemenia recurvalis* | Chenopodiaceae, Cucurbitaceae, Amaranthaceae | 198                           | 20                | 0                               | 0                                            |
| Noctuidae | 11. *Peridromia 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)
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 luctuosa* [n = 37 individuals collected], *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.

**Table 1. (Continued)**

| FAMILY       | HOST SPECIES TESTEDa | FAMILY OF FOOD PLANT FOR HOST LARVAE TESTED | TOTAL NO. OF HOST INSECT USED | NO. OF REPLICATION | TOTAL NO. OF PARASITOID COCOONS | SUCCESSFUL PARASITISM (COCCOON 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 | |
| Lycanidae    | 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 were used in the field, and host larvae of 10 species 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 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, the 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 (4/3π*(L/2)*(W/2)²) (L: total length of larval body, W: width in swollen part of abdomen). However, the calculation was slightly underestimated 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. Euplagia kuehiella    | 15            | 1.8 ± 2.7**               | 0-9   |
| 12. Heliothis 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. kuehiella, 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. kuehiella, 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 larva 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 OVIPPOSITION |
|-------------------------------|------------------------------|----------------------------------------------------------------------------------|
|                               | 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  | 28 | 0  | 28 | 0  | 2  | 9  | —  |
| 5. Heloëla undalis            | 79  | 13 | 13 | 0  | 0  | 22 | 16 | 6  | 0  | 12 | 0  | 12 | 0  | 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  | 0  | 19 | 12 | 7  | 0  | 10 | 0  | 4  | 6  | —  | —  | —  |
| 7. Ephesia kuehniella         | 69  | 10 | 10 | 0  | 0  | 12 | 12 | 0  | 0  | 14 | 14 | 0  | 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 | 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 | 14 | 0  | 24 | 0  | 24 | 0  | 18 | 0  | 18 | 0  | 8  | 0  | 6  | 2  | 11 | 0  | 0  | 11 |
| 15. Autographa nigripesigna   | 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 | 15 | 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  | 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  | —  | —  | —  | —  | —  | —  | —  | —  | —  | —  | —  | —  | —  | —  | —  |

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, χ² | 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) | 18.454711 | <.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 | 3.2842009 | .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 | 7.8008384 | .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 indicates 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
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 Noctuidae, C vestalis survived poorly in H armigera, P panopeali, 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 internixa) 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 Ichneumonidae 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

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**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.
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 even in low-quality (late instar) hosts. 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 (e.g., plants in the families 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 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 reported geographic variation in host acceptance between American and European parasitoid strains of the gregarious

| FACTORS | LIKELIHOOD RATIO, $\chi^2$ | 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) | 11.9295923 | <.0001 |
| (AL-Ha)*DAP | 4.45368918 | .0348 |
| (Mb&Se-Pn&Pr) | 37.6377259 | <.0001 |
| (Mb&Se-Pn&Pr)*DAP | 61.0497225 | <.0001 |
| (Mb&Se-Pn&Pr)*DAP | 6.50624679 | .0107 |
| (Mb&Se-Pn&Pr)*DAP | 0.49321979 | .4825 |
| (AL&Ha-Bm)*DAP | 54.5198877 | <.0001 |
| (AL-Ha)*DAP | 10.5095869 | .0012 |
| (Mb&Se-Pn&Pr)*DAP | 4.5658298 | .0326 |
| (Mb&Se-Pn&Pr)*DAP | 22.8875413 | <.0001 |
| (Mb&Se-Pn&Pr)*DAP | 6.7609709 | .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; Pr, Pieris rapae curcivora; Se, Spodoptera exigua; SL, Spodoptera litura; Tn, Trichoplusia ni.

Table 6. Statistical analysis by stepwise regression based on least Akaike information criterion value.
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] µM³ × 10³) |
|--------------|-----------------------------|---------------------------------------------------|
|              | DAYS AFTER PARASITIZATION (STAGE OF PARASITOID LARVA) | 3d (FIRST INSTAR) | 5d (SECOND INSTAR) | 7d (SECOND INSTAR) |
|              | 3d | 5d | (SECOND INSTAR) | 7d | (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 (6)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)ab | — |
| 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)ab |
| 15. *Autographa nigrisigna* | 51 | 4.1 ± 0.7 (8)ddef | — | — | 42.7 ± 39.8 (25)ab | 752.2 ± 551.5 (18)ab |
| 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)defg | — | 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)cdef | 5.6 ± 1.6 (5)cdef | — | 3.9 ± 2.4 (19)cde | — |
| 23. *Bombyx mori* | 32 | 163.2 ± 173.7 (20)abc | 140.5 ± 208.5 (6)bc | — | 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 suggested 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: reorganized the data and rewrote the whole sentence.

REFERENCES
1. Godfray HCJ. *Parasitoids: Behaviour and Evolutionary Ecology*. Princeton, NJ: Princeton University Press; 1994.
2. Quicke DLJ. Parasitic Wasps. London, England: Chapman & Hall; 1997.
3. Quicke DLJ. Overcoming host immune reaction and physiological interactions with host. In: Quicke DLJ, ed. *The Braconid and Ichneumonid Parasitoid Wasp: Biology, Systematics, Evolution, and Ecology*. Oxford, UK: Wiley Blackwell; 2015: 132–162.
4. Lavine MD, Strand MR. Insect hemocyes and their role in immunity. *Insect Biochem Mol Biol*. 2002;32:1295–1309.
5. Vet LEM, Dicke M. Ecology of inflorescital use by natural enemies in a triphasic context. *Ann Rev Entomol*. 1993;37:141–172.
6. Harvey JA. Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. *Entomol Exp Appl*. 2005;117:1–13.
7. Ode PJ. Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annu Rev Entomol*. 2006;51:57–84.
8. Fox LR, Morrow PA. Specialization: species property or local phenomenon? *Science*. 1981;211:887–893.
9. Gauld ID. The Pimplininae, Xoridinae and Lectrinae (Hymenoptera: Ichneumonidae) of Australia. *Bull Br Mus nat Hist (Ent)*. 1984;49:235–339.
10. Myers LW, Varner T. Factors affecting the host range and diversity of parasitoid complexes in tephrithid fruit flies. *Pistacia*. 1992:289–288–297.
11. Harvey JA, de Embun MGX, Bukovinsky T, Gols R. The roles of ecological fitting, phylogeny and physiological equivalence in understanding realized and fundamental host ranges in endoparasitoid wasps. *J Evol Biol*. 2012;25: 2137–2148.
12. Harvey JA, Sano T, Tanaka T. Differential host growth regulation by the solitary endoparasitoid, *Meteorus palporbicionis* in two hosts of greatly differing mass. *J Insect Physiol*. 2010;56:1178–1183.
13. Harcourt DG. Biology of the diamondback moth, *Plutella xylostella* (Lep.) (Lepidoptera: Plutellidae), in Eastern Ontario. II. Life-history, behaviour, and host relationship. *Can Entomol*. 1957;59:554–563.
14. Velasco LRI. The life history of *Cotesia vestalis* Kurjii (Bracidae), a parasitoid of the diamondback moth. *Philippine Entomol*. 1982;3:355–399.
15. Talekai NS, Shelton AN. Biology, ecology, and management of the diamondback moth. *Ann Rev Entomol*. 1993;38:277–301.
16. Noda T. Parasitoids of the diamondback moth distributed in Japan [in Japanese]. *Shokubutsu-boeki*; 1997:51:20–24.
17. Furlong MJ, Wright DJ. Diamondback moth ecology and management: problems, progress, and prospects. *Ann Rev Entomol*. 2013;58:517–541.
18. Matsuura M. Parasites of the diamondback moth, *Plutella xylostella* (Linnaeus) their species and seasonal fluctuations. *Bull Fac Agr Mie Univ*. 1977;54:45–51.
19. Kawaguchi M, Tanaka T. Biological characteristics of a larval endoparasitoid, *Cotesia vestalis* (Hymenoptera: Braconidae): host stage preference, subsequent sex ratio of progeny and mate location of males. *Appl Entomol Zool*. 1999;34:213–221.
20. Shi Z-H, Lui S-S, Li Y-X. *Cotesia plutella* parasitizing *Plutella xylostella*: host-age dependent parasitism and its effect on host development and food consumption. *BioCon*. 2002;47:499–511.
21. Papp J. A survey of the European species of *Apatelodes fuscus* (Hymenoptera, Braconidae: Microgastrinae). XII. Supplement to the key of the glomeratus-group. *Parasitoid/host list 2. Ann Historico Naturale Mus Nationali Hungarici*. 1990;81:159–203.
22. Nixan GEJ. A revision of the north-western European species of the glomeratus-group of *Apatelodes Forster* (Hymenoptera, Braconidae). *Bull Entomol Res*. 1974;64:453–524.
23. Okada T. Parasitoids of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomaeidae): species and seasonal changes of parasitism in cabbage fields. *Jap J Appl Entomol Zool*. 1989;33:17–23.
24. Pottier RPJ, Poppy GJ, Schuler TH. The role of volatiles from cruciferous plants and pre-flight experience in the foraging behaviour of the specialist parasitoid *Cotesia vestalis*. *Ent Exp Appl*. 1999;93:87–95.
25. Louxide HD, Lushai G, Harvey JA. The evolutionary improbability of “generalism” in nature, with special reference to insects. * Biol J Linn Soc*. 2011;103:1–18.
26. Londale HD, Harvey JA. 2016. The "generalism" debate: misinterpreting the term in the empirical literature focusing on dietary breadth in insects. *Biol J Linn Soc*. 2011;119:265–282.
27. Hirashima Y, Abe M, Tadauchi O, Konishi K, Maeto K. The hymenopteron parasitizing ratio of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomaeiddae) in Japan. *EntoKLA*. 1989;28:63–73.
28. Kaneko J. Parasitic wasps of the silver Y moth, *Autographa gamma* (L.) (Noctuidae: Plusiinae) and the Asiatic common looper, *A. nigripes* (Walker) in Hokkaido, Japan [in Japanese]. *Jap J Appl Entomol Zool*. 1999;37:22–24.
29. Shoji K, Takahayashi J, Yano S, Takafuji A. Flight response to parasitoids toward plant-herbivore complexes: a comparative study of two parasitoid-herbivores systems on cabbage plants. *Appl Entomol Zool*. 2000;34:459–461.
30. Alleyne M, Wiedenmann RN. Suitability of lepidopteran stemborees for parasitization by novel association endoparasitoids. BioControl. 2001;46:1–23.

31. Kurihara M, Tatsuki S, Sutrisno S, Fukami J. Artificial diet for the large cabbage-heart caterpillar, Crocidolomia binotalis (Zell.) (Lepidoptera: Pyralidae). Appl Entomol Zool. 1987;22:232–234.

32. Akaike H. Akaike's information criterion. In: Lovric M, ed. International Encyclopedia of Statistical Science. Berlin, Germany: Springer; 2011:25.

33. Nakamatsu Y, Fuji S, Tanaka T. Larvae of an endoparasitoid, Cotesia kariyai (Hymenoptera: Braconidae), feed on the host fat body directly in the second stadium with the help of teratocytes. J Insect Physiol. 2002;48:1041–1052.

34. Beckage NE. Parasitoids and polydnaviruses. BioScience. 1998;48:305–311.

35. Cui LA, Soldevila I, Webb WA. Relationships between polydnavirus gene expression and host range of the parasitoid wasp Campoplexis sonorensis. J Insect Physiol. 2000;46:1397–1407.

36. Kroemer JA, Webb BA. Polydnavirus genes and genomes: emerging gene families and new insights into polydnavirus replication. Annu Rev Entomol. 2004;49:431–456.

37. Webb BA, Strand MR. The biology and genomics of polydnaviruses. In: Gilbert LI, Iatrou K, Gill SS, eds. Comprehensive Molecular Insect Science. Vol. 6, Oxford, UK: Elsevier; 2005:323–360.

38. Strand MR, Burke GR. Polydnaviruses as symbionts and gene delivery systems. PLoS Pathogens. 2014;8:e1002757.

39. Vinson SB, Iwantsch GF. Host suitability for insect parasitoids. Ann Rev Entomol. 1980;25:397–419.

40. Turechek MJ, Webb BA. Perspectives on polydnavirus origins and evolution. Adv Virus Res. 2002;58:203–254.

41. Gelman DB, Gerling D, Blackburn MB, Hu JS. Host-parasite interactions between whiteflies and their parasitoids. Arch Insect Biochem Physiol. 2005;60:209–222.

42. Rana RL, Dahlman DL, Webb BA. Expression and characterization of a novel teratocyte protein of the braconid, Microplitis croceipes (Cresson). Insect Biochem Mol Biol. 2002;32:1507–1516.

43. Hoy HL, Dahlman DL. Extended in vitro culture of Microplitis croceipes teratocytes and secretion of TSP14 protein. J Insect Physiol. 2002;48:401–409.

44. Barratt BJ, Sutherland M. Development of teratocytes associated with Microctonus ariaioides Loan (Hymenoptera: Braconidae) in natural and novel host species. J Insect Physiol. 2001;47:257–262.

45. Alleyne M, Wiedenmann RN, Diaz RR. Quantification and development of teratocytes novel-association host-parasitoid combinations. J Insect Physiol. 2001;47:1419–1427.

46. Dahlmann DL. Evaluation of teratocytes functions: an overview. Arch Insect Biochem Physiol. 1990;13:159–166.

47. Dahlmann DL. Teratocytes and host/parasitoid interactions. Biol Control. 1991;1:118–126.

48. Ralec AL, Ribule A, Barragan A, Outreman Y. Host range limitation caused by incomplete host regulation in an aphid parasitoid. J Insect Physiol. 2011;57:363–371.

49. Hegazi EM, Khafagi WE. Gregarious development of the solitary endo-parasitoid, Microplitis rufiventris in its habitual host, Spodoptera littoralis. J Insect Physiol. 2008;54:427–438.

50. Brodeur J, Vet LEM. Relationships between parasitoid host range and host defense: a comparative study of egg encapsulation in two related parasitoid species. Physiol Entomol. 1995;20:7–12.

51. Vos M, Vet LEM. Geographic variation in host acceptance by an insect parasitoid: genotype versus experience. Evol Ecol Res. 2004;6:1021–1035.