Intraspecific Competition Between Adult Females of the Hyperparasitoid *Trichomalopsis apanteloctena* (Hymenoptera: Chelonidae), for Domination of *Cotesia kariyai* (Hymenoptera: Braconidae) Cocoons

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ABSTRACT The development of parasitoid wasps is dependent on the finite resources contained in a single item of resource (=host) that is frequently not much larger than the adult parasitoid. When the costs of egg production are high, and host distribution is highly aggregated, parasitoid females may spend prolonged periods guarding their eggs and host resources as an adaptive strategy to optimize their inclusive fitness. Here, we examine aggressive interactions between the females of the secondary hyperparasitoid *Trichomalopsis apanteloctena* (Crawford) (Hymenoptera: Chelonidae), for control of cocoon clusters of their primary parasitoid host *Cotesia kariyai* (Watanabe) (Hymenoptera: Braconidae). Generally, larger female hyperparasitoids were more successful at defending cocoon clusters than smaller female hyperparasitoids. However, when first encountering host cocoons, larger females behaved more aggressively toward conspecific wasps than smaller females. After occupation of a host cocoon cluster, females of similar size rarely engaged in physical combat, but both females primarily exhibited threatening behavior toward each other. However, larger females usually displaced smaller females which had initially occupied cocoon clusters. Some small females chewed through the outer cocoon silk layer to avoid being displaced by larger females and these wasps were able to continue parasitizing cocoons of *C. kariyai*. Extended bouts of aggression tended to reduce the number of eggs laid by the guarding female because of disruption of oviposition behavior. The relationship between the size of host cocoons and body mass in *T. apanteloctena* was also examined. The size of hyperparasitoid progeny was strongly correlated with host size. However, the relationship between maternal size, the number of matured eggs in her ovarioles and body mass in her offspring was not significant.

KEY WORDS competition, idiobiont, occupation, ovipositional resource

The consumption and assimilation of nutrients is essential for organisms to maintain metabolic activity for vital life functions such as locomotion, flight and reproduction. In situations where nutrients are scarce or of low quality, intense intra- and/or interspecific competition for access and control of these resources can lead to the establishment of “dominance hierarchies” among species and individuals occupying a guild (Sutherland 1996). One group of organisms in which resources for development are exceedingly limited is parasitoid wasps. Parasitoids develop in, or on, the bodies of other arthropods (usually insects), and their development is crucially dependent on the limited resources contained in a single host individual that is frequently not much larger than the parasitoid itself. By contrast, most other predatory arthropods may need to feed on several to many prey to attain maturity. Thus, parasitoids are under strong selection pressure to optimize the use of these limited resources (Slansky 1986).

Among parasitoids, there is considerable variation in constraints imposed by resource availability during larval development. For example, some gregarious endoparasitoids develop in large hosts, such as mature caterpillars, and consume only a small fraction of it before pupating (Harvey 2000). Alternatively other parasitoids may encounter severe resource limitations during their development, particularly if this occurs in very small or starved hosts. One group of parasitoids that is strongly constrained by host size is solitary secondary hyperparasitoids. These parasitoids develop in fully cocooned prepupa and pupae of their primary parasitoid hosts. Some species of secondary hyperparasitoids have been found to exhibit high host use efficiencies. For example, body mass in *Lyssibia nana* Gravenhorst, a solitary hyperparasitoid of *Cotesia glomerata* (L.) pupae, is often >90% that of adult *C.
glomerata emerging from healthy cocoons of comparable mass (Harvey et al. 2006).

Because host resources are often scarce and are of high nutritional value, mechanisms have evolved in female parasitoids that enable them to monopolize these resources. For example, egg parasitoids are well known to compete for host egg masses intraspecifically through such processes as sperm competition (van den Assem et al., 1980) and female–female competition (Godfray 1994). Some female parasitoids actively defend host patches until it is ensured that their progeny have completed their development. The gregarious parasitoid Hysopus pallidus (Askew) remains with her brood for up to 48 h after oviposition, enabling the eggs to hatch (Zaviezo and Mills 2000). Similarly, brood guarding has been observed in another gregarious parasitoid, the bethylid Goniozus nephantidis (Muesbeck) (Hardy and Blackburn 1991). In both of these primary parasitoid species, adult female parasitoids produce small numbers of large, yolky “anhypotropic” eggs that require significant protein investment per egg, secured via destructive “host-feeding” behavior (Jervis and Kidd 1986). Moreover, because the eggs are large, the handling time (required to oviposit a single egg into the host) also is protracted. Alternatively, many parasitoid species produce very large numbers of tiny, yolkless ‘hypotropic’ eggs that may be produced rapidly and injected quickly into the host (Jervis and Kidd 1986). As reproduction is much less costly in these parasitoids, brood guarding has never been observed.

Trichomalopsis apanteloctena (Crawford) (Hymenoptera: Pteromalidae) is a solitary, secondary hyperparasitoid that has a fairly broad host range (Kamijo and Grisell 1982). One of its preferred hosts is the gregarious endoparasitoid Cotesia kariyai (Watanabe) (Hymenoptera: Braconidae). After emerging from caterpillars of their host, Pseudaletia separata (L.) (Lepidoptera: Noctuidae), larvae of C. kariyai construct a cocoon cluster that is composed of 20–140 individual cocoons (Tanaka et al. 1992). The cocoon cluster is composed of an inner cocoon layer that encloses each individual parasitoid pupa and an outer cocoon layer that covers the entire cocoon brood. T. apanteloctena is an ectoparasitic idiobiont that lays a single egg on each host prepupa or pupa by perforating the silk layers with the ovipositor. Preceding oviposition, the wasp injects permanently paralyzing venom into the host (Nakamatsu and Tanaka 2004). Cocoon clusters of C. kariyai are heterogeneously distributed in the environment and thus represent a resource that is highly aggregated for hyperparasitoids.

In a previous study, we found that female T. apanteloctena wasps monopolize broods of C. kariyai cocoons over several days and that they aggressively respond to attempts by conspecific females to gain access to the broods (Naoto et al., 2009). It was found that the temporal dynamics of egg maturation in T. apanteloctena are closely synchronized with the size of a single parasitoid brood, such that female hyperparasitoids are able to parasitize almost a full brood of C. kariyai cocoons over the course of several days. During this time attending wasps physically attack other females that approach the cocoons of the host (Naoto et al., 2009). However, little is known about the means by which female hyperparasitoids first acquire host resources when more than a single female simultaneously competes for access to cocoon of C. kariyai. Here, we show how competition between females leads to dominance hierarchies in T. apanteloctena.

Materials and Methods

Insect Cultures. All insects were reared at 25 ± 1°C and a photoperiod of 16:8 (L:D) (long-day) h (Nakamatsu and Tanaka 2004). Colonies of the primary parasitoid C. kariyai and the hyperparasitoid T. apanteloctena have been maintained in the laboratory at Nagoya University (NU), Japan, for >150 generations over the 8 yr since their initial collection in cornfields in Kanoya, Kagoshima Prefecture, Japan. C. kariyai emerged from larvae of P. separata, which have subsequently been used as a host for the parasitoid at NU. The P. separata culture is maintained on artificial diet (INSECTA-LF, Nihon Noshan, Kanagawa, main ingredients; mulberry [Morus spp.], leaf and defatted soybean [Glycine max (L.) Merr.]). Both laboratory colonies of P. separata and C. kariyai are added once every year from Kanoya cornfields, Zea mays L. After emerging from their host, larvae of C. kariyai construct cocoons which form an aggregated cluster that is covered with an additional layer of soft silk. Natural brood sizes of C. kariyai collected in the Kanoya cornfields are ~60–80. T. apanteloctena was identified by K. Kamijo of Hokkaido Forest Experiment Station. Female hyperparasitoids were isolated immediately after emergence from host cocoons and kept for 1 d with 2–3-d old males for mating. Wasps of both species were kept in plastic tubes (3.5 cm in diameter, 15 cm in length), with a cotton plug containing absorbed 2–3% sugar solution.

Fate of C. kariyai Cocoons Exposed to T. apanteloctena Females. To examine the fate of host cocoon clusters exposed to females, we clarified the rate of parasitism and host feeding over the course of 24 h. Caterpillars in the first day of the final (=sixth) instar of P. separata were parasitized by C. kariyai, and 1-d-old cocoon clusters were individually placed into petri dishes (9 cm in diameter, 1.5 cm in height) in which a single 2–3-d-old mated female hyperparasitoid was released. The dishes were covered with lids to prevent the wasp from escaping. To confirm oviposition or host feeding by the hyperparasitoid, each C. kariyai cocoon from clusters was dissected using fine forceps on the sixth day after the commencement of occupying the cluster. This day was based on adult eclosion of C. kariyai, which generally is completed by the fifth day after egression and pupation from P. separata. The entire outer silk layer was removed using the forceps, and then the inner individual cocoons were carefully removed and dissected on glass slides; eggs and young larvae of the hyperparasitoid and host injected with venom were counted in each cocoon.
Observation of Competition between Females of *T. apanteloctena* for Host Cocoon Cluster. To determine whether there are antagonistic interactions between female hyperparasitoids, a pilot experiment was conducted in which four female wasps were simultaneously given access to the same cocoon cluster of *C. kariyai* over 24 and 48 h. The aim was to determine whether the wasps shared a host as oviposition resource with each other or if the cluster was dominated by a single female after the times had elapsed (*n* = 9 replicates). As stated above, the experiment was conducted using petri dishes with a cotton plug containing absorbed 2–3% sugar solution as food. Either 24 h or 48 h after the release of the females, only one or two *T. apanteloctena* females were found on the host clusters. In five of nine (e.g., 55.6%) of the dishes, only one female of four remained alive in after 48 h had elapsed, even when food was readily available. This suggests that a one female killed the others and thus dominated the cocoon clusters of *C. kariyai*.

**Ranking of *T. apanteloctena* Females for Control of Cocoon Clusters of *C. kariyai***. The aim of this experiment was to determine whether there is a relationship between adult female body size and the exclusion of other hyperparasitoid females on host cocoon clusters. Observations that females frequently sparred with each other using their mandibles in face-to-face at encounters predicted that the size of head capsule was a reliable indicator of body size. As expected, the head capsule width in female hyperparasitoids was positively correlated with the body length from head to caudal tip (*n* = 31, *R*^2^ = 0.8371, *y* = 0.2345*x* + 1.2566; Nakamatsu and Tanaka, 2004).

To verify the importance of body size in structuring dominance hierarchies in *T. apanteloctena*, large hyperparasitoid females were added to arenas containing single host cocoon clusters that had been controlled by single, small females (*n* = 14 replicates). It was predicted that the difference in the body size of hyperparasitoid females may enable them to establish a dominance hierarchy, with the larger females displacing smaller females from the host cocoon clusters.

To clarify the ranking of hyperparasitoid wasps, five females with differently marked colors were placed into a single petri dish with a fresh host cocoon cluster of *C. kariyai*. Competition was observed among the five females on the host cocoon until one female wasp was able to dominate the cocoon brood. Before experiments, to ensure the motivation of individual females for oviposition, *T. apanteloctena* female wasps were released individually into petri dishes (9 cm in diameter) containing single cocoon clusters of *C. kariyai*. Approximately 24 h later, the female wasps that were sitting on top of cocoons were collected from the dishes to be used for experiments. Females with low motivation to oviposit were excluded by this method. Five females that had previously been found to exhibit high motivation to oviposit were simultaneously released into a petri dish with one cocoon cluster for ≈4 h. Furthermore, to determine the second ranking female in the group, a second fresh host cocoon cluster was supplied at this point to the same petri dishes, and the behavior of the female wasps was observed until a second female dominated the new brood. Each female was individually marked by painting the thorax with different colors of water-soluble paints (Takara-Tomy Co. Ltd., Tokyo, Japan) so that they could be easily recognized. This experiment was replicated 10 times.

Furthermore, to determine whether the aggressive tendencies in *T. apanteloctena* females changed before and after the ranking hierarchy was established, five color-marked females were simultaneously released into arenas containing single cocoon clusters of *C. kariyai* in experiment 1. In experiment 2, four females were released in a petri dish with one cocoon cluster already occupied with one large female.

**Relationship between Female Head Capsule Width and the Number of Mature Eggs in the Ovaries.** Hyperparasitoids were carefully dissected on glass slides using fine forceps after removing the head capsules by using a fine forceps and a dissecting knife. Each head capsule was then attached on a slide glass with sticky tape on both-side (3M, Scotch). It was then was measured using a light microscope with a micrometer. The ovaries were excised from the abdomen and counted; their length also was determined (±1 mm) under a stereomicroscope by using a calibrated slide. Many eggs in the ovarioles were whitish in color were >360 mm in length. These were classified as “mature eggs.” Eggs under 360 mm were deemed to be in various stages of maturation (Y.N., unpublished data).

**Results**

Fate of *C. kariyai* Cocoons Exposed to *T. apanteloctena* Females. The number of *C. kariyai* cocoons that were used for oviposition and host feeding by hyperparasitoid females over the first 24 h is shown in Table 1. On average, ≈22% of a cocoon clusters were used for oviposition, whereas an additional 13% were injected with venom. The injection of venom was based on the number of hosts that exhibited developmental arrest after stinging. Thirty-five percent of host broods were used either for host feeding or oviposition by hyperparasitoid females. Venom-injected hosts (where no eggs were laid) seemed to be used only for host-feeding purposes, although the actual function of the venom injection was not determined. Hyperparasitoid females were frequently observed to insert and remove the ovipositor, injecting venom, and then to drink hemolymph that oozed from the host at the site of the wound.

Observation of Competition between Females of *T. apanteloctena* for Host Cocoon Cluster. One day after their release into the arenas, one or two (at most) female hyperparasitoids were observed sitting on host...
cocoon clusters. Within 2 d, usually only a single female remained on the cluster; the other wasps had died. Of the nine separate cocoon clusters that were monitored, the breakdown was as follows 2 d later: 1) five cocoon clusters were dominated by a single female wasp; 2) two cocoon clusters were shared between two females; and 3) two cocoon clusters were unoccupied (all of the female wasps were dead). This was probably the result of aggressive fighting behavior that was observed among the female wasps for access to cocoon clusters (Table 2).

**Fighting Behavior between Females of *T. apanteloctena* on Cocoon Clusters of *C. kariyai***

Females of *T. apanteloctena* often exhibited aggressive tendencies toward other females, characterized by changes in body posture, e.g., abdominal bending in the shape of the letter “C,” and the use of large, biting mandibles (Figs. 1, 1). We occasionally observed one female physically attacking another using their mandibles after the initiation of threatening posture. This was especially prevalent in contests involving two females of equivalent size (Figs. 1 and 2). Aggressive behavior was photographed under a stereomicroscope with low magnification.

**Ranking of *T. apanteloctena* Females for Control of Cocoon Clusters of *C. kariyai***

In 12 of the 14 arenas (>85.7%), large females displaced small females for control of the cocoon clusters. In nine of the arenas, the small females survived for only 1 or 2 d, suggesting that they had been attacked and killed by the larger females. In the remaining two arenas, cocoon clusters of *C. kariyai* were found to be occupied by both a large and a smaller female.

In the ranking experiments, the largest individual among the five females released into a petri dish dominated the host cocoon brood in all but one replicate (Table 3). In the one exception (9), the dominant female was the second largest in terms of head capsule width. In all cases, the second female to dominate the new cocoon clusters was the next largest among the remaining competitors (Table 3). The top ranking female hyperparasitoids, in terms of size, often fought other females that attempted to gain access to cocoon clusters of *C. kariyai*. Moreover, the frequency of second ranking females to engage in contests was slightly lower than that of the top ranking (=dominant) females (Table 3). However, *T. apanteloctena* wasps that lost contests with other females still occasionally exhibited threatening postures when they encountered other hyperparasitoid females (Fig. 1, 1).

Fighting behavior was observed significantly more often when females were simultaneously released than when a cocoon cluster was already controlled by a dominant female (Table 4). Once a cocoon cluster

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**Table 1. Rate of parasitization and host feeding for a host cocoon cluster when one hyperparasitoid female occupied one cluster for 24 h**

| Female no. | No. individual cocoons in one cluster | % oviposited hosts (no.) in one cluster | % hosts injected with venom (no.) | % hosts used by hyperparasitoid (total no.) |
|------------|---------------------------------------|----------------------------------------|-----------------------------------|-------------------------------------------|
| 1          | 40                                    | 27.5 (11)                              | 17.5 (7)                          | 45.0 (18)                                 |
| 2          | 104                                   | 13.5 (14)                              | 6.7 (7)                           | 20.2 (21)                                 |
| 3          | 52                                    | 11.5 (6)                               | 9.6 (5)                           | 21.2 (11)                                 |
| 4          | 47                                    | 36.2 (17)                              | 12.6 (6)                          | 45.9 (23)                                 |
| 5          | 56                                    | 12.5 (7)                               | 21.4 (12)                         | 33.9 (19)                                 |
| 6          | 20                                    | 65.0 (13)                              | 10.0 (2)                          | 75.0 (15)                                 |
| 7          | 51                                    | 11.8 (6)                               | 9.5 (3)                           | 21.6 (11)                                 |
| 8          | 42                                    | 2.4 (1)                                | 35.7 (15)                         | 38.1 (16)                                 |
| 9          | 49                                    | 26.5 (13)                              | 4.1 (2)                           | 30.6 (15)                                 |
| 10         | 93                                    | 21.5 (20)                              | 6.5 (6)                           | 25.0 (26)                                 |
| 11         | 106                                   | 10.4 (11)                              | 9.4 (10)                          | 19.8 (21)                                 |
| Avg        | 60.0 ± 25.1                           | 21.7 (10.8 ± 5.5)                      | 13.0 (7.4 ± 3.6)                  | 34.8 (18.2 ± 4.7)                         |

Numbers in parentheses means the number of hosts used.

**Table 2. Cocoon-occupation behavior over 2 d by four *T. apanteloctena* females**

| Individual no. of *C. kariyai* cocoon cluster | 1 d after releasing | 2 d after releasing |
|-----------------------------------------------|---------------------|---------------------|
|                                               | No. wasps on *C. kariyai* cocoon cluster | No. wasps away from *C. kariyai* cocoon cluster | Dead | Alive |
| 1                                             | 2                   | 1                   | 1    | 1     |
| 2                                             | 1                   | 2                   | 1    | 1     |
| 3                                             | 2                   | 0                   | 0    | 0     |
| 4                                             | 1                   | 0                   | 3    | 1     |
| 5                                             | 1                   | 0                   | 3    | 1     |
| 6                                             | 2                   | 0                   | 2    | 2     |
| 7                                             | 2                   | 0                   | 2    | 2     |
| 8                                             | 2                   | 0                   | 2    | 2     |
| 9                                             | 1                   | 1                   | 2    | 0     |

Four *T. apanteloctena* female was released in petri dish placed a *C. kariyai* host cocoon cluster in center for 2 d.
was dominated by a single large female hyperparasitoid (experiment 2), the fighting frequency decreased to \(<5\%\), although the number of wasps exhibiting a threatening posture was actually higher than in experiment 1 (Table 4). Top ranking females were observed to patrol the entire surface of the cocoon cluster, and still repelled subordinate females by exhibiting threatening postures to those that approached the clusters or tried to mount them. However, subordinate females usually did not approach cocoon clusters once a dominance hierarchy had been established.

Although the difference was not statistically significant, fighting behavior showed a small tendency to reduce the number of eggs laid by the dominant female hyperparasitoid (Table 5). This suggests that guarding behavior confers a cost to the dominant female. Almost all females that have been added to arenas were later found to be dead and away from cocoon clusters.

![Fig. 1. Threatening (1) and fighting (2) postures demonstrated by T. apantelocena. The arrow shows large mandibles that are agape in the female hyperparasitoids (M). (Online figure in color.)](image)

![Fig. 2. Relationship between head capsule width of hyperparasitoid (millimeters) and the number of mature (=ovulated) eggs in the ovaries of T. apantelocena. Eggs that are white in color and \(>0.36\) mm lengthwise were judged as mature (=ovulated).](image)

**Table 3. Comparison of female head capsule width (millimeters) from first to third instar and size-rank in each replication**

| Observation no. | First     | Second    | Third and below |
|-----------------|-----------|-----------|-----------------|
| 1               | 0.832     | 0.713     | 0.703 ± 0.047   |
| 2               | 0.830     | 0.697     | 0.685 ± 0.063   |
| 3               | 0.816     | 0.737     | 0.734 ± 0.019   |
| 4               | 0.902     | 0.752     | 0.685 ± 0.040   |
| 5               | 0.792     | 0.792     | 0.671 ± 0.013   |
| 6               | 0.790     | 0.776     | 0.675 ± 0.061   |
| 7               | 0.776     | 0.776     | 0.713 ± 0.034   |
| 8               | 0.772     | 0.772     | 0.782 ± 0.017   |
| 9               | 0.762     | 0.712     | 0.719 ± 0.012   |
| 10              | 0.749     | 0.737     | 0.724 ± 0.028   |
| Total no.       | 10        | 10        | 29              |
| **Avg** &       | 0.792 ± 0.028a | 0.756 ± 0.036a | 0.707 ± 0.041b |
| **No. fighting**| 14.2 ± 5.8a | 11.8 ± 5.5a | 7.2 ± 4.0b      |

* Different lowercase letter in average line means significantly different at \(P < 0.01\) with one-way ANOVA (\(F_{2,46} = 20.84\)).

b Different lowercase letter in a line means significantly different at \(P < 0.01\) with one-way ANOVA (\(F_{2,46} = 9.60\)).
Table 4. Frequency of fighting before or after occupation on the host cocoon-cluster when a female encountered interspecific female competitors

| Exp 1<sup>a</sup> | Exp 2<sup>b</sup> | Total no. encounter | No. female wasps occupying | Avg no. threatening per each female | Avg no. fighting | Rate of behavior leading from threatening to fighting |
|------------------|------------------|---------------------|-----------------------------|-----------------------------------|------------------|---------------------------------------------------|
| 29               | 112              | 4.1 ± 1.8           | 2.0 ± 1.0                   | 48.3                              | 2.9              |

<sup>a</sup> Experiment 1: Five females marked with different color were released in a petri dish with one host cocoon cluster placed in center. Frequency of threatening and fighting on cocoon cluster were recorded.

<sup>b</sup> Experiment 2: Four females marked with each different color were released in each petri dish with one host cocoon cluster already occupied with one female.

**Relationship between Female Head Capsule Width and the Number of Mature Eggs in the Ovaries.** Larger females were more successful in competition for domination of host cocoons than smaller females. One possible factor in mediating aggressive behavior is that larger female wasps possess higher egg loads than smaller wasps, thus increasing their “motivation to oviposit” via stretch receptors in the ovaries. However, the relationship between head width in female hyperparasitoids and the number of mature eggs in their ovaries was not significant (Fig. 2).

**Relationship of Head Capsule Width Mothering Female T. apanteloctena and Offspring Size.** The relationship between the size of mother wasps and their progeny (correlation coefficient, $R^2$ = 0.2 for female, 0.18 for males in Fig. 3) was not significant. As in many idiobionts, body size in T. apanteloctena was apparently constrained more by the total amount of resources in host pupae of C. kariyai.

In this experiment, the number of host cocoons in each brood was counted instead of measuring body size of C. kariyai prepupae or pupae, because it was very difficult to separate individual cocoons from the cluster without damaging the pupae. However, body mass of C. kariyai wasp was weakly but also inversely correlated with the number of individual cocoons per brood (Fig. 4). However, the head capsule width of female hyperparasitoids was not correlated with the number of individual cocoons per brood (Fig. 4).

**Small T. apanteloctena Females Gain Access to Host Cocoons via “Sneaking” Behavior.** Although large female wasps usually won aggressive encounters with small conspecics for domination of host cocoon clusters, small “losing” females occasionally gained access to cocoons by perforating the outer silk layer with their mandibles. Females that were found under the outer silk layer had significantly smaller head capsules those females guarding the cocoon clusters (Table 6). Small females were able to hide under the silk and frequently parasitized pupae of C. kariyai. Dominant females were never observed in antagonistic encounters with small females that were effectively hidden under the outer silk layer.

**Discussion**

Females of the hyperparasitoid wasp T. apanteloctena aggressively defended cocoon clusters of their host, C. kariyai, from attack by conspecific females. Encounters between two females for access to cocoon clusters were characterized by ritualistic behavior, whereby wasps exhibited distinctly aggressive body postures (e.g., like the letter “C”) and in many cases these contests led to actual physical combat. Fighting incurred the use of the mandibles and usually involved two females of approximately the same size. Alternatively, smaller females usually avoided fighting and were much more easily driven away from host cocoon clusters by larger females. Fighting behavior was strictly correlated with a body size hierarchy: the largest, top-ranking females engaged in fighting behavior much more often than the second largest and ranking females, and so on. Once a top-ranking female dominated a cocoon cluster, there was a notable shift in her behavior from fighting to ritualistic aggression, and this usually was directed toward the nearest ranking female of similar size. These results suggest that female T. apanteloctena are probably able to recognize size differences between one another after a period of antagonistic interactions.

Table 5. Effect of interspecific hyperparasitoid competitors on the number of eggs laid in single cocoon clusters

| Treatment | No. host cocoon clusters | No. eggs laid per each cluster | No. hyperparasitoids that died away from host cocoons (%) |
|-----------|--------------------------|-------------------------------|--------------------------------------------------------|
| 1 + 1<sup>a</sup> | 10                       | 29.4 ± 10.4a                  | 9 (90.0)                                                |
| 1 + 0<sup>b</sup> | 6                        | 333 ± 9.8a                    |                                                        |

<sup>a</sup> 1 + 1: one female wasp was released in a petri dish placed one cocoon cluster of C. kariyai, then another female as a competitor was released in the same petri dish next day.

<sup>b</sup> 1 + 0: one female was released in petri dish with one cocoon cluster and stayed for 2 d. The number of hyperparasitoids egressed was counted. Statistical analysis by a Student’s t-test was performed between each other at 0.01 level.
broadly opposite “r-selected” reproductive traits. For example, most koinobionts are endoparasitoids that have higher fecundities than idiobionts and koinobionts are characterized by species producing tiny, ostensibly yolkless hydropic eggs that are often laid in a few seconds into the host (Jervis and Kidd 1986). Large females of the koinobiont Biosteres longicaudatus Fischer are able to dominate smaller females in patches of their host, Anastresa suspense Loew (Lawrence 1981). Most importantly, factors facilitating the evolution of resource guarding in parasitoids are complex and involve a number of factors relating to host distribution and parasitoid ecophysiology. Interestingly, territorial behavior also has been observed in male parasitoids of Nasonia vitripennis (Walker) (van den Assem et al. 1980).

Although a size-ranking hierarchy was established in T. apanteloctena, with the largest females usually winning in aggressive interactions, smaller females were occasionally able to parasitize hosts. These small females lost aggressive competition with larger conspecifics but still gained access to hosts by chewing through the outer silk web covering the cocoons and hiding from the dominant females in this “refuge.” Similarly, the secondary hyperparasitoid L. nana is often able to avoid brood-attending dominants by chewing through an outer silk web of its host C. glomerata (Harvey et al., 2006; M. Kos, unpublished data). In the koinobiont B. longicaudatus, small females were also found to avoid larger females in host patches through rapid “hit-and-run” oviposition behavior (Lawrence 1981).

Field observations of C. kariyai in Kanoya reveal that parasitism rates of P. separata are variable but can be sometimes quite low (data not shown). When suitable hosts are scarce, cocoons thus represent a valuable resource for hyperparasitoids such as T. apanteloctena, and this might also help to explain the importance of resource guarding in this species. It is likely that other species of secondary hyperparasitoids that exploit heterogeneously distributed hosts (e.g., other gregarious Cotesia species) also might be expected to exhibit aggressive behavior against other hyperparasitoids. For example, in the western Palearctic, cocoons of C. glomerata are attacked by several species of closely related hyperparasitoids in the family Ichneumonidae (subfamily Cryptinae), including L. nana and Gelis agilis (F.) (Schwarz and Shaw 2000).

**Fig. 3.** Relationship between head capsule width (millimeters) in female T. apanteloctena and in their progeny. Each correlation coefficient ($r^2$) and regression line is shown in the figure.

**Fig. 4.** Relationship between host body mass of C. kariyai and the number of individual cocoons per brood.
In spite of their phylogenetic affinity, there are striking differences in the morphology and reproductive biology of these parasitoids. *G. agilis* is wingless, host feeds, produces very small numbers of offspring daily, lays large eggs, and has a long life span, whereas these traits are reversed in *L. nana* (Harvey 2008). Although this has not yet been examined, these differences suggest that brood guarding is much more likely to occur in *G. agilis* than *L. nana*.

Although larger females were competitively superior to smaller females, this was not correlated with the number of mature eggs in the ovarioles of *T. apantelectena*. Furthermore, the size of hyperparasitoid progeny was not significantly linked with maternal size. Body size in parasitoids, in particular idiobionts, is often a function of host size at oviposition (Arthur and Wylie, 1959; Corrigan and Lashomb, 1990; Harvey et al., 1998, 2006; Harvey et al., 2008). Because idiobionts represent a "fixed" amount of resource, larger hosts are assumed to be of higher quality because they contain more resources than small hosts. This is confounded, however, by age-related changes in quality (Harvey et al. 2006).

In summary, we have reported that females of the secondary hyperparasitoid *T. apantelectena* engage in aggressive interactions for access to and control of cocoon clusters of their host *C. kariyai*. Aggression is based on a series of ritualistic behaviors that occasionally lead to fighting among females of similar size. Ultimately, domination of a host cocoon cluster is based on a host size hierarchy among competing females. However, smaller females may still be able to parasitize hosts and to avoid combat by chewing though the outer silk web overlaying the cocoons and by crawling through the perforation where they are not detected by larger females. Although brood and resource guarding has been observed in only a few species of parasitoids (mostly idiobionts), it would be interesting to compare behavior among a guild of (hyper) parasitoids attacking a common host stage of the same species, to see whether such behavior is ubiquitous. Because there may be considerable variation in life-history traits even among parasitoids within defined guilds, it might then be possible to elucidate a specific trait or suite of trait that facilitate the evolution of aggression in species the different within the guild.

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