

SEQUENTIAL RAPID ADAPTATION OF INDIGENOUS PARASITOID WASPS TO THE INVASIVE BUTTERFLY *PIERIS BRASSICAE*

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The introduction of a new species can change the characteristics of other species within a community. These changes may affect discontinuous trophic levels via adjacent trophic levels. The invasion of an exotic host species may provide the opportunity to observe the dynamics of changing interspecific interactions among parasitoids belonging to different trophic levels. The exotic large white butterfly *Pieris brassicae* invaded Hokkaido Island, Japan, and quickly spread throughout the island. Prior to the invasion, the small white butterfly *P. rapae* was the host of the primary parasitoid *Cotesia glomerata*, on which both the larval hyperparasitoid *Baryscapus galactopus* and the pupal hyperparasitoid *Trichomalopsis apanteroctena* depended. At the time of the invasion, *C. glomerata* generally laid eggs exclusively in *P. rapae*. During the five years following the invasion, however, the clutch size of *C. glomerata* in *P. rapae* gradually decreased, whereas the clutch size in *P. brassicae* increased. The field results corresponded well with laboratory experiments showing an increase in the rate of parasitism in *P. brassicae*. The host expansion of *C. glomerata* provided the two hyperparasitoids with an opportunity to choose between alternative hosts, that is, *C. glomerata* within *P. brassicae* and *C. glomerata* within *P. rapae*. Indeed, the pupal hyperparasitoid *T. apanteroctena* shifted its preference gradually to *C. glomerata* in *P. brassicae*, whereas the larval hyperparasitoid *B. galactopus* maintained a preference for *C. glomerata* in *P. rapae*. These changes in host preference may result from differential suitability of the two host types. The larval hyperparasitoid preferred *C. glomerata* within *P. rapae* to *C. glomerata* within *P. brassicae*, presumably because *P. brassicae* larvae attacked aggressively, thereby hindering the parasitization, whereas the pupal hyperparasitoid could take advantage of the competition-free resource by shifting its host preference. Consequently, the invasion of *P. brassicae* has changed the host use of the primary parasitoid *C. glomerata* and the pupal hyperparasitoid *T. apanteroctena* within a very short time.

KEY WORDS: *Cotesia glomerata* (L.), host range evolution, hyperparasitoids, interspecific interactions, *Pieris rapae crucivora* (Boisduval), tri-trophic levels.

Interspecific interactions within a community are characterized by a complex combination of bottom-up effects (Ehrlich and Raven 1964; Carroll and Boyd 1992; Hunter and Price 1992), top-down effects (Price et al. 1980; Sato and Ohsaki 1987; Bernays and Graham 1988; Reznick et al. 1990; Ohsaki and Sato 1999), competition among species of the same trophic level (Denno et al. 1995;

Söderbäck 1995; Martel et al. 2001; Chase et al. 2002), and indirect interspecific interactions (Holt 1977; Menge 1995; van Nouhuys and Hanski 2000). Numerous studies have examined interactions between adjacent trophic levels, including recent studies that have emphasized the importance of indirect interactions in multitrophic systems. Such indirect interactions can be altered when a new

species is introduced to a community (Cox 2004). To understand the significance of the effect of interactions on community structure, researchers have studied multitrophic-level systems (e.g., Nyström et al. 2001). There are two types of process that affect the course of interspecific interactions: the effect of population-density dynamics (the interaction chain) and the effect of modifying interactions between two other component species (interaction modification) (Wootton 1994). Many studies have focused on the first process (Menge 1997), whereas few have concentrated on the second (e.g., Schmitz et al. 1997; Masters et al. 2001).

The process of natural selection, in terms of adaptation to environmental changes caused by either natural or artificial agents, can occur quickly (Thompson 1998, but see, e.g., Knowlton et al. 1993). Changes in any trophic level may have effects beyond just the adjacent or dependent trophic levels. The invasion of exotic species may provide an opportunity to study changes that occur in interspecific interactions (e.g., May and Anderson 1983; Magurran et al. 1992; Mauricio and Rausher 1997; Abrahamson et al. 2003). Except for a few cases of artificial introduction, however, it is difficult to determine when an exotic species was first introduced because of the time lag between establishment and the appearance of ecological effects (Cox 2004). Assuming that interspecific interactions sometimes change rapidly, the conflicts among species that caused the renewal of interactions may have already disappeared (Connell 1974). Therefore, we need to compare interactions both before or just after invasion and after some time has passed.

The distribution expansion of the invasive butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae) has been recorded since the beginning of its invasion. *Pieris brassicae* arrived in northern Japan from Siberia in approximately 1995. The butterfly was discovered on the west coast of Hokkaido Island and quickly spread to the east coast. By 2000, it ranged over the entire island and over the northern tip of Honshu Island (Ueno 2001). In Europe, both *P. brassicae* and *P. rapae* (L.) are important pests of crucifers, and both are parasitized by the gregarious parasitoid wasp *Cotesia glomerata* L. (Hymenoptera: Braconidae) (Laing and Levins 1982). The same wasp species exists in Japan, where it originally parasitized the Japanese small white butterfly *P. rapae crucivora* Boisduval (Lepidoptera: Pieridae), before the introduction of *P. brassicae* (Sato 1976). Laboratory experiments performed in 1999 showed that *C. glomerata* did not lay eggs in *P. brassicae* caterpillars, despite always inserting the ovipositor (Sato and Ohsaki 2004). However, field collections found that 0–2.0, 21.2, and 3.5–16.7% of *P. brassicae* caterpillars in 1997, 1998, and 1999, respectively, were parasitized by *C. glomerata* (Sato and Ohsaki 2004). These data suggest that the wasp began to use *P. brassicae* as a host within a short period.

The primary parasitoid *C. glomerata* is also parasitized by several hyperparasitoid wasps. The total rate of both larval and

pupal hyperparasitism of *C. glomerata* in western Japan sometimes reaches > 60% of all cocoons (Matsuzawa 1958). The most dominant pupal hyperparasitoid is *Trichomalopsis apante-roctena* (Crawford [Matsuzawa 1958]; Hymenoptera: Pteromalidae), which sometimes parasitizes > 40% of all *C. glomerata* cocoons. Little is known regarding the parasitism rate of the larval hyperparasitoid *Baryscapus galactopus* (Ratzeburg; Hymenoptera: Eulophidae). If *C. glomerata* began to use *P. brassicae* as a host following its invasion, the host use of these two hyperparasitoids may have also changed.

Therefore, we investigated how the invasion of *P. brassicae* affected the host use of indigenous parasitoid wasps belonging to two trophic levels (parasitoid and hyperparasitoid). Wasps were collected from six census sites for seven years to observe their behavioral changes. First, we compared the number of eggs laid (clutch size) by the primary parasitoid *C. glomerata* in *P. brassicae* and *P. rapae* caterpillars. We also determined the proportion of individuals that emerged per cocoon cluster and the dry weight of adult female wasps to assess the quality of *P. brassicae* and *P. rapae* as hosts for *C. glomerata*. In addition, we studied the host preference of two hyperparasitoid wasps, *B. galactopus* and *T. apante-roctena*, by letting them choose between host wasp *C. glomerata* that has parasitized *P. rapae* or *P. brassicae*. We also examined parasitism and hyperparasitism rates in the field for comparison with the laboratory experiments. Finally, we discuss the mechanisms that may be responsible for the alteration of interspecific interactions that occur upon the introduction of a nonnative invasive species to a community.

Materials and Methods

MATERIALS

The invasive *P. brassicae* and the indigenous *P. rapae* are sympatric and occur simultaneously in Hokkaido, Japan. The primary parasitoid wasp *C. glomerata* oviposits many eggs into the first to third instars of *P. brassicae* and *P. rapae* caterpillars. The larvae of *C. glomerata* egress from fifth-instar caterpillars and form a cluster of cocoons beside the host caterpillar.

Two hyperparasitoid wasps parasitize different stages of the primary wasp *C. glomerata*. *Baryscapus galactopus* parasitizes larvae of the primary wasp within the body of the host caterpillar, whereas *T. apante-roctena* parasitizes the pupae of the primary wasp. The former is gregarious, the latter is solitary, and both parasitize various wasp species, including *C. glomerata* (larval hyperparasitoid: Gaines and Kok 1999; pupal hyperparasitoid: Kamijo and Grissell 1982). Both hyperparasitoids hatch from the pupae of the host wasp; therefore, their resource requirements overlap somewhat because *T. apante-roctena* favors newly pupated host wasps, which may contain developing *B. galactopus* individuals.

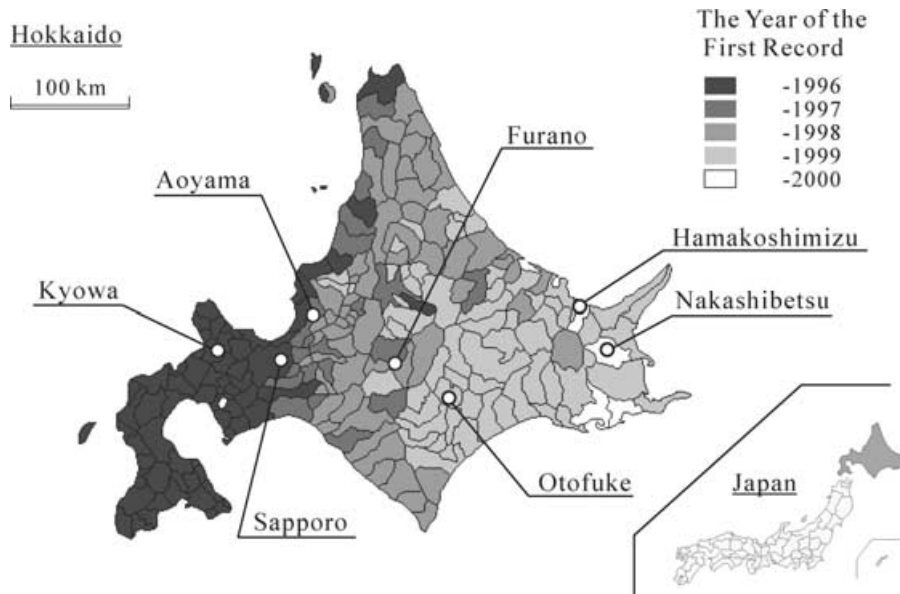


Figure 1. Map showing the changing distribution of *Pieris brassicae*, indicating the year the species was first reported in each municipality in Hokkaido, Japan (adapted from Ueno 2001). Circles indicate the seven census sites.

ESTIMATED NUMBER OF GENERATIONS SINCE *P. BRASSICAE* INVASION AT EACH CENSUS SITE

The expansion of the distribution of *P. brassicae* has been well documented (Ueno 2001; Fig. 1). We chose the following census sites in seven areas that were invaded by *P. brassicae* at different times: Sapporo (1995), Kyowa (1996), Aoyama (1997), Furano (1998), Otofuke (1999), Nakashibetsu (2000), and Hamakoshimizu (2000). We collected wasps periodically from each of these census sites.

We estimated the number of generations per year at each census site using the developmental zero (10.0, 6.9, and 8.9°C for eggs, caterpillars, and pupae, respectively), total effective temperature (54, 212, and 140 degree-days, respectively) (Davies and Gilbert 1985), and critical day length (13 h 50 min; Hashimoto and Hachiya 1999). Among the above data, developmental zero and total effective temperature of *P. brassicae* eggs were substituted for those of *P. rapae* eggs because the developmental characteristics of these two *Pieris* species do not differ greatly (Davies and Gilbert 1985). The time to sexual maturity has been reported as 3–14 days (Feltwell 1982); thus, we used seven days for our calculations. The average temperature at each census site was determined using climatic data from the Japan Meteorological Agency for 1971–2000. Day-length data were obtained from the National Astronomical Observatory of Japan. We added 1 h to the total day length to accommodate for dawn and dusk. The sensitive period of diapause occurred at the fifth-instar stage for *P. brassicae* (Feltwell 1982; Hashimoto and Hachiya 1999), and the photoperiod declined below the critical day length for diapause on 10 September. We defined the final generation of the year as the last generation

that could develop to the pupal stage before the average temperature dropped below caterpillar developmental zero. If caterpillars could not develop to pupae, the previous generation was the final generation. The calculated number of generations corresponded well with those observed at Sapporo in 2005 (S. Tanaka, unpubl. data).

Because *C. glomerata* has one generation per host generation the number of generations of *P. brassicae* reflects that of the parasitoid wasp. *Cotesia glomerata* also parasitizes the pierid butterflies *Aporia crataegi adherbal* and *Pieris napi nesis*; however, only a small fraction of the *C. glomerata* population uses these butterflies (rates of parasitism are estimated to be less than 10 and 12%, respectively; N. Ohsaki and Y. Sato, pers. comm.). Because of their scarcity, we excluded these species from the analysis under the assumption that this would not affect our calculations. Using the cumulative number of generations of *P. brassicae* as an index, we could compare the clutch sizes of various *C. glomerata* populations that were collected from different sites in different years. It is possible, however, that some hyperparasitoid wasps could use another host species that has a different number of generations than *C. glomerata* so the number of generations of *P. brassicae* may not reflect that of the hyperparasitoids. As such, we ranked the hyperparasitoid populations from the census sites according to their length of coexistence with *P. brassicae*, and used this rank in the statistical analyses. We defined the cumulative number of generations at each census site as the sum of generations from the first generation of the year of the invasion of *P. brassicae* to the last generation before we collected parasitoids at each site (Table 1).

Table 1. Estimated periods of coexistence with *Pieris brassicae* for indigenous parasitoid wasps. The periods of coexistence were determined based on the estimated number of generations of *P. brassicae*. (A) Estimated time of coexistence for the primary parasitoid *Cotesia glomerata*. (B) Estimated time of coexistence for the two hyperparasitoids: *Baryscapus galactopus* and *Trichomalopsis apanteroetena*. The estimated number of generations of *P. brassicae* may not be directly related to the estimated time of coexistence for both hyperparasitoids because they are generalists. We ranked each hyperparasitoid population according to the estimated time of coexistence as indicated by the numbers in parentheses. The upper columns are estimates from sequential experiments for the same population, and the lower columns are estimates from experiments comparing populations.

	Parasitoid	Site	Year of invasion	Collected year	Generations (per year)	Generations passed after the invasion (order)	
(A)	Within a population	<i>C. glomerata</i>	Sapporo	1995	1999	3	15
		<i>C. glomerata</i>	Sapporo	1995	2000	3	18
		<i>C. glomerata</i>	Sapporo	1995	2001	3	21
		<i>C. glomerata</i>	Sapporo	1995	2002	3	24
	Among populations	<i>C. glomerata</i>	Hamakoshimizu	2000	2001	2	4
		<i>C. glomerata</i>	Otofuke	1999	2001	3	9
		<i>C. glomerata</i>	Furano	1998	2001	3	12
		<i>C. glomerata</i>	Aoyama	1997	2001	3	15
		<i>C. glomerata</i>	Kyowa	1996	2001	3	18
		<i>C. glomerata</i>	Sapporo	1995	2001	3	21
(B)	Within a population	<i>B. galactopus</i>	Furano	1998	2002	3	15
		<i>B. galactopus</i>	Furano	1998	2005	3	24
		<i>T. apanteroetena</i>	Furano	1998	2002	3	15
		<i>T. apanteroetena</i>	Furano	1998	2005	3	24
	Among populations	<i>B. galactopus</i>	Nakashibetsu	2000	2002	2	6 (1)
		<i>B. galactopus</i>	Otofuke	1999	2002	3	12 (2)
		<i>B. galactopus</i>	Furano	1998	2002	3	15 (3)
		<i>B. galactopus</i>	Kyowa	1996	2001	3	18 (4)
		<i>T. apanteroetena</i>	Otofuke	1999	2002	3	12 (1)
		<i>T. apanteroetena</i>	Furano	1998	2002	3	15 (2)
	<i>T. apanteroetena</i>	Kyowa	1996	2001	3	18 (3)	
	<i>T. apanteroetena</i>	Sapporo	1995	2002	3	24 (4)	

COLLECTION AND TREATMENT OF MATERIALS

All of the butterflies and wasps were collected from cabbage fields at each census site in late August to late September. They were then reared in the laboratory at $25 \pm 2^\circ\text{C}$ and L14:D10 in 1999, and $25 \pm 2^\circ\text{C}$ and L16:D8 in other years.

Nonparasitized caterpillars were obtained by rearing eggs from the Sapporo population on cabbage leaves in plastic cups (12 cm in diameter, 5 cm in height). These eggs were obtained from female butterflies that had been reared under the same conditions.

We obtained the primary parasitoid *C. glomerata* when it emerged from fifth-instar caterpillars of *P. rapae* collected from cabbage fields at each census site. The caterpillars were reared on fresh cabbage leaves in a cardboard box (23 cm long \times 26 cm wide \times 30 cm high). After egression of *C. glomerata*, each cluster of wasp cocoons was cut from the cardboard box and reared in a separate test tube (1.8 cm diameter, 18 cm long). We used clusters of < 50 cocoons because larger clusters often contained several families. The emerged wasps were given filter paper soaked in 10–20% sugar solution.

The two hyperparasitoid wasps were obtained from *C. glomerata* cocoon clusters collected from cabbage fields at each census site. The *C. glomerata* cocoon clusters were reared in test tubes as above. Hatched hyperparasitoid individuals were transferred to new test tubes for each cluster. The emerged wasps were given filter paper soaked in sugar solution.

We collected wasps from the seven census sites (Fig. 1). *Cotesia glomerata* was collected at all sites except Nakashibetsu. At Sapporo, we collected *C. glomerata* in four sequential years (1999–2002). Wasps used to determine the eclosion rate per cocoon cluster were collected in 2000; those used for dry weight measurements were collected in 1999; all were from Sapporo. Performance and average clutch size in *P. brassicae* and *P. rapae* were measured for different sets of wasps. The hyperparasitoids were collected in 2001, 2002, and 2005. The larval hyperparasitoid *B. galactopus* was collected at Kyowa in 2001, Nakashibetsu, Otofuke, and Furano in 2002, and Furano in 2005. The pupal hyperparasitoid *T. apanteroetena* was collected at Kyowa in 2001, Otofuke, Furano, and Sapporo in 2002, and Furano in 2005. We collected

both hyperparasitoid wasps at Furano in 2005 and compared them with those collected in 2002 for host preference.

GRADUAL CHANGE IN *C. GLOMERATA* CLUTCH SIZE

To evaluate *C. glomerata*'s acceptance of the two caterpillar species as hosts, we conducted an oviposition test in covered glass petri dishes (9 cm in diameter, 2 cm in depth). Two *C. glomerata* wasps from the same caterpillar (i.e., assumed siblings) were randomly selected from each cocoon cluster. The number of cocoon clusters is given in the legend of Figure 2. These wasps were naive females that had emerged three to seven days before the start of the experiments and were kept in individual test tubes (1.2 cm in diameter, 7.5 cm in length) and fed sugar solution under standardized conditions. For each pair of sister wasps, one was provided a *P. brassicae* caterpillar and the other by a *P. rapae* caterpillar. We then determined the resulting wasp clutch size in each caterpillar species.

In each petri dish, a first-instar caterpillar was placed on a piece of cabbage leaf (2 × 2 cm) on which five or six first-instar caterpillars of the same species had fed for 12 h. The leaf was placed on wet filter paper (4 × 4 cm). To control for caterpillar size, similar-sized late first-instar *P. rapae* and mid-first-instar *P. brassicae* were used for the experiments. In each trial, a naive female wasp was introduced to the dish, and then removed just after oviposition. In most cases, oviposition behavior (i.e., from finding the host caterpillar to withdrawing the ovipositor) was completed within 1 min. In the case of *P. brassicae*, however, the insertion of the ovipositor into the host body sometimes lasted for over 1 min (Sato and Ohsaki 2004); we counted such cases as one trial. The parasitized caterpillar was maintained for 24 h on a fresh piece of cabbage leaf in a plastic tube (0.9 cm in diameter, 5 cm in

length) plugged with wet cotton wool. This protocol allowed us to easily count the parasitoid wasp eggs because they expanded rapidly within the caterpillar (le Masurier 1990). We dissected the caterpillar, stained the eggs with acetic fuchsin, and counted the eggs under a dissecting microscope to determine *C. glomerata* clutch sizes. We used regression analysis to determine the effect of the number of generations since the invasion of *P. brassicae* on *C. glomerata* clutch size in both species of caterpillar, both across years at a single site (Sapporo) and among sites that varied in the time since the *P. brassicae* invasion.

PERFORMANCE OF *C. GLOMERATA* IN DIFFERENT HOST CATERpillARS

To assess the quality of *P. brassicae* and *P. rapae* as hosts for *C. glomerata*, we measured the eclosion rate of wasps per cocoon cluster and the dry weight of female wasps emerging from both *P. brassicae* and *P. rapae*. These were obtained by rearing third-instar caterpillars of *P. brassicae* and *P. rapae* collected from the Sapporo population. This protocol allowed the caterpillars to grow free from intraspecific resource competition. It also prevented parasitism of *C. glomerata* larvae by *B. galactopus* because *B. galactopus* tends to prefer *C. glomerata* larvae in fifth-instar caterpillars.

The number of emerged wasps was counted three days after the first wasp emerged from each cluster. Data were arcsine transformed for the statistical analyses. Female body weight was measured for five individuals randomly selected from each cluster. The wasps were euthanized in a freezer within 24 h of hatching, and then dried for three days in a desiccator at 45°C. The total dry weight of the five wasps (within 10 μg) was measured using an electric balance, and the mean weight per individual was calculated for each cluster. We used a general linear model (GLM)

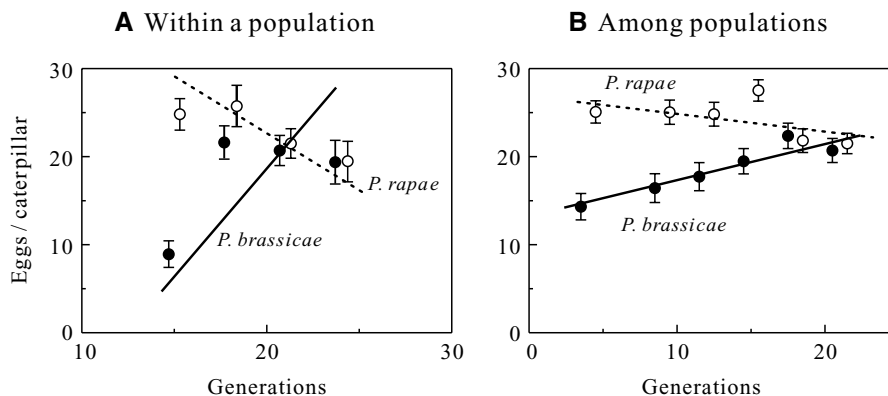


Figure 2. Spatiotemporal changes in the number of eggs laid by *Cotesia glomerata* in *Pieris brassicae* and *P. rapae* caterpillars following the invasion of *P. brassicae* on Hokkaido. (A) Number of eggs laid by *C. glomerata* collected from Sapporo in four consecutive years (1999–2002). We measured 31, 28, 30, and 16 wasp families for 15, 18, 21, and 24 generations since *P. brassicae* invaded Sapporo, respectively. (B) Number of eggs laid by *C. glomerata* collected from six census sites in 2001: Hamakoshimizu (25), Otofuke (21), Furano (22), Aoyama (27), Kyowa (22), and Sapporo (30). Numbers in parentheses indicate the number of wasp families used in oviposition experiments. ●, number of eggs laid in *P. brassicae*; ○, number of eggs laid in *P. rapae*. Data are reported as mean ± SE. The number of generations of *C. glomerata* following the arrival of *P. brassicae* is reported in Table 1.

with the number of cocoons per cluster as a covariate to determine whether the eclosion rate or body weight of *C. glomerata* differed according to host species.

PARASITISM RATES IN THE FIELD

To examine host exploitation by *C. glomerata* in the field, we investigated its parasitism rates of *P. brassicae* and *P. rapae*. Given that *C. glomerata* parasitizes first- to third-instar caterpillars, we collected fourth- and fifth-instar caterpillars from the census sites and dissected them to determine the number of parasitized caterpillars. We collected caterpillars from cabbage and mustard fields each autumn from 1999 to 2005 and compared parasitism rates between years for each host plant and each host caterpillar using likelihood ratio tests. For the *P. brassicae* and *P. rapae* caterpillars collected in mustard fields, we compared the rates of parasitism between 1999 and 2005 with likelihood ratio tests, after calibrating the caterpillar density to per 300 m². In addition, we collected *P. rapae* caterpillars at Hamakoshimizu, Otofuke, Furano, Aoyama, and Kyowa in 2001, and used logistic regression to test whether parasitism rates differed among census sites according to the duration of coexistence with *P. brassicae*.

HOST PREFERENCE OF HYPERPARASITIDS

The host preferences of hyperparasitoid wasps were examined in host-choice tests using the larval hyperparasitoid *B. galactopus* and the pupal hyperparasitoid *T. apantelectena*. The hosts were placed 15 cm apart in covered plastic cases (20 cm long × 13 cm wide × 8.5 cm high). The hyperparasitoids were then introduced to the center of the plastic case. When a hyperparasitoid mounted a host and assumed the typical posture for ovipositor insertion, we assumed that the host had been chosen. Both hyperparasitoid species moved slowly and sometimes rested for long periods; we excluded individuals that had not chosen a host after 3 h of observation.

As target hosts of the larval hyperparasitoid *B. galactopus*, we provided one active early final-instar caterpillar of each of *P. brassicae* and *P. rapae* that had been parasitized by *C. glomerata*, and placed each on a separate piece of cabbage leaf (2 × 2 cm). The pupal hyperparasitoid *T. apantelectena* was provided with one cluster of *C. glomerata* cocoons placed beside a dying caterpillar of each of *P. brassicae* and *P. rapae*. These cocoons had been spun one to two days before the test. We used cocoon clusters of average size, containing 20–40 cocoons each. We determined that the hyperparasitoids had chosen a host when *B. galactopus* assumed a typical ovipositing posture over the body of the host caterpillar and when *T. apantelectena* inserted an ovipositor into the host cocoon. We compared Furano populations collected in 2002 with those collected in 2005 with respect to host preference for both *B. galactopus* and *T. apantelectena* using a likelihood ratio test. The numbers of experiments conducted to compare host

preference were 16 and 23 for 2002 and 2005 with *B. galactopus*, and 43 and 34 for 2002 and 2005 with *T. apantelectena*. We also tested whether the host preference of both hyperparasitoids had changed in the time since *P. brassicae* introduction using logistic regression. The number of replicates for each hyperparasitoid is noted in Figure 4.

HYPERPARASITISM RATES IN THE FIELD

We investigated the hyperparasitism of *C. glomerata* in the field. The hyperparasitism rates of both *B. galactopus* and *T. apantelectena* were compared for clusters of *C. glomerata* cocoons collected from different host caterpillars, using likelihood ratio tests. The rate of hyperparasitism in *P. rapae* was also recorded at various sites in which the hyperparasitoids had coexisted with *P. brassicae* for different lengths of time. Clusters of *C. glomerata* cocoons for which the host caterpillars had been determined were collected from cabbage fields, and the clusters were reared in separate test tubes. After three weeks, when the majority of hyperparasitoids had emerged from the cocoons, we counted the number of adult hyperparasitoids and the number of cocoons with exit holes. Because *B. galactopus* is a gregarious wasp, we considered the number of cocoons with pinholes to represent the number of cocoons parasitized by *B. galactopus*. The hyperparasitoid that had egressed from the cocoon was easily identified based on the size of the egression hole. We were able to test the effect of host caterpillar on the rate of hyperparasitism for the Sapporo population of *P. rapae* in the fall of 2003; however, the reliability was thought to be poor in comparison to other years because we could not collect wasps at the same site.

Results

GRADUAL CHANGE IN CLUTCH SIZE OF *C. GLOMERATA*

The clutch size of *C. glomerata* per host caterpillar gradually and consistently increased for *P. brassicae* ($F_{1,103} = 17.6448$, $P < 0.0001$), whereas it decreased for *P. rapae* ($F_{1,103} = 4.5623$, $P = 0.0351$) in the Sapporo population (Fig. 2A). Thus, the clutch size in the two species of caterpillar changed in opposite directions following the invasion of *P. brassicae*. The same tendencies were observed among populations that varied in time since colonization by *P. brassicae*, and the clutch size increased in *P. brassicae* ($F_{1,145} = 16.7025$, $P < 0.0001$) and decreased in *P. rapae* ($F_{1,145} = 4.9860$, $P = 0.0271$; Fig. 2B).

PERFORMANCE OF *C. GLOMERATA* IN DIFFERENT HOST CATERPILLARS

A GLM with host species, size of the cocoon cluster, and their interaction as explanatory variables was used to explain the eclosion rate of *C. glomerata* per cocoon cluster ($F_{3,73} = 6.2309$, $P = 0.0008$, Fig. 3A). Although host species did not affect the rate of

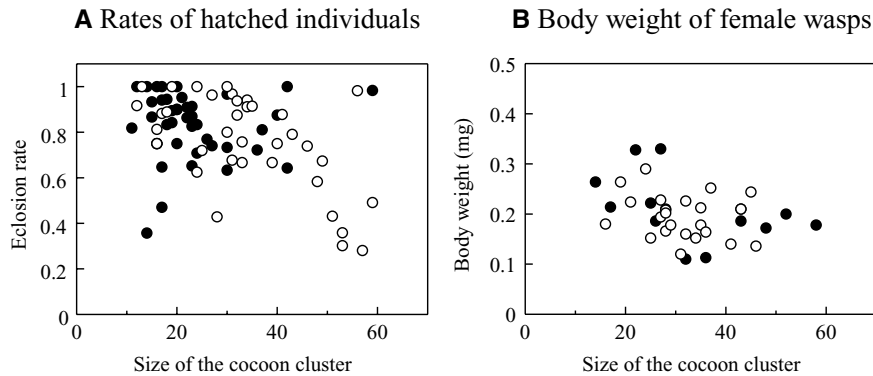


Figure 3. Eclosion rate of *Cotesia glomerata* per cocoon cluster and dry weight of adult females from the two host caterpillar species. (A) Eclosion rate per cluster. The number of cocoon clusters that were provided for comparison of emergence rates was 36 for *Pieris rapae* and 41 for *P. brassicae*. (B) Average body weight of female *C. glomerata*. We used 23 cocoon clusters that were collected from *P. rapae* and 14 clusters that originated from *P. brassicae* to measure the body weight of wasps. • *C. glomerata* in *Pieris brassicae*; ○ *C. glomerata* in *P. rapae*.

emergence, the rate decreased as the size of the cocoon cluster increased, especially in *P. rapae* (effect test; host: $df = 1, F = 0.0690, P = 0.7935$; cocoon cluster size: $df = 1, F = 5.9642, P = 0.0170$; interaction: $df = 1, F = 6.4353, P = 0.0133$).

We also fitted the weight of *C. glomerata* to a GLM with host species, size of the cocoon cluster, and their interaction as explanatory variables; however, the model failed to account for the weight of wasps ($F_{3,33} = 1.9752, P = 0.1368$, Fig. 3B). The host species and the interaction were not significant, but the weight of *C. glomerata* decreased as the size of the cocoon cluster increased (effect test; host: $df = 1, F = 1.1434, P = 0.2927$; cocoon cluster size: $df = 1, F = 4.2133, P = 0.0481$; interaction: $df = 1, F = 0.2176, P = 0.6439$).

PARASITISM IN THE FIELD

In the field, the parasitism rate of *P. brassicae* increased, whereas that of *P. rapae* decreased on both host plants examined over

several years (Table 2A). This tendency was the same when the frequencies of each host species in the field were considered (Table 2B). The rates of parasitism of *P. rapae* did not differ among populations that coexisted with *P. brassicae* for various lengths of time (logistic regression, $df = 1, \chi^2 = 0.0447, P = 0.8326$; Table 3).

HOST PREFERENCE OF HYPERPARASITOIDS

For hyperparasitoid populations collected at Furano in 2002 and 2005, the larval hyperparasitoid *B. galactopus* preferred host wasps inside the *P. rapae* caterpillar in both years; this preference did not change, regardless of how long they coexisted with *P. brassicae* ($df = 1, \chi^2 = 3.649, P = 0.0561$). Some *B. galactopus* attempted to parasitize host wasps inside the *P. brassicae* caterpillar, but failed, presumably because they were thrown off or bitten by the caterpillar. The pupal hyperparasitoid *T. apante-roctena* did not show a preference for clusters of wasp cocoons

Table 2. Chronological change in parasitism rates by *Cotesia glomerata* from the Sapporo population in the field. (A) Rates of parasitism of *Pieris brassicae* and *P. rapae* on cabbage. (B) Rates of parasitism of *P. brassicae* and *P. rapae* on mustard. The numbers of host caterpillars collected in mustard fields were converted to per 300 m². The rates are compared among the same host plant species.

	Host	Year	Host plant	Host caterpillar		df	Likelihood ratio χ^2	P
				n	% parasitism			
(A)	<i>P. brassicae</i>	1999	Cabbage	75	6.67	3	30.2215	<0.0001
	<i>P. brassicae</i>	2001	Cabbage	235	13.62			
	<i>P. brassicae</i>	2004	Cabbage	403	23.08			
	<i>P. brassicae</i>	2005	Cabbage	486	26.75	1	43.5431	<0.0001
	<i>P. rapae</i>	2001	Cabbage	191	60.21			
	<i>P. rapae</i>	2004	Cabbage	137	24.09			
(B)	<i>P. brassicae</i>	1999	Mustard	243	22.63	1	17.362	<0.0001
	<i>P. brassicae</i>	2005	Mustard	488	37.70			
	<i>P. rapae</i>	1999	Mustard	1243	77.59	1	81.489	<0.0001
	<i>P. rapae</i>	2005	Mustard	341	52.00			

Table 3. Comparison of parasitism rates among *Cotesia glomerata* populations that coexisted with *Pieris brassicae* for various time periods. In this case, we used only *P. rapae* as the host caterpillar.

Host	Collection site	Generations	Host caterpillar	
			<i>n</i>	Parasitism (%)
<i>P. rapae</i>	Hamakoshimizu	4	62	48.39
<i>P. rapae</i>	Otofuke	9	34	85.29
<i>P. rapae</i>	Furano	12	56	53.57
<i>P. rapae</i>	Aoyama	15	50	86.00
<i>P. rapae</i>	Kyowa	18	59	42.37
<i>P. rapae</i>	Sapporo	21	–	–

that egressed from the two caterpillar species in 2002, but it preferred cocoons egressed from *P. brassicae* in 2005 ($df = 1$, $\chi^2 = 5.468$, $P = 0.0194$).

A similar tendency was observed among the populations. *Baryscapus galactopus* still preferred *C. glomerata* in *P. rapae* caterpillars 20 generations after *P. brassicae* had invaded ($df = 3$, $\chi^2 = 3.0982$, $P = 0.3767$; Fig. 4A). Conversely, *T. apantelecta* tended to parasitize *C. glomerata* cocoon clusters from *P. brassicae* more frequently as the period of coexistence with *P. brassicae* increased ($df = 3$, $\chi^2 = 10.5724$, $P = 0.0143$; Fig. 4B).

HYPERPARASITISM IN THE FIELD

Hyperparasitism by *T. apantelecta* was higher than that by *B. galactopus* in cocoons from *P. brassicae* ($df = 1$, $\chi^2 = 53.262$, $P < 0.0001$), whereas *B. galactopus* hyperparasitism was higher than that of *T. apantelecta* in cocoons from *P. rapae* ($df = 1$, $\chi^2 = 66.702$, $P < 0.0001$; Table 4). Hyperparasitism by *B. galactopus* increased in cocoons reared in *P. rapae*, whereas hyperparasitism by *T. apantelecta* decreased. *Trichomalopsis apantelecta* was the most common pupal hyperparasitoid at each census site, and both *B. galactopus* and *T. apantelecta* were the most frequent hyperparasitoids in each developmental stage of *C. glomerata*.

Discussion

The invasion of the large white butterfly *P. brassicae* (*P. brassicae*) to Hokkaido Island, Japan, around 1995 (Ueno 2001) dramatically changed the behavior of the primary parasitoid wasp *C. glomerata* toward the potential host *P. brassicae* within a short time. In oviposition experiments, the average clutch size of *C. glomerata* on *P. brassicae* gradually increased from 0 (in Sapporo; Sato and Ohsaki 2004) to about 20 eggs (Fig. 2A,B) within approximately 20 generations following invasion of this new host. The average clutch size of *C. glomerata* on *P. brassicae* in Europe

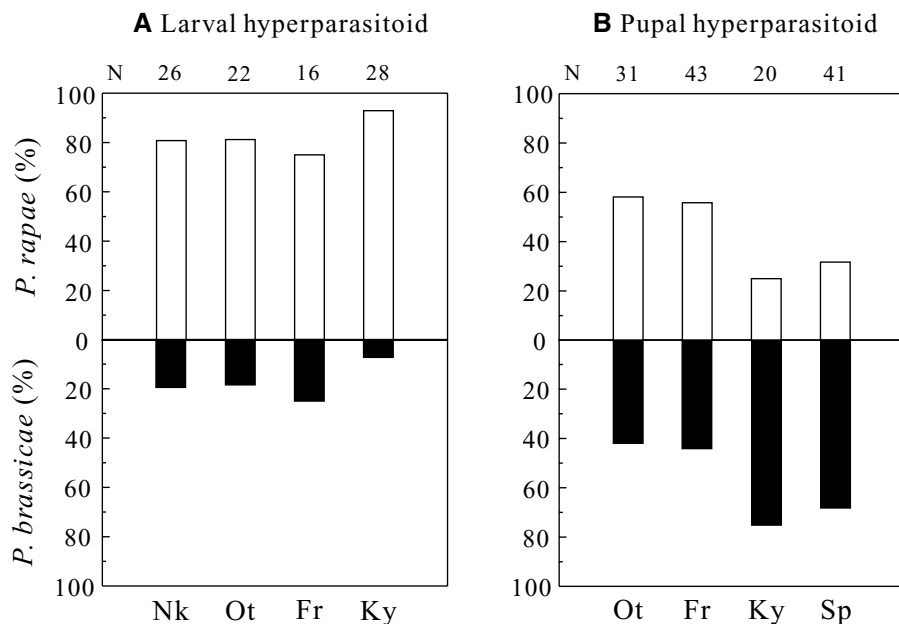


Figure 4. Spatial comparison of the host preference of the two hyperparasitoid species among four census sites. (A) Host preference of the larval hyperparasitoid *Baryscapus galactopus*. (B) Host preference of the pupal hyperparasitoid *Trichomalopsis apantelecta*. Open and black bars indicate the proportion of individuals that chose host wasps that parasitized *Pieris rapae* and *P. brassicae*, respectively. The total length of each set of bars equals 100% and the numbers indicate the sample size. Census sites: Nk, Nakashibetsu; Ot, Otofuke; Fr, Furano; Ky, Kyowa; Sp, Sapporo. The census sites are ranked according to the duration of coexistence with *P. brassicae*, increasing from left to right.

Table 4. Rates of hyperparasitism (number of parasitized cocoons/total number of cocoons) by two hyperparasitoids in the field. Pupal hyperparasitoids are divided into two classes: *Trichomalopsis apanteroctena* and other species.

Year	Collection site	Host caterpillar	Number of cocoons	% hyperparasitism			% Dead	
				Larval	Pupal			
					<i>T. apanteroctena</i>	Others		
2001	Kyowa	<i>P. rapae</i>	792	5.81	31.06	3.16	40.03	27.65
2003	Sapporo	<i>P. rapae</i>	361	26.59	10.53	0	37.12	37.40
2003	Sapporo	<i>P. brassicae</i>	474	6.33	31.01	20.04	57.38	24.05

is 27–30 eggs (Brodeur et al. 1998), so that of *C. glomerata* in Japan may increase in the future. The mechanisms governing the changes in average clutch size of *C. glomerata* may be explained as follows. Immediately after *P. brassicae* invasion, most *C. glomerata* did not oviposit in *P. brassicae*, although some did. Over time, the frequency of individuals not ovipositing in *P. brassicae* gradually decreased, whereas the frequency of those ovipositing in *P. brassicae* increased. As a result, there was an increase in the average clutch size in *P. brassicae*. Given these facts, we conclude that *C. glomerata* adapted to use *P. brassicae* following *P. brassicae* invasion. Vos and Vet (2004) argued that clutch size is not an important determinant of *C. glomerata* fitness in North America, because it is easily affected by physiological or experimental conditions. However, when we calculated the mean clutch size, including cases when no eggs were found (i.e., *C. glomerata* inserted the ovipositor but did not oviposit), the difference in clutch size seemed to correspond with the level of adaptation, according to the progress of the recent generation.

Different qualities of *P. brassicae* and *P. rapae* as host species may have caused changes in the average clutch size of *C. glomerata* in the two species. The average mass of a *P. brassicae* caterpillar was more than twice that of *P. rapae* under optimal (i.e., unparasitized) conditions (Davies and Gilbert 1985), although caterpillars parasitized by *C. glomerata* reduced this difference (Harvey 2000). When the density of *C. glomerata* eggs inside caterpillars was high, survival decreased in *P. rapae*, but did not change in *P. brassicae* (Fig. 3A), although the average dry weight of emerged adult *C. glomerata* females did not differ significantly between the caterpillar species (Fig. 3B). Thus, these potential differences in body size of *P. brassicae* and *P. rapae* may affect the conditions of the parasitoid larvae, because a given clutch size would result in more intense resource competition among larvae within *P. rapae* caterpillars than within *P. brassicae* caterpillars. Therefore, the *P. brassicae* invasion may have acted in favor of *C. glomerata*, which oviposited in *P. brassicae*, and may have led to a decrease in the number of wasps that did not oviposit in *P. brassicae*.

It is interesting that immediately following *P. brassicae* invasion, the level of parasitism of *P. brassicae* was low (e.g., 0–2.0%

in 1997; Sato and Ohsaki 2004), although further research in subsequent years showed that parasitism of *P. brassicae* increased over time (e.g., 26.8–37.7% in 2005, Table 2). On the other hand, the parasitism rate in the indigenous host species *P. rapae* decreased during the same period, whereas there was no significant decrease in the parasitism rate of *C. glomerata* against *P. rapae* among populations. It should be noted that parasitism levels are expected to fluctuate with environmental factors such as season, location, and host-plant abundance (Ohsaki and Sato 1990; van Driesche and Bellows 1996; Gratton and Welter 1999). Even so, the enhanced parasitism rate of *P. brassicae* by *C. glomerata* seems to have been affected by the increasing density of *P. brassicae* in the field, and the opposite may be true for the reduced parasitism rate of *P. rapae* by *C. glomerata*. The results of the experiment indicated that natural selection imposed by the increased density of *P. brassicae* favored the *C. glomerata* genotype that could use *P. brassicae* over the other *C. glomerata* genotype that could not. In the field, the occurrence of the two host species overlapped largely, both in time and space, providing ample opportunities for *C. glomerata* to choose between the two species. The alternative explanation is phenotypic plasticity in the host selection of *C. glomerata* in response to the increasing and decreasing availability of *P. brassicae* and *P. rapae* caterpillars, respectively, through oviposition experience (e.g., Geervliet et al. 1998; Vos and Vet 2004). It should be noted, however, that the latter hypothesis alone cannot explain the results of the laboratory experiment, because we tested the host preference of naive *C. glomerata* wasps. We suggest that both of the above processes may be involved in the observed host shift of *C. glomerata* in nature. The rapid increase in the population size of *P. brassicae* likely played a role in the adaptation of *C. glomerata*. *Pieris brassicae* was rare at the start of its invasion (Hachiya 1997), but its numbers increased rapidly. Random sampling of first- to third-instar caterpillars of *P. brassicae* and *P. rapae* on 40 cabbages in Sapporo in late July 2005 resulted in 707 *P. brassicae* caterpillars, 4.6 times as many as those of *P. rapae* (153 individuals) (S. Tanaka, unpubl. data), and *P. brassicae* now occurs throughout Hokkaido. This rapid increase and spread of *P. brassicae* can be expected to have induced the change in *C. glomerata* host-use through bottom-up effects.

The host preferences of the two hyperparasitoid wasps were also affected by the invasion of *P. brassicae*. The larval hyperparasitoid *B. galactopus* parasitized *C. glomerata* larvae within *P. rapae* caterpillars, whereas it rarely parasitized *C. glomerata* larvae within *P. brassicae* caterpillars; this did not change over the course of our observations (Fig. 4A). This may be because *P. brassicae* caterpillars attack *B. galactopus* individuals when they attempt to parasitize the wasps within the caterpillar (S. Tanaka, pers. obs.). In contrast, *B. galactopus* is able to freely parasitize the larvae of *C. glomerata* within *P. rapae* caterpillars without being attacked. Therefore, the host choice by *B. galactopus* may reflect the behavior of the more violent host caterpillar *P. brassicae*, resulting in high parasitism pressure of *B. galactopus* on *C. glomerata* within *P. rapae* caterpillars.

In contrast, the pupal hyperparasitoid *T. apantelecta* gradually showed a preference for cocoons of *C. glomerata* that had egressed from *P. brassicae* over those that had egressed from *P. rapae* after the invasion of *P. brassicae* (Fig. 4B). This trend was observed both within and among populations. One factor causing this change in host preference may have been competition with *B. galactopus* for host wasps. *Baryscapus galactopus* is thought to be able to out-compete *T. apantelecta* because it can access an earlier developmental stage of the host (Gaines and Kok 1999). Therefore, by choosing host cocoons from *P. brassicae*, *T. apantelecta* escapes this competition for resources, resulting in an advantage for *T. apantelecta*. In addition to *B. galactopus*, many other hyperparasitoid species that attack pupae were observed on the cocoons of *C. glomerata*, and several species of hyperparasitoid wasp often egressed from the same cluster of *C. glomerata* cocoons, indicating the potential for intense competition. As a result, *T. apantelecta* may have rapidly adapted to use *C. glomerata* from the novel host caterpillar. *Trichomalopsis apantelecta* uses a broad range of host species, possibly allowing it to adapt alongside *C. glomerata* as the host tendency of *C. glomerata* changed from *P. rapae* to *P. brassicae*. Neither host size nor quality was expected to affect the host preference of *T. apantelecta* in experiments because the cocoon clusters that we provided were of similar size, and *C. glomerata* did not differ in body size between the host caterpillar species (but see Harvey 2000). However, we cannot reject the possibility of size effects.

The observed rates of hyperparasitism in the field may also support the results of our host preference tests. The parasitism rate of each hyperparasitoid species that emerged from *C. glomerata* cocoon clusters differed between host caterpillars, even though the cocoons were collected from the same place at the same time (Table 4). The proportion of *T. apantelecta* that emerged from *C. glomerata* cocoons from *P. brassicae* caterpillars was higher than that from *C. glomerata* cocoons from *P. rapae* caterpillars, despite the observation that the proportion of *B. galactopus* that emerged from the latter was higher than that from the former. We

also compared hyperparasitism rates between populations that had coexisted with *P. brassicae* for different durations. The proportion of each hyperparasitoid differed, even though the *C. glomerata* cocoons had all egressed from *P. rapae* caterpillars, such that the proportion of *T. apantelecta* was lower in the population that had coexisted with *P. brassicae* for relatively long periods. Although the hyperparasitism rate in the field would be affected by various environmental factors (Matsuzawa 1958), these results partly corresponded with those from the laboratory experiments.

The rapid adaptation of *C. glomerata* to *P. brassicae* and subsequent change of host preference of *T. apantelecta* suggests the possibility that hyperparasitism pressure forced *C. glomerata* to change its host use. Hyperparasitoid wasps appear to be an important predator of *C. glomerata* in Europe (Laing and Levins 1982), North America, and Japan (Matsuzawa 1958). Many species of Ichneumonidae, Pteromalidae, and Eulophidae are known to target *C. glomerata*, with hyperparasitism levels sometimes exceeding 60% of cocoons. Phytophagous insects are reported to selectively use host plants in which they are rarely parasitized (Sato and Ohsaki 1987; Brown et al. 1995; Feder 1995; Ohsaki and Sato 1999). Therefore, parasitism pressure may cause the prey to take advantage of novel host species in which they are less likely to be targeted (Brown et al. 1995; Feder 1995; Gratton and Welter 1999; Ohsaki and Sato 1999). Likewise, hyperparasitoids may cause *C. glomerata* to switch to *P. brassicae* caterpillars.

The evolutionary scenario we propose for changes in host use by the primary parasitoid (*C. glomerata*) and the two hyperparasitoids (*B. galactopus* and *T. apantelecta*) is summarized in Figure 5. After the invasion of *P. brassicae*, a portion of the *C. glomerata* population began to use *P. brassicae* caterpillars as hosts. Exploitation of *P. brassicae* likely increased the fitness of *C. glomerata* because it avoided the larval hyperparasitoid *B. galactopus*, which in turn caused the rate of parasitism of *P. brassicae* to increase. However, this host range expansion by *C. glomerata* provided the pupal hyperparasitoid *T. apantelecta* with a potential new, unexploited host that was unsuitable for its competitor, *B. galactopus*. Consequently, *T. apantelecta* began to use *C. glomerata* cocoons from *P. brassicae*, thus taking advantage of the change in host use by *C. glomerata*.

The invasion of the exotic butterfly *P. brassicae* led to dynamic evolutionary changes within <10 years, both with respect to host use by the primary parasitoid *C. glomerata*, and the host preference of the two hyperparasitoids *B. galactopus* and *T. apantelecta*. Although the direct cause of the changes in host use by *C. glomerata* was likely the invasion of *P. brassicae*, hyperparasitism pressure may also have contributed to the rapid adaptation of *C. glomerata* to the new host species. Host use by the hyperparasitoids was also affected by both direct competitive interactions (for *T. apantelecta*) and indirect interactions caused by interference by host caterpillars (for *B. galactopus*). By examining

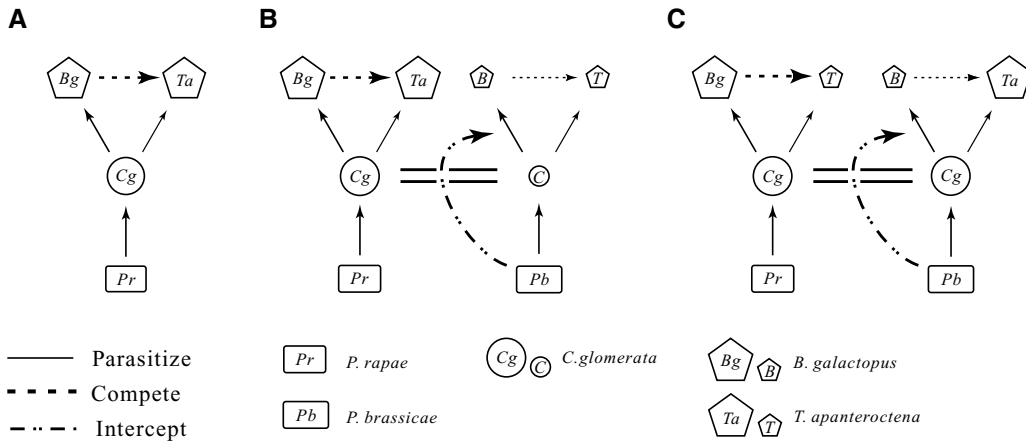


Figure 5. Flowchart of changing interspecific interactions. The size of each symbol corresponds to the total number of individuals. (A) The primary parasitoid *Cotesia glomerata* parasitizes only the native *Pieris rapae*, and two hyperparasitoid wasps, that is, the larval hyperparasitoid *Baryscapus galactopus* and the pupal hyperparasitoid *Trichomalopsis apanteroctena*, parasitize *C. glomerata*. *Baryscapus galactopus* may be superior to *T. apanteroctena* in terms of competition for resources. (B) After the arrival of the exotic butterfly *P. brassicae*, some *C. glomerata* individuals begin to parasitize *P. brassicae*. A small number of hyperparasitoids of *C. glomerata* parasitize *C. glomerata* from *P. brassicae* as well; however, the pressure by hyperparasitoids is relatively low in this phase. (C) Renewal of interspecific interactions. In parasitizing *P. brassicae*, *C. glomerata* escapes *B. galactopus* because of the aggressive behavioral response of *P. brassicae* caterpillars to *B. galactopus* approach. If *T. apanteroctena* parasitizes *C. glomerata* egressed from *P. brassicae*, *T. apanteroctena* can avoid resource competition with *B. galactopus*. Indeed, *T. apanteroctena* primarily attacks *C. glomerata* from *P. brassicae*. Therefore, each hyperparasitoid wasp may eventually segregate its host use upon *C. glomerata* from different host butterflies.

interspecific interactions within multiple trophic levels, we were able to observe other dynamic interactions that might have been missed by observing only direct interactions within one trophic level.

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