Guild Structure and Coexistence Mechanisms in the Parasitoid Assemblage Associated with a Leafminer, *Coptotriche japoniella* (Lepidoptera, Tischeriidae), on an Evergreen Tree, *Eurya japonica* (Theaceae)

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ABSTRACT The parasitoid assemblage associated with a lepidopteran leafminer, *Coptotriche japoniella* (Tischeriidae), on an evergreen tree, *Eurya japonica* (Theaceae), was studied in the center of Japan to explore parasitoid coexistence mechanisms. The leafminer supported 12 parasitoid species. Eight abundant or common species were classified into five guilds according to their koinobiont/idiobiont mode and host-instar utilization pattern: early larval koinobiont, mid-larval idiobiont, mid-larval–late larval idiobiont, late larval–pupal idiobiont, and pupal idiobiont. The early larval koinobiont (*Orgilus kumatai*) and mid-larval idiobiont (*Achrysocharoides* sp.) seemed to be specialized on the host, whereas the members of the other guilds had a wide host range. The mid-larval–late larval (*Cirrospilus diallus* and *Pnigalio* sp.) or late larval–pupal idiobionts (*Chrysocharis albipes*, *Apleurotropis kumatai*, and *Pleurotroppopsis japonica*) facultatively hyperparasitized half of spinning larvae or pupae of the early larval koinobiont. These results suggest that parasitoid coexistence in this assemblage is greatly promoted by high levels of facultative hyperparasitism by idiobionts with wide host ranges on the dominant koinobiont.

KEY WORDS host-instar utilization, host range, hyperparasitism, idiobiont/koinobiont mode, parasitism

Resource partitioning between competitive species has been regarded as a major coexistence mechanism in ecological communities (Begon et al. 2006). It can be performed by specialization on distinct resources and different resource utilization in space and time. In two parasitoids, *Encarsia perniciosi* (Tower) and *Aphytis melinus* (DeBach), which are specialized on California red scale *Aonidiella aurantii* (Maskell), the former prefers hosts on stems, whereas the latter prefers those on leaves and fruits (Yu et al. 1990). Twelve monophagous parasitoid species attacking galls of a midge, *Giraudiella inclusa* Frauenfeld, on a common reed, *Phragmites australis* (Cavanilles), divide into species attacking the first host generation and species attacking the second to fourth host generations and also exhibit different preferences for the position of the galls on host plant shoots (Tscharntke 1992).

However, neither spatial nor temporal resource partitioning may greatly contribute to coexistence of parasitoids attacking a univoltine leafminer. Because the leafminers use only leaf blades of their food plants, their parasitoids can hardly perform spatial resource partitioning for host utilization. Previous studies showed that those parasitoids often differ in host in-

stars they exploit (Ujiye 1980, Pschorn-Walcher and Altenhofer 1989, Kato 1994, Sato 1995, Ujiye et al. 1996), thereby they are supposed to partition the host temporally. However, temporal resource partitioning among parasitoids within a host generation does not necessarily ensure parasitoids coexistence. Briggs et al. (1993) and Briggs (1993) pointed out that a parasitoid attacking a certain host instar will decrease the abundance of later-instar hosts available for other parasitoids. Temporal resource partitioning according to host generation within a year may promote parasitoid coexistence (Barrett and Jorgensen 1986), but it can be disregarded in parasitoids attacking univoltine leafminers.

Theoretical studies have dealt with systems of two parasitoid species attacking a common host species to develop population dynamic models that make the coexistence of the parasitoids possible (Hassell and Varley 1969, May and Hassell 1981, Hogarth and Diamond 1984, Kakehashi et al. 1984, Godfray and Waage 1991, Briggs 1993, Briggs et al. 1993). These models except Briggs et al. (1993) and Briggs (1993) included some forms of density dependence that caused intraspecific competition to exceed interspecific competition. Conversely, Briggs and her co-workers showed that two parasitoids attacking successive host

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instars can coexist without assumption of density dependence (1) if the host population is composed of a mixture of different instars (Briggs et al. 1993) or (2) if the later parasitoid attacks hosts that have already been parasitized by the early parasitoid (Briggs 1993). Briggs (1993) also found that the late parasitoid was driven extinct by the early parasitoid unless it had a hyperparasitic capability. This means that the early parasitoid has a preemptive advantage over the late parasitoid. Borer (2006) assessed the validity of the Briggs' model by using a host–parasitoid system consisting of California red scale and its two specialist parasitoids, in which the idiobiont ectoparasitoid *A. melinus* can secondarily parasitize hosts containing a larva of the koinobiont endoparasitoid *E. perniciosi.* However, the model failed to predict parasitoid coexistence when parameterized with empirically derived values, suggesting that facultative hyperparasitism alone was unlikely to maintain coexistence of the parasitoids.

The parasitoid assemblages associated with lepidopteran leafminers of Gracillariidae, Tischeriidae, and Nepticulidae have been studied on Takamado Hill and its vicinities, Nara, Japan (Sato 1995, Sato et al. 2002). In this study, we dealt with parasitoids attacking a lepidopteran leafminer, *Coptotriche japoniella* Puplesis and Diŝks (Tischeriidae). *C. japoniella* is a univoltine leafminer. The adults emerge in early to mid-June. The females lay eggs on 1-yr-old or older leaves. The eggs hatch in August, and the larvae soon mine the leaf and become the fifth instar by the end of November. The fifth-instar larvae overwinter and grow into the sixth instar in mid- to late April. While feeding on leaf tissue, larvae excrete frass from the mine. The sixth-instar larvae pupate within the mine in mid- to late May (Oishi and Sato 2008).

In the study, first, we made regular sampling and rearing of host leafminers in 3 successive yr to obtain quantitative data of parasitoids for abundance, host-instar utilization, primary parasitism, hyperparasitism, and multiparasitism. Second, we delineated guild structure using parasitism mode and host-instar utilization pattern. Finally, we attempted to explain parasitoid coexistence not from spatial or temporal resource partitioning but host range and facultative hyperparasitism.

Materials and Methods

Study Area and Life History of *C. japoniella.* This study was conducted in a red pine (*Pinus densiflora*) wood on Takamado Hill, Nara, central Japan (34°40′ N, 135°52′ E, 400 m a.s.l.). The floor is dominated by an evergreen tree, *E. japonica,* which is the sole food plant of *C. japoniella* in this area.

Sampling and Rearing of *C. japoniella* and Parasitoids. The 2003 and 2004 generations of *C. japoniella* were examined by sampling leafminers once or twice a month in January and April to June 2004 for the 2003 generation and November and December 2004 and February to June 2005 for the 2004 generation. The generation year was defined as the year when eggs

were laid. At each sampling, 25–30 leaves containing intact mine(s), the surface of which was neither torn open nor had an emergence hole of adult parasitoid, were randomly collected from 8 to 15 *E. japonica* trees each.

Leaves were individually put in a plastic case in which a sheet of moistened filter paper was laid and were kept in an incubator at a temperature of 18°C and a photoperiod of 14 L:10 D. Plastic cases were checked for emergence of adult moths and parasitoids every third to fourth day. When an adult parasitoid emerged, the mine was dissected to determine the host instar by comparing the head width to reference specimens (Oishi and Sato 2008).

Parasitoids reared from *C. japoniella* were examined for four types of parasitism mode, i.e., solitary/gregarious, ecotoparasitism/endoparasitism, idiobiont/koinobiont, primary/secondary/tertiary, and specialist/generalist, in the following ways.

Solitary/Gregarious. When more than one parasitoid adults emerged from a mine, the parasitoids were regarded as gregarious; when not, they were regarded as solitary.

Ectoparasitism/Endoparasitism. When we noticed that mines expelled no frass during rearing, we put them on a glass stage from under which light was irradiated to find out whether a parasitoid larva was outside or inside the host with a microscopic binocular. In the former case, the parasitoid was regarded as an endoparasitoid and in the latter case as an ectoparasitoid.

Idiobiont/Koinobiont. When a parasitoid adult emerged from a mine that had expelled a fair amount of frass before the host was killed, it was regarded as koinobiont; when not, it was regarded as idiobiont.

Primary/Secondary/Tertiary. We dissected mines after an adult parasitoid emerged, finding out whether the parasitoid had preyed on a cocoon or pupa of a different parasitoid. When there was no parasitoid cocoon or pupal carcass by the pupal exuviae from which the parasitoid emerged, the parasitoid was regarded as primary; when there was a carcass, it was regarded as secondary; and when there were two carcasses, it was regarded as tertiary. Because hyperparasitism, or secondary and tertiary parasitism, is difficult to detect in early to late larval hosts, its levels should have been underestimated in this study.

Specialist/Generalist. When the same parasitoid species as emerged was recorded from other leafminer species in the study area and its vicinities (Sato 1995, Sato et al. 2002; H.S., unpublished data), the parasitoid was regarded as generalist; otherwise, judgment was suspended.

Leaves that decayed before moth or parasitoid emergence were dissected. When there was frass expelled from mines in a rearing case and no dead parasitoid larva or pupa in the mine, the host was considered to be alive at the time of sampling and have died from unknown causes during rearing. When there was neither frass in a rearing case nor parasitoid in the

Table 1. Parasitoids reared from *C. japoniella* in 2003 and 2004 generations

Family Subfamily Species	Progeny allocation	Endoparasitism or ectoparasitism	Koinobiont or idiobiont	Primary or secondary parasitism	Genralist or specialist	No. of hosts killed by the parasitoid[a] (%)	
						2003	2004
Ichenumonidae							
Pimplinae							
1. *Itoplectis* sp.	Solitary	Endoparasitism	Idiobiont	Primary	Generalist	28 (4.7)	1 (0.2)
Cryptinae							
2. *Gelis areator* (Panzer)	Solitary	Endoparasitism	Idiobiont	Primary	Generalist	0 (0.0)	1 (0.2)
Braconidae							
Rogadinae							
3. *Orgilus kumatai* Watanabe	Solitary	Endoparasitism	Koinobiont	Primary	Specialist?	368 (62.3)	236 (49.0)
Eulophidae							
Elachertinae							
4. *Cirrospilus ?lyncus* Walker	Solitary	Ectoparasitism	Idiobiont	Secondary	Generalist	0 (0.0)	1 (0.2)
5. *Cirrospilus diallus* Walker	Solitary	Ectoparasitism	Idiobiont	Primary, secondary	Generalist	57 (9.6)	58 (12.0)
Eulophinae							
6. *Pnigalio* sp.	Solitary	Endoparasitism	Idiobiont	Primary, secondary	Generalist	14 (2.4)	2 (0.4)
Tetrastichinae							
7. *Tetrastichus* sp.	Gregarious	Endoparasitism	Idiobiont	Primary	Generalist	4 (0.7)	0 (0.0)
Entedontinae							
8. *Apleurotropis kumatai* (Kamijo)	Solitary	Endoparasitism	Idiobiont	Primary, secondary	Generalist	2 (0.3)	14 (2.9)
9. *Pleurotroppopsis japonica* (Kamijo)	Solitary	Endoparasitism	Idiobiont	Primary, secondary	Generalist	1 (0.2)	33 (6.8)
10. *Chrysocharis albipes* (Ashmead)	Solitary	Endoparasitism	Idiobiont	Primary, secondary	Generalist	71 (12.0)	68 (14.1)
11. *Achrysocharoides* sp.	Solitary	Endoparasitism	Idiobiont	Primary	Specialist?	18 (3.0)	40 (8.3)
12. *Asecodes erxias* (Walker)	Solitary	Ectoparasitism	Idiobiont	Primary	Generalist	0 (0.0)	1 (0.2)
Unidentified eulophids[b]						28 (4.7)	27 (5.6)
Total						591 (100.0)	482 (100.0)

[a] Including one multiparasitized host in 2004.

[b] Parasitoids undetermined to genus or species level because of immamture death, adult loss, or poor specimen conditions.

mine, the host was considered to have been dead at the time of sampling and was excluded from analysis.

Parasitoids were identified by K. Kamijo (Bibai, Hokkaido, Japan; Eulophidae), K. Maeto (Kobe University, Japan; Braconidae), and K. Konishi (National Agricultural Research Center for Hokkaido Region, Sapporo, Japan; Ichneumonidae). Voucher specimens of all parasitoids were deposited in the Institute of Entomological Systematics, Faculty of Agriculture, Hokkaido University, Hokkaido, Japan.

The percentage parasitism by a parasitoid species was defined as $N_p/N_m \times 100$, where N_p is the number of leafminers killed by the species, and N_m is the number of leafminers reared. Hyperparasitized hosts were treated as those killed not by primary parasitoids but by hyperparasitoids because all hyperparasitoids obtained in this study were facultative.

Cluster Analysis. A similarity dendrogram of parasitoids in terms of host-instar utilization was drawn by a cluster analysis on the basis of arcsine-transformed proportions of parasitism at host instars. Euclidean distance and unweighted pair group method with arithmetic average (unweighted pair-group method with arithmetic average) were used as similarity metric and clustering linkage, respectively. The statistical procedure was performed by SPSS ver. 11.5 (SPSS 2002).

Results

Species Composition. A total of 3,133 larvae or pupae of *C. japoniella* were reared, and 34.2% of them were parasitized. Twelve parasitoid species were recorded, including one koinobiont endoparasitoid, eight idiobiont endoparasitoids, and three idiobiont ectoparasitoids (Table 1). *Tetrastichus* sp. was the sole gregarious parasitoid.

The most abundant species was the sole koinobiont *Orgilus kumatai* Watanabe, followed by *Chrysocharis albipes* (Ashmead) and *Cirrospilus diallus* Walker (Table 1). The rank abundance of these species was the same in the two host generations. *Pnigalio* sp., *Apleurotropis kumatai* (Kamijo), *Pleurotroppopsis japonica* (Kamijo), and *Achrysocharoides* sp. were regarded as common species. Their rank abundance differed between 2003 and 2004 host generations. For *Achrysocharoides* sp., which overwinters as larva in the host, the apparent difference in relative abundance between the host generations was mainly caused by more frequent sampling in the 2004 generation during the winter. The other common parasitoids did not overwinter as larvae. Thus, the difference in the rank abundance of the common parasitoids except *Achrysocharoides* sp. between the host generations was not caused by the sampling effect.
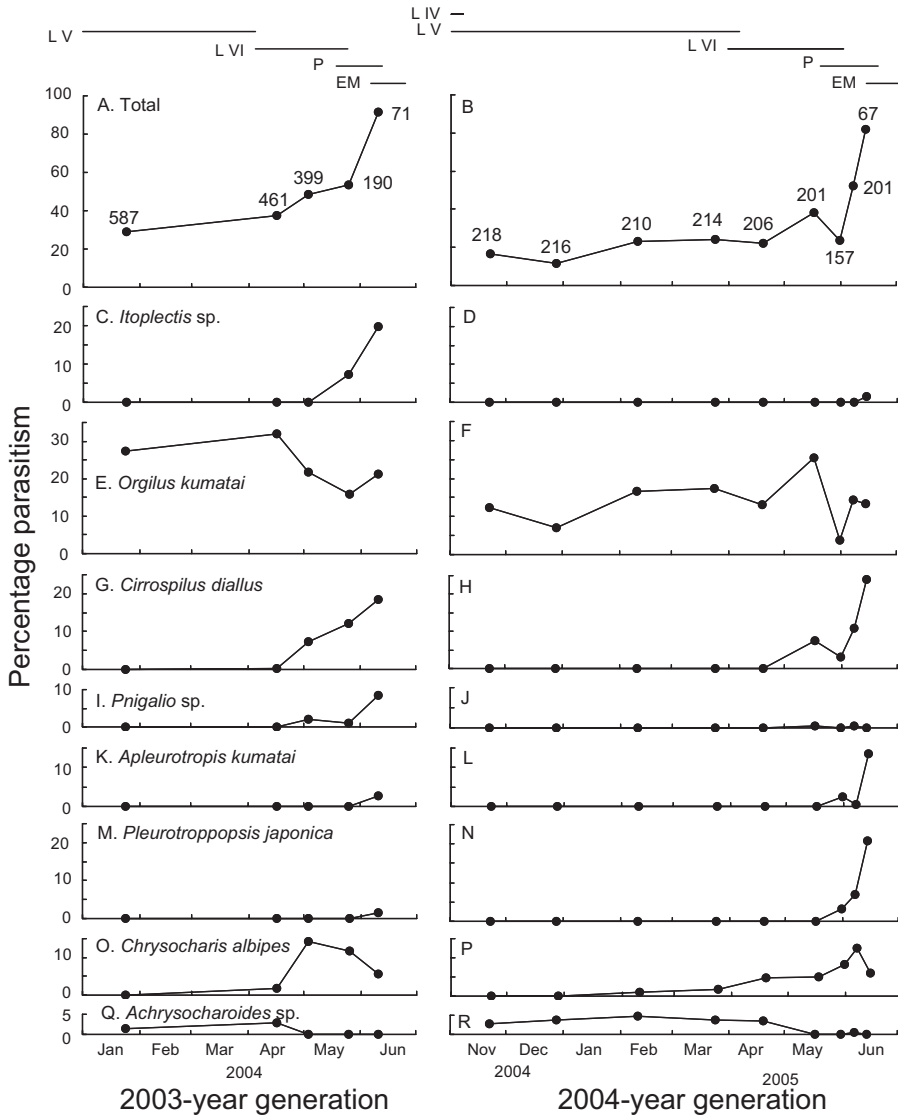
Fig. 1. Percentage parasitism of *C. japoniella* by all species and each of eight abundant or common parasitoids in 2003 and 2004 host generations. Numbers by solid circles in A and B indicate the number of *C. japoniella* reared. Horizontal bars at the head show instar periods of *C. japoniella* (Oishi and Sato 2008). L IV, fourth-instar larva; L V, fifth-instar larva; P, pupa; EM, adult emergence.

Of eight abundant or common parasitoids, *C. diallus, C. albipes, P. japonica,* and *A. kumatai* were certainly generalists. *O. kumatai* has been reared only from *C. japoniella* in the study area, although it was recorded from gracillariid leafminers in Hokkaido, northern Japan (Watanabe 1968). *Achrysocharoides* sp., *Pnigalio* sp., and *Itoplectis* sp. have probably not been recorded until this study.

Percentage Parasitism and Host Utilization. The percentage parasitism of *C. japoniella* was somewhat low (10–15%) in November and December and remained in the range of 25–30% until April (Fig. 1A and B). It increased rapidly after mid-April. In June, 50–90% of *C. japoniella* were parasitized. Relatively low

percentages in November and December were in part attributable to the fact that it was quite difficult to find parasitoid larvae in hosts dead from leaf decay and unknown causes while they were reared.

Temporal host utilization of eight abundant or common parasitoids was expressed by percentage parasitism at the sampling dates (Fig. 1C–R). The koinobiont *O. kumatai* had already attacked hosts in November and killed them in late May to early June (Fig. 1E and F). *Achrysocharoides* sp. was recorded from hosts from November to April (Fig. 1Q and R), and thus its temporal host utilization fully overlapped with that of *O. kumatai. C. diallus,* and *C. albipes* attacked hosts in May to June (Fig. 1G, H, O, and P), whereas *Itoplectis*
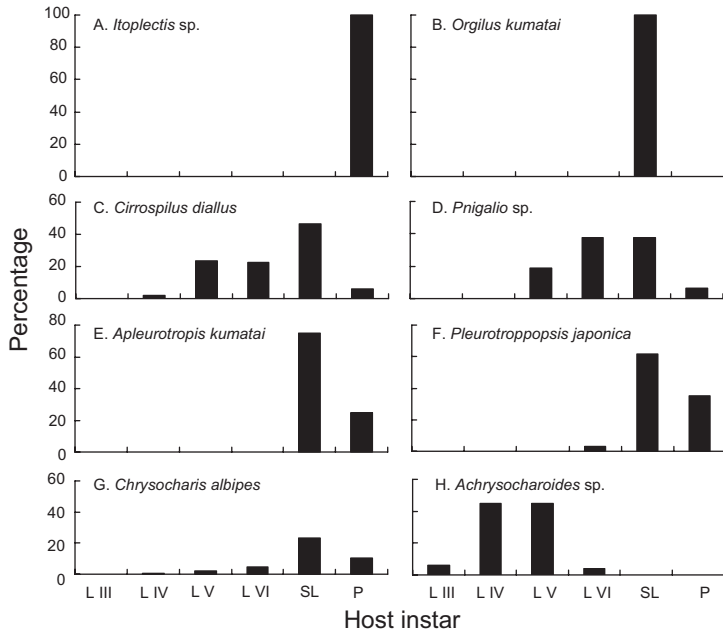
Fig. 2. Frequency distribution of individuals that emerged from different instars of *C. japoniella* in eight abundant or common parasitoids. Data of 2003 and 2004 host generations were combined. L III, third-instar larva; L IV, fourth-instar larva; L V, fifth-instar larva; L VI, sixth-instar larva; SL, spinning larva; P, pupa.

sp., *A. kumatai*, *Pnigalio* sp., and *P. japonica* did mainly in June (Fig. 1C, D, and I–N).

Host-instar utilization was represented by the frequency distribution of individuals that emerged from different host instars (Fig. 2). The adults of the koinobiont *O. kumatai* emerged exclusively from cocoons of the host. All idiobionts but *Itoplectis* sp. attacked and killed immatures of two or more instars. Using cluster analysis on the basis of host-instar utilization pattern, eight abundant or common species were classified into five guilds: early larval koinobiont (*O. kumatai*), mid-larval idiobiont (*Achrysocharoides* sp.), mid-larval–late larval idiobiont (*C. diallus* and *Pnigalio* sp.), late larval–pupal idiobiont (*C. albipes*, *A. kumatai* and *P. japonica*), and pupal idiobiont (*Itoplectis* sp.) (Fig. 3).

Hyperparasitism and Multiparasitism. Secondary and/or tertiary parasitoids, namely hyperparasitoids, included six eulophid species, *C. diallus*, *Pnigalio* sp., *P. japonica*, *A. kumatai*, *C. albipes*, and *C.* ?*lyncus* (Table 1). These species except *C.* ?*lyncus* were also primary parasitoids. *C.* ?*lyncus* has been recorded from various species of leafminers in the study area (Sato et al. 2002; H.S, unpublished data). Thus, all the hyperparasitoids reared in this study were considered to be facultative.

Quantitative analysis of hyperparasitism was carried out in May and June (Fig. 4) because facultative hyperparasitoids were active in this period (Fig. 1). Tertiary parasitism was found in the 2003 host generation (Fig. 4A) and not in the 2004 host generation (Fig. 4B). Facultative hyperparasitoids primarily parasitized 37.6% of *C. japoniella* and secondarily parasitized

19.8% of *O. kumatai* in the 2003 generation. In contrast, in the 2004 generation, secondary parasitism (37.5%) exceeded primary parasitism (25.4%). All parasitized *O. kumatai* were of spinning larvae for pupation or pupae within the cocoon. This does not mean that facultative hyperparasitoids preferred these stages of *O. kumatai*; instead, it is merely because of difficulty in detecting hyperparasitism in mid- and late larval hosts containing an immature larva of *O. kumatai*. As far as spinning larvae and pupae of *O. kumatai* were concerned, 55.6 (=30/54) and 53.8% (=58/104) of them were parasitized in the 2003 and 2004 generations, respectively. Facultative hyperparasitoids did not frequently suffer secondary or tertiary parasitism: secondary parasitism, 2.8 and 3.5% of primary parasitoids in the 2003 and 2004 host generations, respectively: tertiary parasitism, 6.7% of secondary parasitoids in the 2003 host generation.

Multiparasitism, which was defined as emergence of two or more different parasitoid species from a single host, was observed in only one host in the 2004 host generation. In that case, a *C. albipes* adult and a *C. diallus* adult emerged from the host.

Variation in Sex Ratio According to Primary/Secondary Parasitism Mode. Each facultative hyperparasitoid, *C. diallus*, *P. japonica*, *C. albipes*, *Pnigalio* sp., or *A. kumatai*, showed a higher proportion of females in secondary parasitism than in primary parasitism, although this was statistically insignificant in the last two species (Table 2). Sex ratios of three primary parasitoids, *Itoplectis* sp., *O. kumatai*, and *Achrysocharoides* sp., were significantly male-biased, significantly female-biased, and unbiased, respectively.
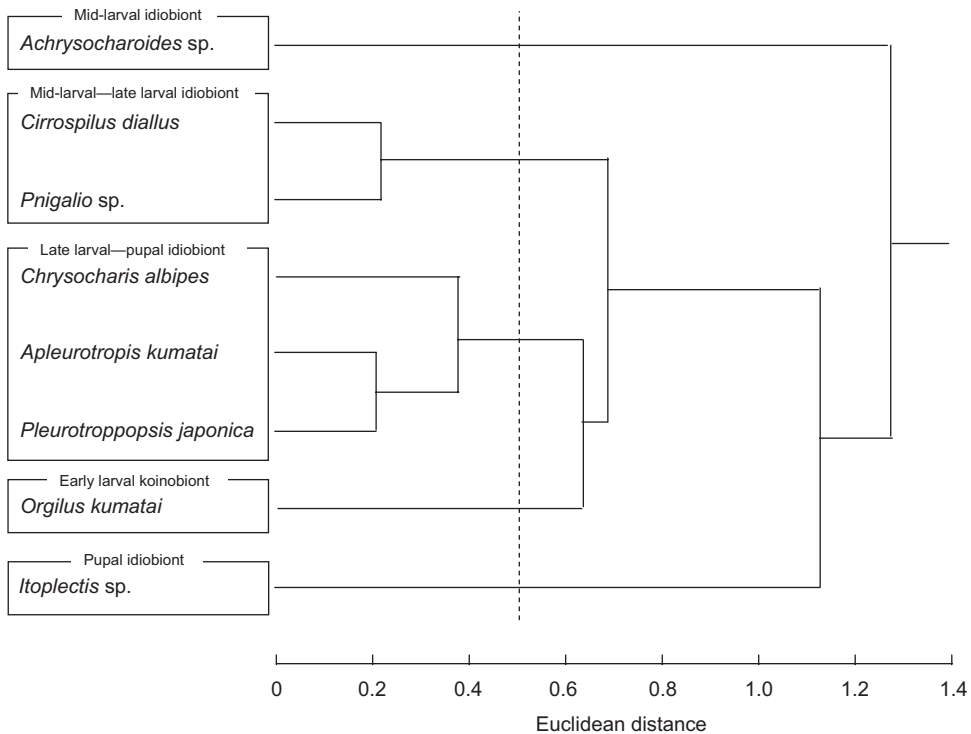
Fig. 3. A similarity dendrogram of eight abundant or common parasitoids on the basis of host-instar utilization pattern. Five guilds are classified at the level of 0.5 Euclidean distance.

Discussion

Species Richness and Composition. A total of 12 species in the parasitoid assemblage associated with *C. japoniella* is about twice as high as the average calculated by Hawkins (1994) for 55 dipteran and lepidopteran leafminers in North America and Europe. This species richness is, however, not as high as others obtained from local populations of *Phyllonorycter* leafminers (Gracillariidae) (Ujiye 1980, Maier 1982, 1984, Barrett and Jorgensen 1986, Sato 1990, Godfray et al. 1995, Sato et al. 2002). Sato (1990) recorded seven parasitoids from *Tischeria quercifolia* Kuroko and *T. decidua* Wocke on two deciduous oaks, *Quercus dentata* and *Q. crispula*, in the Ishikari Coast, Hokkaido, Japan. Compared with these tischeriids, *C. japoniella* seems to harbor a rich parasitoid fauna. However, this could be because of the difference in sample size. The sample size of this study was ≈50 times as large as that of Sato (1990). In fact, Jordan (1995) recorded 12 parasitoid species from a population of *T. ekebladella* (Bjerkander) on a deciduous oak, *Q. robur*, in northern Germany by 3-successive-yr sampling. Thus, the parasitoid species richness on *C. japoniella* is not especially high.

Orgilus kumatai is the sole koinobiont and numerically dominates in the parasitoid assemblage on *C. japoniella.* The numerical dominance of the sole koinobiont is also found in the parasitoid assemblage on a dipteran holly-leafminer, *Phytomyza ilicicola* Loew; the koinobiont is *Opius striativentris* Gahan (Kahn and

Cornell 1989, Braman and Pendley 1993, Eber et al. 2001). According to Hawkins (1994), on average, koinobionts comprise ≈33% in a parasitoid assemblage associated with a leafminer in North America and Europe. By comparison, the proportions of koinobionts in parasitoid assemblages on *C. japoniella* and *P. ilicicola* are very low. This is likely to be related with long larval periods of the hosts. The host larvae feed through summer to winter and pupate in spring or early summer, and thus their larval periods are 8–9 mo. Like *O. kumatai* and *O. striativentris*, most koinobionts of leafminers oviposit on eggs or early-instar larvae and spend their larval period within the host until the start of host pupation (Askew and Shaw 1986). Accordingly, possible koinobionts exploiting *C. japoniella* or *P. ilicicola* would be required to regulate the development of the host and to overcome host immune responses for such a long period. This requirement would act as a constraint on host use for koinobionts, so that koinobionts that can exploit *C. japoniella* or *P. ilicicola* would be numbered.

The numerical dominance of *O. kumatai* or *O. striativentris* in the assemblage would be responsible for a narrow window of vulnerability to hyperparasitism by idiobionts. Host larvae in summer would be too small in size for most idiobionts to complete their development. Parasitoid activity is relatively low from autumn to spring because of low temperatures. Thus, *O. kumatai* and *O. striativentris* would be exposed to hyperparasitism for only a short period relative to their
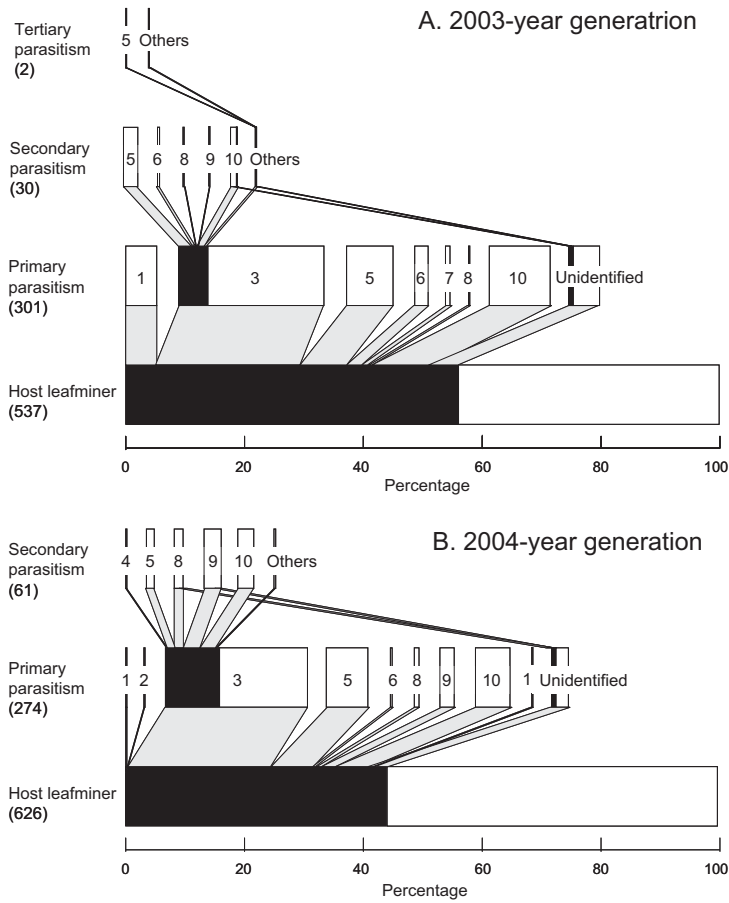
Fig. 4. Relative frequency of primary, secondary, and tertiary parasitism in 2003 and 2004 host generations. Solid and open bars indicate parasitized and unparasitized individuals, respectively. Numbers within the bars correspond with species code numbers in Table 1. Numbers in parentheses are sample sizes.

long larval periods, so that they would dominate in the assemblages.

Host Range. Of eight abundant and common species, *C. diallus*, *C. albipes*, *P. japonica*, and *A. kumatai* are generalists in the study area. Although *Pnigalio* sp.

and *Itoplectis* sp. have not been recorded from the study area (Sato 1995, Sato et al. 2002; H.S., unpublished data), they seem to have a wide host range. Marked differences in their relative abundance between the two host generations imply that they may

Table 2. Sex ratio of abundant or common species in primary and secondary parasitism

	Primary parasitism				Secondary parasitism			
	Male	Female	Proportion of females	P^a	Male	Female	Proportion of females	P^a
Itoplectis sp.	22	3	0.120	[b]	—	—	—	—
Orgilus kumatai	242	312	0.563	[c]	—	—	—	—
Cirrospilus diallus	48	36	0.429	NS	2	25	0.926	[b]
Pnigalio sp.	9	5	0.357	NS	1	1	0.500	NS
Apleurotropis kumatai	0	6	1.000	[d]	2	7	0.778	NS
Pleurotroppopsis japonica	5	10	0.667	NS	2	14	0.875	[c]
Chrysocharis albipes	78	33	0.297	[b]	2	22	0.917	[b]
Achrysocharoides sp.	29	25	0.463	NS	—	—	—	—

Parasitoids in secondary parasitism are those emerged from *O. kumatai*.
[a] Binomial test (H_0: $P = 0.5$).
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.
NS, not significant.

exploit *C. japoniella* facultatively when the host is abundant and other hosts are unavailable or rare. Their male-biased sex ratios, although statistically insignificant in *Pnigalio* sp., are also suggestive of dependence on other hosts, because the male-biased sex ratio indicates that the host is not optimal for the parasitoid (Askew and Shaw 1979).

Achrysocharoides sp. and *O. kumatai* are likely to be specialized on *C. japoniella* in this study area. Further explanation for *O. kumatai* may be required. The original description of *O. kumatai* is based on specimens reared from two gracillariids, *Caloptilia magnoliae* Kumata and *Gracillaria albicapitata* Issiki (Watanabe 1968), both of which occur in northern Japan and highlands of central Japan (Kumata 1982). Larval habits of *C. magnoliae* and *G. albicapitata* are considerably different from that of *C. japoniella* in that they live as leafminers until third instar and afterward as leaf rollers, overwintering as pupae. Hence, it would be difficult for the koinobiont *O. kumatai* to exploit both gracillariids and *C. japoniella* as hosts. If so, *O. kumatai* would form a complex consisting of different species attacking gracillariids or *C. japoniella*.

The host range of parasitoids seems to be linked with host-instar utilization. Specialist parasitoids, *O. kumatai* and *Achrysocharoides* sp., attack early or midinstar larvae of the host, whereas generalist parasitoids, *C. diallus*, *C. albipes*, *Pnigalio* sp., *P. japonica*, *A. kumatai*, and *Itoplectis* sp., attack late-instar larvae or pupae. This trend is found in parasitoids attacking *Phyllonorycter elmaella* Doganlar and Mutuura in western North America (Barrett and Jorgensen 1986).

Coexistence Mechanisms. A parasitoid exploiting early instars of the host will diminish the abundance of late instars that other parasitoids can exploit. This leads us to predict that parasitoids compete for the host, even though they partition the host according to its instar in the way of niche differentiation. Briggs (1993) has developed a theoretical model that promotes the coexistence of two parasitoids sharing a single host and attacking different host instars: one is a koinobiont that attacks the egg stage of the host and emerges from the last-instar larva, and the other is an idiobiont that attacks hosts whether or not they have been previously parasitized by the koinobiont. In this model, the two parasitoids can coexist because hosts parasitized by the koinobiont serve as an additional resource for the idiobiont. A similar interaction may be found between koinobiont and idiobiont parasitoids in the present system, where the koinobiont *O. kumatai* was frequently attacked by idiobionts (Fig. 4). Mechanisms for the coexistence of parasitoids deserve to be considered in detail using the guild concept.

To begin with, we consider coexistence of early larval koinobiont (or *O. kumatai*), mid-larval–late larval idiobiont, and late larval–pupal idiobiont. *O. kumatai* would always have a preemptive advantage over those idiobionts unless hosts parasitized by *O. kumatai* are attacked by the idiobionts. In contrast, 20% or more of *O. kumatai* in May and June suffered facultative parasitism by the idiobionts (Fig. 4). This sug-

gests that hyperparasitism promotes the coexistence of *O. kumatai* and the idiobionts as the Briggs' model predicts. One may, however, doubt whether the Briggs' model, which deals with two parasitoid species, can be applied in this system, which consists of multiple species. In the "aggregation model of species coexistence" (Shorrocks and Rosewell 1986, Sevenster 1996), multiple-species systems are converted into two-species system consisting of one species and one "super-species" as an amalgamation of facultative hyperparasitoids. Following this, we can apply the Briggs' model to our system.

Facultative hyperparasitoids seem to use *O. kumatai* as a suitable host as well as *C. japoniella*. This is confirmed by the fact that each parasitoid exhibited a female-biased sex ratio when emerged from *O. kumatai;* the sex ratio is useful in assessing host quality for the parasitoid species (Askew and Shaw 1979). High quality of *O. kumatai* as a host presumes that facultative hyperparasitoids benefit from hyperparasitism by excluding *O. kumatai* without reducing their fitness. Brodeur (2000) mentioned that facultative hyperparasitoids should behave as secondary parasitoids when primary hosts are rare or unavailable. Clearly, his view is not true in this system.

The Briggs' model also presents a condition for the coexistence of two parasitoid species: if the host and koinobiont have the same larval period susceptible to parasitism by the idiobiont, the idiobiont must have a lower attack rate on hosts that were already parasitized by the koinobiont than on unparasitized hosts. Accordingly, a question arises as to whether or not this system fulfills the condition. Attack rates have to be substituted for by percentage parasitism, because they can hardly be obtained in the field. The percentage of *O. kumatai* parasitized secondarily by idiobionts in May and June was lower than that of *C. japoniella* parasitized primarily by idiobionts in the 2003 host generation but vice versa in the 2004 host generation (Fig. 4). The difference between the generations can be attributed to the lack of samples in early May 2005, when we could not determine whether hosts attacked by idiobionts had been already parasitized by *O. kumatai.* Thus, addition of samples in early May should have reduced the proportion of hyperparasitized hosts in the 2004 generation. If so, this system might fulfill the condition the Briggs' model requires.

The above argument is based on an implicit assumption that idiobionts always consume koinobionts in a shared host (Godfray 1994, Mills 1994). To the contrary, Borer (2002) has shown that the idiobiont *A. melinus* survives at a lower rate on California red scale containing a more developed larva of the koinobiont *E. pernicios*, suggesting that the outcome of interference competition between the parasitoids in the host should depend on the developmental stage of the koinobiont. We did not evaluate the survival rate of idiobionts on the hosts containing young larvae of the koinobiont *O. kumatai*. Nevertheless, considering the fact that >50% of spinning larvae or pupae of *O. kumatai* were consumed by idiobionts, hyperparasitism cannot be ignored as a coexistence mechanism for

O. kumatai and those idiobionts. More intensive studies, particularly on hyperparasitism in mid- to late larval host instars, are necessary to test the Briggs' model for the present assemblage.

Next, we consider the coexistence of mid-larval–late larval idiobiont, late larval–pupal idiobiont, and pupal idiobiont, all of which consist of only generalists. Mid-larval–late larval idiobionts can attack hosts earlier than the others, having a preemptive advantage. Similarly, late larval–pupal idiobionts have an advantage over pupal idiobionts. Late larval–pupal idiobionts and pupal idiobionts, however, remain in the assemblage. This can be explained by the fact that they are generalists. These idiobionts may exploit *C. japoniella* facultatively when the host is abundant, and other hosts are unavailable or rare as noted earlier. Although hyperparasitism among these idiobionts might be another possible mechanism, it hardly contributes to coexistence because of its fairly low frequency (Fig. 4).

Finally, we consider interactions of the mid-larval idiobiont *Achrysocharoides* sp. with other parasitoids. *Achrysocharoides* sp. is preemptively superior to the other idiobionts but inferior to the koinobiont *O. kumatai.* There are two possible reasons why *Achrysocharoides* sp. cannot exclude the other idiobionts: (1) the latter are generalists and (2) they have much higher parasitism rates than the former. Possibility 2 is suggested theoretically by Briggs (1993). However, the coexistence of *Achrysocharoides* sp. and *O. kumatai* might be maintained by a high mortality of *O. kumatai* from hyperparasitim by idiobionts.

Although several reviews have indicated that hyperparasitism is an important role in structuring parasitoid communities (Force 1974, Stiling and Rossi 1994, Sullivan and Völkl 1999, Brodeur 2000), few studies have carried out quantitative investigation of hyperparasitism in parasitoid assemblages. Recently, however, two studies have done that. First, Langellotto et al. (2006) examined the frequency of hyperparasitism in a parasitoid assemblage associated with gall-making midges using stable isotope analysis, but did not consider how facultative hyperparasitism contributes to parasitoid coexistence in the assemblage. Second, Borer (2006) showed that the coexistence of *A. melinus* and *E. perniciosi*, specialists on California red scale, was unlikely to be maintained by facultative hyperparasitism alone, suggesting that spatial heterogeneity may play an important role in coexistence through differential preferences of each species for hosts on stems and leaves of trees (Yu et al. 1990, Borer 2004). In the parasitoid assemblage associated with *C. japoniella*, we presume that not only high facultative hyperparasitism by idiobionts on the dominant koinobiont but also that their wide host ranges should promote parasitoid coexistence.

Acknowledgments

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