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Intra- and interspecific host discrimination by host-seeking larvae of coleopteran parasitoids

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Abstract Intraspecific host discrimination is widespread in solitary parasitoids whose adult females forage for and evaluate host suitability, whereas interspecific discrimination is less common. In some parasitoid species, mostly Diptera and Coleoptera, the larva performs the last step of host searching. It has been suggested that host discrimination will rarely occur in such host-seeking larvae because their low mobility results in a low host encounter rate. We determined the extent to which the larvae of *Aleochara bilineata* Gyllenhal (Coleoptera: Staphylinidae), a solitary parasitoid of aggregated Diptera pupae: (1) discriminated between unparasitized hosts and hosts parasitized by conspecifics; (2) used semiochemical cues to discriminate; (3) were influenced by life expectancy, presence of conspecifics and host availability in their host acceptance decision; and the extent to which (4) *A. bilineata* and *A. bipustulata* L., a species exploiting the same hosts and occurring sympatrically, showed interspecific host discrimination. *A. bilineata* larvae were able to discriminate between unparasitized hosts and hosts parasitized by conspecifics in a choice experiment. Such behavior has never previously been described for a coleopteran parasitoid or for a parasitoid species whose larvae perform host searching. Host discrimination in this species was not based on the presence of visual or tactile cues (e.g., entrance holes) but rather on chemical cues. The life expectancy of *A. bilineata* larvae was significantly shorter in the presence than in absence of hosts, and older larvae had lower parasitism success than young larvae in a 24-h experiment. However, the host acceptance decision of *A. bilineata* larvae was not influenced by larval age or the presence of conspecifics when the ratio of hosts per larva was greater than or equal to 1. When hosts were scarce, the degree of superparasitism increased significantly with the number of foraging conspecifics and the age of the larvae. Both species of *Aleochara* showed intra- and interspecific host discrimination in a choice experiment. In contrast to *A. bipustulata*, *A. bilineata* larvae more frequently parasitized hosts parasitized by *A. bipustulata* than those parasitized by conspecifics. We suggest that host discrimination will be frequent in solitary parasitoids with host-seeking larvae when hosts are aggregated.

Key words *Aleochara* spp. · Olfactory marker · Host discrimination · Superparasitism · Multiparasitism

Introduction

In contrast to predators, insect parasitoids do not remove attacked individuals from the habitat and therefore these individuals may be encountered again. Since parasitized hosts often have a much lower fitness value than unparasitized hosts (van Baaren et al. 1995), it is adaptive for female parasitoids to distinguish between these host categories. The ability of female parasitoids to distinguish parasitized from unparasitized hosts is called host discrimination (Roitberg and Mangel 1988). Host discrimination has a strong selective advantage because females can avoid wasting eggs and time when rejecting a parasitized host is faster than ovipositing (Bakker et al. 1985). Intraspecific host discrimination (the ability to recognize hosts parasitized by conspecifics) is common in hymenopteran parasitoids (van Lenteren 1981; van

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Alphen and Visser 1990) and rare in dipteran parasitoids (van Lenteren 1981; Feener and Brown 1997), having been recorded in only two tachinid species (López et al. 1995). It has never been studied in coleopteran parasitoids. On the other hand, interspecific host discrimination (the ability to recognize hosts parasitized by another parasitoid species) has been demonstrated only in few parasitoid species, mostly Hymenoptera (Vinson and Ables 1980; van Baaren et al. 1994). Intra- and interspecific host discrimination have been studied only in species where the adult female parasitoid actively searches for the host and evaluates its condition. However, in some species of hymenopteran parasitoids (restricted to the two chalcidoid families Perilampidae, Eucharitidae and the ichneumonid subfamily Eucerotinae) (Godfray 1994), and in most dipteran and coleopteran parasitoid species (Eggleton and Belshaw 1992), the first-instar larva searches for the host. Host-seeking larvae could evolve the ability to discriminate between unparasitized and parasitized hosts, but it has been assumed that this behavior may not be as advantageous as in species where females search for hosts (Feener and Brown 1997), because of the low frequency of host encounter by host-seeking larvae that results from their limited dispersal ability. However, for parasitoid species whose hosts are aggregated, host-seeking larvae may have a host encounter rate high enough to make host discrimination worthwhile. In addition, the fitness gain from host discrimination would be high in host-seeking larvae, because an individual larva has only one opportunity of entering a host whereas female parasitoids may spread their progeny over several hosts.

Although most adult female parasitoids discriminate between hosts, superparasitism (multiple attacks of a host by the same parasitoid species) frequently occurs in nature (van Lenteren 1981). Female parasitoids face both physiological (egg load, life expectancy, movement capacity, experience, ability to learn) (van Alphen and Visser 1990 and references therein) and environmental constraints (presence or absence of competitors, distribution and density of hosts, level of host patch exploitation) (Bakker et al. 1985; Hubbard et al. 1987; Visser et al. 1990), and superparasitism may be the optimal behavior to maximize fitness gain under certain combinations of these factors. Even if host-seeking larvae can discriminate between unparasitized hosts and hosts parasitized by conspecifics, we may expect that constraints such as movement capacity, life expectancy and environmental factors will also affect superparasitism decisions by these larvae.

Aleochara bilineata (Gyll.) (Coleoptera: Staphylinidae) is a generalist predator as an adult (Read 1962) and immature stages develop inside puparia of some species of Diptera (Anthomyiidae: *Delia radicum* L., *D. platura* Meig., *D. antiqua* Meig., *D. floralis* Fall., *D. planipalpus* Stein, *Pegomyia hyoscyami* Curtis, *P. cepetorum* Maede; Muscidae: *Musca domestica* L.; Calliphoridae: *Calliphora erythrocephala* Meig.) (Klimaszewski 1984). In areas where crucifer crops are grown, the cabbage maggot, *D. radicum*, is one of the major hosts of *A. bilineata* (Wishart et al. 1957). The immature stages of *D. radicum* are often aggregated (Mukerji and Harcourt 1970), and several dozen larvae can be found in the root of a single cabbage plant resulting in aggregation of pupae around the roots. *A. bilineata* females oviposit on the soil near infested cabbage plants (Fuldner 1960). Newly emerged larvae seek cabbage maggot pupae in soil and chew small holes into puparia through which they enter to feed on pupae. *A. bilineata* is a solitary parasitoid and although several first-instar larvae may enter a cabbage maggot puparium, only one will complete development, the supernumerary larvae being eliminated through physical combat (L. Royer, personal observations). The first larva to enter a puparium probably has an advantage over subsequent larvae, because it has the opportunity to feed on the pupa before initiating a fight (L. Royer, personal observations; Fuldner 1960). *A. bipustulata* L. occurs sympatrically with *A. bilineata* in nature, exploiting the same host species. In this system, we may expect intra- and interspecific host discrimination to be advantageous for host-seeking larvae of *A. bilineata*.

In this paper, we present evidence of intraspecific host discrimination by the host-seeking larvae of *A. bilineata* and evidence suggesting that host discrimination is based on chemical cues. We also examine the effect of larval age and the ratio of host per larva on intraspecific host discrimination of *A. bilineata*. Finally, we determine the extent to which *A. bilineata* and *A. bipustulata* can distinguish between hosts parasitized by conspecifics and by the other species.

Materials and methods

Intraspecific host discrimination

Insect rearing

Colonies of *D. radicum* and *A. bilineata* were established using field-collected cabbage maggot puparia from Ste-Clotilde, Québec, Canada, and maintained at 20 ± 0.5°C, 70 ± 5% relative humidity (R.H.) under a 18 h light:6 h dark photoperiod. Both colonies were restocked twice each year. Cabbage maggot adults were provided with a source of water, a 10% honey solution, and a mixture of 50:50 soya flour and brewer's yeast, as well as half a rutabaga (*Brassica napus* var. *napobrassica* (L.) Reichb.) lying on 2 cm of moist sand, as an oviposition site. The oviposition sites were replaced twice each week, and cabbage maggots developed in rutabagas until pupation. All cabbage maggot puparia used in the following experiments were medium-sized (about 5 mm long and 2 mm wide), and at the beginning of the phanerocephalic stage (this stage is visually equivalent to what is ordinarily called a pupa in other insect orders, and follows the head evagination) (Fraenkel and Bhaskaran 1973).

Males and females of *A. bilineata* were reared in 35-ml cups (Solo Cup Co., model P100, Chicago, USA) containing a thin layer of moist sand (as the oviposition site) and provided with cabbage maggot third-instar larvae as a food source. Every 3–4 days, eggs were extracted from the sand by flotation and were placed individually in polyethylene capsules (Beem capsules, size 3, Ted Pella Inc., USA). Capsules were grouped in closed 35-ml cups on damp

Whatman no. 1 filter papers (R.H. ≥ 90%) until eclosion, which was monitored daily to obtain first-instar larvae of a known age. When older first-instar larvae were needed, they were kept as previously described in closed cups for the required period of time. All experiments were done under the same conditions of temperature and photoperiod as the rearing.

In all parasitism experiments, cabbage maggot puparia were placed in a 35 ml cup over 1 ml of sand moistened with 0.3 ml of distilled water and then covered with loose moist sand (2 ml of sand + 0.3 ml of distilled water). In experiments where a single cabbage maggot puparium was required, it was placed at the center of the cup; puparia were placed *c.* 1 cm apart at the cup center when two puparia were needed; otherwise, puparia were placed *c.* 2 mm from the cup wall at an equal distance from each other and oriented in the axis of the cup radius. *A. bilineata* larvae were individually placed on a 25-mm^2 piece of damp filter paper deposited on the sand surface at the cup center. Larvae that did not immediately crawl into the sand were replaced, to avoid any bias in parasitism success due to manipulation injury. Cups were capped and kept under experimental conditions for the required period of time. At the end of the exposure period, cabbage maggot puparia were extracted from the sand and the presence of *A. bilineata* larvae was checked by rendering the puparium transparent by using a transillumination unit for brightfield/darkfield.

Discrimination capability

Intraspecific host discrimination by *A. bilineata* larvae was evaluated in a choice experiment by introducing one newly emerged larva (≤24 h) in a cup where one unparasitized cabbage maggot puparium and one puparium parasitized less than 24 h earlier by a conspecific were buried in the sand. Because host discrimination could occur before or during penetration of the puparium by the larva, which may take up to 36 h (Colhoun 1953; Fuldner 1960), groups of puparia were exposed during a period of 24 and 72 h. Thirty larvae were tested in each time treatment.

To determine whether the presence of entrance holes was used by *A. bilineata* larvae to discriminate between unparasitized and parasitized hosts, one newly emerged larva was offered one intact puparium and one puparium artificially pierced with a pin at the abdominal-central extremity on the dorsal surface, where natural entrance holes usually occurred (Fuldner 1960; Royer et al. 1998). To check whether *A. bilineata* larvae used semiochemicals originating from conspecifics during host discrimination, one newly emerged larva was given a choice between one intact cabbage maggot puparium and one puparium pierced by a conspecific that was removed before it entered the puparium. Each of these experiments was performed 30 times, and puparia were examined after 24 h.

Effect of the parasitoid's age on host discrimination

The life expectancy of *A. bilineata* larvae was estimated by daily observations of larvae kept individually in polyethylene capsules (R.H. ≥ 90%) with (n = 134) and without (n = 124) cabbage maggot puparia until all larvae died. Under these conditions, where the puparium is not buried in sand, *A. bilineata* larvae do not enter host puparia. Mortality was noted daily.

We also determined the parasitism success of *A. bilineata* larvae of different ages. One unparasitized cabbage maggot puparium was buried in sand and a single larva, aged from 0 (≤24 h) to 4 days, was placed on the sand surface. The parasitism success of each individual was evaluated after 24 h. Ten larvae of each age class were grouped to calculate a percentage parasitism, and the group was considered as a replicate. Ten replicates were done.

A choice experiment was used to evaluate the effect of *A. bilineata* larval age on host discrimination. One unparasitized and one parasitized cabbage maggot puparium were offered to individual larvae of age 0 (≤24 h), 2 or 4 days for either 24 or 72 h. Thirty individuals were tested for each age and time treatment.

The effect of *A. bilineata* larval age on superparasitism when hosts were scarce (host/larva ratio = 0.1) was investigated by placing ten larvae, either 0 (≤24 h) or 2 days old, on the surface of the sand where a single unparasitized cabbage maggot puparium was buried. Puparia were examined after 72 h to determine the number of larvae that had penetrated each puparium. This experiment was replicated ten times.

Effect of the host/larva ratio on host discrimination

The effect of competitors on host discrimination was investigated by placing ten newly emerged *A. bilineata* larvae on the surface of the sand in a 35-ml cup containing 20 cabbage maggot puparia (host/larva ratio = 2). After 72 h, the puparia were examined and the number of larvae that had penetrated each cabbage maggot puparium was counted. Results of five tests were pooled.

To further explore the effect of competitors on parasitism success and discrimination decisions of *A. bilineata* larvae, ten newly emerged larvae were given access to ten cabbage maggot puparia (host/larva ratio = 1) for 1, 8, 24, 48 or 72 h. Parasitism and superparasitism levels were evaluated. Each time treatment was repeated ten times, except for the 1-h treatment which had six replicates.

Host/larva ratios lower than 1 were obtained by either decreasing host availability or increasing the number of competing larvae. The effect of host availability was studied by placing ten newly emerged *A. bilineata* larvae on the surface of the sand where one, two, four, six, eight, or ten cabbage maggot puparia were buried. The effect of parasitoid density when hosts were scarce was determined by placing 10, 20, 30 or 40 newly emerged *A. bilineata* larvae on the surface of sand that contained a single cabbage maggot puparium. Each of these experiments was replicated ten times, and puparia were examined after 72 h to determine the number of larvae that penetrated each puparium.

Interspecific discrimination

The following experiments were done in another laboratory using feral insects and slightly different rearing methods.

Insect rearing

Colonies of cabbage maggot, *A. bilineata* and *A. bipustulata* were established using field-collected cabbage maggot puparia from La Rimbaudais, Brittany (France), and maintained at 20 ± 1°C under a 16 h light:8 h dark photoperiod. Cabbage maggot rearing was similar to the procedure described in the previous section. *A. bilineata* and *A. bipustulata* adults were kept in an apparatus similar to that described by Hertveldt et al. (1984) and provided with minced beef as a food source. Eggs were incubated on damp fabric pieces in petri dishes (80–90% R.H.), and larvae were reared on cabbage maggot puparia. All experiments were done at 20 ± 1°C.

For these experiments, the parasitism procedure described above was used, except that the cabbage maggot puparia were placed over 2 ml of sand moistened with 1.0 ml of distilled water and then covered with loose moist sand (2 ml of sand + 0.3 ml of distilled water). Moreover, cups were kept in complete darkness.

Discrimination capability

The intra- and interspecific discrimination capability of both *A. bilineata* and *A. bipustulata* larvae was evaluated in a choice experiment. One newly emerged larva (≤8 h) of either *A. bilineata* or *A. bipustulata* was placed in a cup where two cabbage maggot puparia had been buried. After a period of 96 h, the parasitism success was checked, and both puparia were buried again in the sand. A second larva of either *A. bilineata* or *A. bipustulata* was placed on the sand surface and exposed for 96 h. Sixty-four replicates were performed for each possible combination of both species.

The avoidance of superparasitism and multiparasitism (multiple attacks of a host by different parasitoid species) by both *A. bilineata* and *A. bipustulata* larvae was also evaluated. One larva was given access to a single cabbage maggot puparium (96 h) and subsequently a second larva was given access to the parasitized host for an equally long period. This was done for all four combinations of the two *Aleochara* species for the first and second larva. Thirty larvae were tested for each combination.

Statistical analysis

In choice experiments, the proportions of larvae entering the two offered hosts were compared to a theoretical ratio of 50:50 using a chi-square test. The effect of treatments on a larva's choice was determined by the analysis of contingency tables fitted by log-linear models (Sokal and Rohlf 1981). Normally distributed data were analyzed using linear and curvilinear regressions, *t*-tests, and ANOVA followed by a Fisher's PLSD test, whereas Kruskal-Wallis tests followed by STP tests for multiple comparisons of means were used to analyze non-parametric data (Sokal and Rohlf 1981). When several larvae were placed in a cup, the distribution of the number of larvae per puparium in each treatment was compared to a Poisson distribution with the same mean, as an estimate of λ, using a likelihood goodness of fit (LGF) test. The actual statistical test used for each data set is indicated in the Results section.

Results

Intraspecific host discrimination

A. bilineata larvae were able to discriminate between parasitized and unparasitized hosts. When offered an unparasitized and a recently (≤24 h) parasitized cabbage maggot puparium, most larvae entered unparasitized puparia (24 h: $\chi^2 = 16.20$, $P < 0.005$; 72 h: $\chi^2 = 10.71$, $P < 0.005$), and their host choice was similar in the two time treatments (contingency table: $G = 1.05$, $P > 0.05$) (Fig. 1A). Moreover, out of the ten larvae that did not enter a puparium in the 24-h treatment, three were found piercing the cuticle of unparasitized puparia at the end of the allocated period. No such attempt was observed in the 72-h treatment.

When *A. bilineata* larvae were offered an intact and a punctured unparasitized cabbage maggot puparium for a 24-h period, significantly more larvae selected artificially pierced puparia ($\chi^2 = 3.52$, $0.05 < P < 0.10$) and puparia pierced by a conspecific ($\chi^2 = 4.17$, $P < 0.05$) than intact puparia (Fig. 1B). Moreover, most larvae penetrated the pierced puparium by using the artificial (87.5%) ($\chi^2 = 9.00$, $P < 0.005$) and the natural (80.0%) ($\chi^2 = 7.20$, $P < 0.01$) entrance holes that were already present.

Effect of the parasitoid's age on host discrimination

The life expectancy of *A. bilineata* larvae was significantly shorter in the presence of cabbage maggot puparia (5.2 ± 0.1 days) than in the absence of hosts (6.1 ± 0.2 days) ($t = 4.63$, $P < 0.0001$) (Fig. 2A).

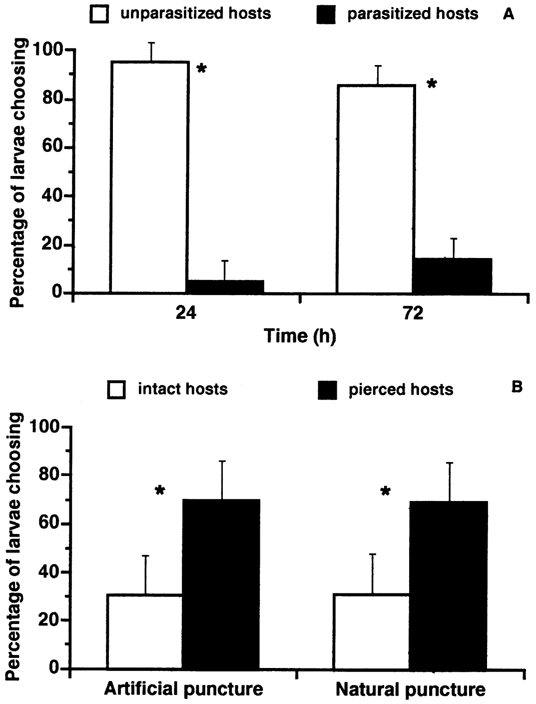

Fig. 1 Percentage of *Aleochara bilineata* larvae that entered **A** unparasitized and parasitized cabbage maggot puparia during a period of 24 h ($n = 30$) and 72 h ($n = 30$), **B** intact cabbage maggot puparia and puparia pierced artificially ($n = 30$) or by a conspecific ($n = 30$). An *asterisk* indicates that distributions of larvae differed from random at $P = 0.05$ (chi-square test); *error bars* correspond to the 95% confidence limits for percentage

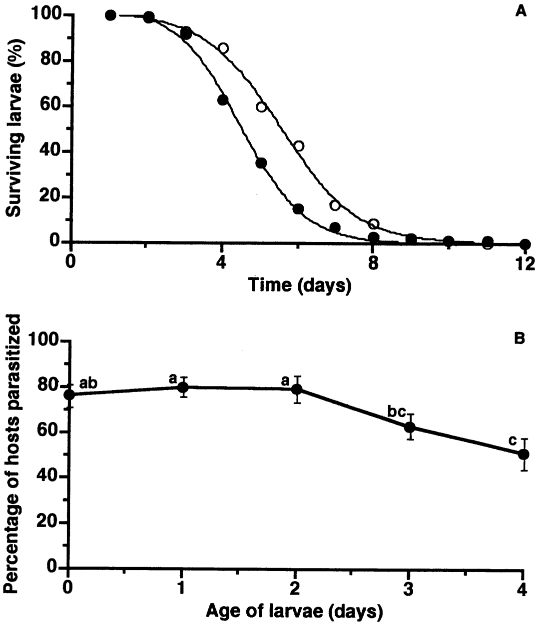

Fig. 2 **A** Survival of *A. bilineata* larvae in the absence (*open circles*) $\{y = 1.02/[1 + \exp(-5.44 + 0.99x)]$, $r^2 = 0.99$, $n = 124\}$ and in the presence of cabbage maggot puparia (*filled circles*) $\{y = 1.04/[1 + \exp(-5.23 + 1.18x)]$, $r^2 = 0.99$, $n = 134\}$. **B** Age-specific parasitism incidence (%) of cabbage maggot puparia by *A. bilineata* larvae ($n = 500$); means (±SE) followed by different *letters* are significantly different at $P = 0.05$ (ANOVA followed by a Fisher's PLSD test)

When larvae of different ages were confined individually with a single cabbage maggot puparium for 24 h, the level of parasitism remained constant at about 80% for 2 days and gradually decreased to 51% for larvae of age 4 days at the beginning of the experiment ($F = 5.03$, $P < 0.05$) (Fig. 2B).

A. bilineata larvae of age 0, 2 and 4 days offered both unparasitized and parasitized puparia significantly preferred the unparasitized puparium in all age treatments during both the 24 h (0 days old: $\chi^2 = 26.00$, $P < 0.005$; 2 days old: $\chi^2 = 16.67$, $P < 0.005$; 4 days old: $\chi^2 = 11.00$, $P < 0.005$) (Fig. 3A) and 72 h (0 days old: $\chi^2 = 27.00$ $P < 0.005$; 2 days old: $\chi^2 = 14.29$, $P < 0.005$; 4 days old: $\chi^2 = 9.78$, $P < 0.005$) choice tests (Fig. 3B). The choice of the larvae was independent of both time and age treatments (contingency table: $G = 11.30$, $P > 0.05$) (Fig. 3B). The parasitism success of *A. bilineata* larvae was not independent of the time and age treatments (contingency table: $G = 33.36$, $P < 0.0001$). The 4-day-old larvae (37%) successfully entered a puparium less frequently than 0-day-old (87%) and 2-day-old (80%) larvae during the 24-h treatment, and less frequently than all larvae (0 days old: 90%, 2 days old: 93.3%, 4 days old: 77%) of the 72-h treatments (partition of contingency table: $G = 32.65$, $P < 0.0001$).

When ten *A. bilineata* larvae were competing for a single puparium, the number of *A. bilineata* larvae per puparium increased significantly from 2.1 ± 0.3 to 3.1 ± 0.2 for larvae of age 0 and 2 days, respectively ($t = 2.39$, $P < 0.05$).

Effect of the host/larva ratio on host discrimination

During a 72-h period, the distribution of the number of larvae per puparium differed significantly from a Poisson distribution with the same mean (LGF test: $\chi^2 = 8.11$, $P < 0.025$; and variance/mean ratio = 0.68) when the ratio of hosts to larva equaled 2 (Fig. 4A), indicating that larvae did not enter puparia at random but discriminated between unparasitized and parasitized hosts. With a ratio of 1 host per larva, after 1 h, 10.0 ± 4.5% of the *A. bilineata* larvae were found on a puparium but none of these larvae had penetrated the cabbage maggot puparium (Fig. 4B), which was not even pierced. Parasitism increased significantly with time ($F = 36.34$, $P < 0.0001$), reaching a maximum value of 81% after 48 h (Fig. 4B). Low levels of superparasitism were noted in the 48- and 72-h treatments (Kruskal-Wallis test: $H = 8.78$, $P > 0.05$) (Fig. 4B), but the distributions of the larvae in the puparia were significantly different from Poisson distributions (LGF test: 8 h: $\chi^2 = 4.33$, df = 1, $P < 0.05$; 24 h: $\chi^2 = 22.44$,

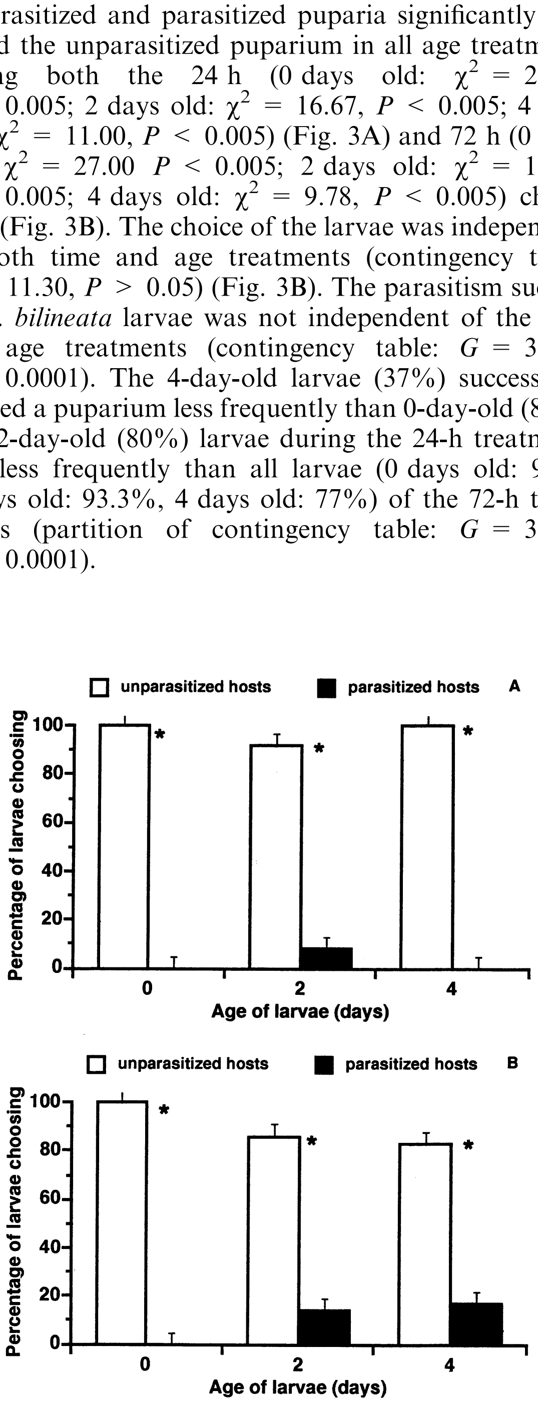

Fig. 3 Effect of age of *A. bilineata* larva on discrimination between unparasitized and parasitized cabbage maggot puparia during a period of **A** 24 ($n = 90$) and **B** 72 h ($n = 90$). An *asterisk* indicates that distributions of larvae differed from random at $P = 0.05$ (chi-square test); *error bars* correspond to the 95% confidence limits for percentage

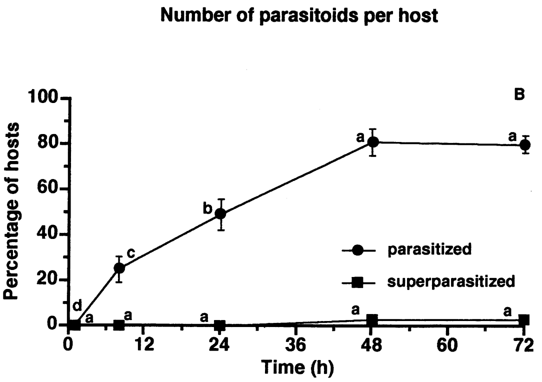

Fig. 4 **A** Comparison between observed distribution of *A. bilineata* larva in hosts and Poisson distribution ($n = 50$). **B** Effect of competitors on the incidence (%) of parasitism (*circle*) and superparasitism (*square*) ($n = 460$). Means (± SE) followed by different *letters* are significantly different at $P = 0.05$ [ANOVA followed by a Fisher's PLSD test (parasitism) and Kruskal-Wallis test followed by a STP test (superparasitism)]

$df = 2$, $P < 0.005$; 48 h: $\chi^2 = 86.68$, $df = 3$, $P < 0.005$; 72 h: $\chi^2 = 83.27$, $df = 3$, $P < 0.005$; all variance/mean ratios < 1) at each time interval.

The number of available hosts influenced host acceptance by *A. bilineata* larvae. As the ratio of hosts per larva decreased, the levels of parasitism (Kruskal-Wallis test: $H = 21.19$, $P < 0.001$) and superparasitism (Kruskal-Wallis test: $H = 34.15$, $P < 0.0001$) increased significantly (Fig. 5A). At low ratios of host/larva (0.1 and 0.2), no significant difference was found between a Poisson distribution with the same mean and the experimental outcome (LGF both $P > 0.05$), whereas at high ratios of host/larva (>0.4) the experimental distributions deviated significantly from Poisson distributions (LGF all $P < 0.005$; all variance/mean ratios<1). The number of larvae per puparium was significantly higher in treatments with a ratio of hosts per larva of 0.1 and 0.2 than in those with higher hosts/larva ratios (Kruskal-Wallis test: $H = 32.89$, $P < 0.0001$) (Fig. 5B).

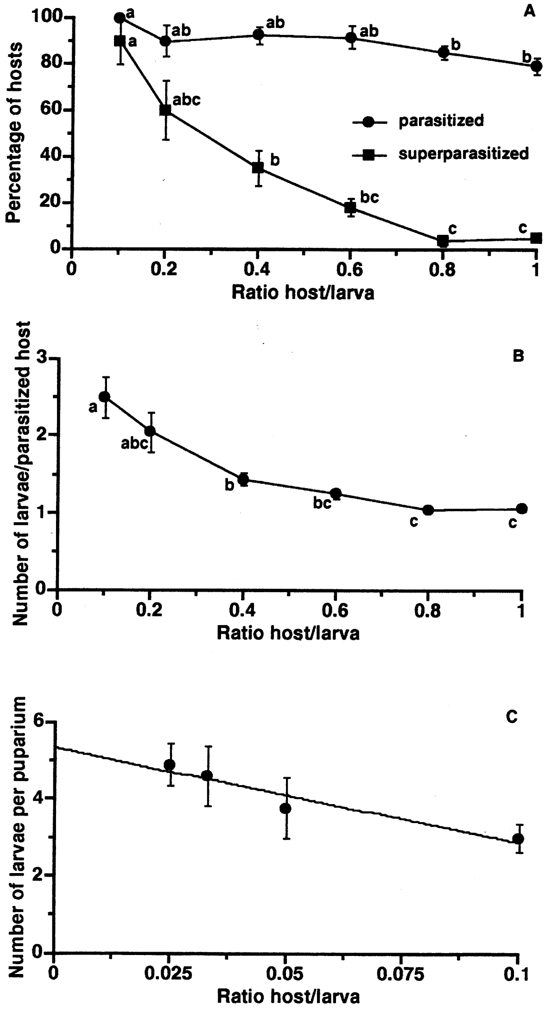

Fig. 5 Effect of the ratio of hosts to larva **A** on the incidence (%) of parasitism (*circle*) and superparasitism (*square*) ($n = 600$), and **B** on the number of larvae per parasitized puparium ($n = 600$). Means ($\pm$SE) followed by different *letters* are significantly different at $P = 0.05$ (Kruskal-Wallis test followed by a STP test). **C** Effect of the ratio of hosts per larva on the number of larvae per puparium when a single host was available ($n = 1000$)

When the number of available hosts was limited, the number of newly emerged larvae that penetrated a cabbage maggot puparium during a 72 h period increased linearly as the ratio of hosts per larva decreased ($y = 5.36 - 24.56x$; $r^2 = 0.13$; $P_{\text{slope}} < 0.05$) (Fig. 5C).

Interspecific discrimination

Larvae of both *A. bilineata* and *A. bipustulata* preferentially penetrated the unparasitized puparia when they were given access to an unparasitized puparium and a puparium parasitized (≤96 h) by a conspecific (*A. bilineata*: $\chi^2 = 23.17$, $P < 0.005$; *A. bipustulata*: $\chi^2 = 21.33$, $P < 0.005$) (Fig. 6A) or by a larva of the other species (*A. bilineata*: $\chi^2 = 18.75$, $P < 0.005$; *A. bipustulata*: $\chi^2 = 14.29$, $P < 0.005$) (Fig. 6B). Moreover, there was no difference in the choice distribution between the two species in intraspecific (LGF test: $\chi^2 = 0.12$, $P > 0.05$) (Fig. 6A) and interspecific ($\chi^2 = 0.12$, $P > 0.05$) (Fig. 6B) situations, as well as between intra- and interspecific choice (LGF test: *A. bilineata*, $\chi^2 = 0.37$, $P > 0.05$; *A. bipustulata*, $\chi^2 = 0.18$, $P > 0.05$) for each species (Fig. 6A,B).

A. bilineata larva having access to a single parasitized puparium penetrated more frequently the puparia parasitized by an *A. bipustulata* larva than by a conspecific (LGF test: $\chi^2 = 16.88$, $P < 0.005$); whereas *A. bipustulata* larvae penetrated both types of parasitized puparia in the same proportions (LGF test: $\chi^2 = 2.54$, $P > 0.05$) (Fig. 6C). Therefore, *A. bilineata* larvae multiparasitized more frequently than *A. bipustulata* larvae when there was interspecific competition (LGF test: $\chi^2 = 6.67$, $P < 0.025$), although no difference in superparasitism was observed between the two *Aleochara* species when there was only intraspecific competition (LGF test: $\chi^2 = 1.88$, $P > 0.05$).

Discussion

In contrast to adult female parasitoids, *A. bilineata* and *A. bipustulata* larvae, seeking subterranean hosts, accept a host only once during their lifetime. The "decision" to accept a particular host thus determines the future fitness of the larva, because in such solitary parasitoids, only one larva can develop in each cabbage maggot puparium. Entrance into an already parasitized host results in a fight to the death. Our results clearly demonstrate that *A. bilineata* and *A. bipustulata* larvae discriminate between unparasitized and parasitized hosts in laboratory experiments.

A. bilineata larvae preferred both artificially and naturally punctured puparia that did not contain a larva compared to intact puparia in a choice experiment. Wright and Müller (1989) suggested that *Aleochara* sp.

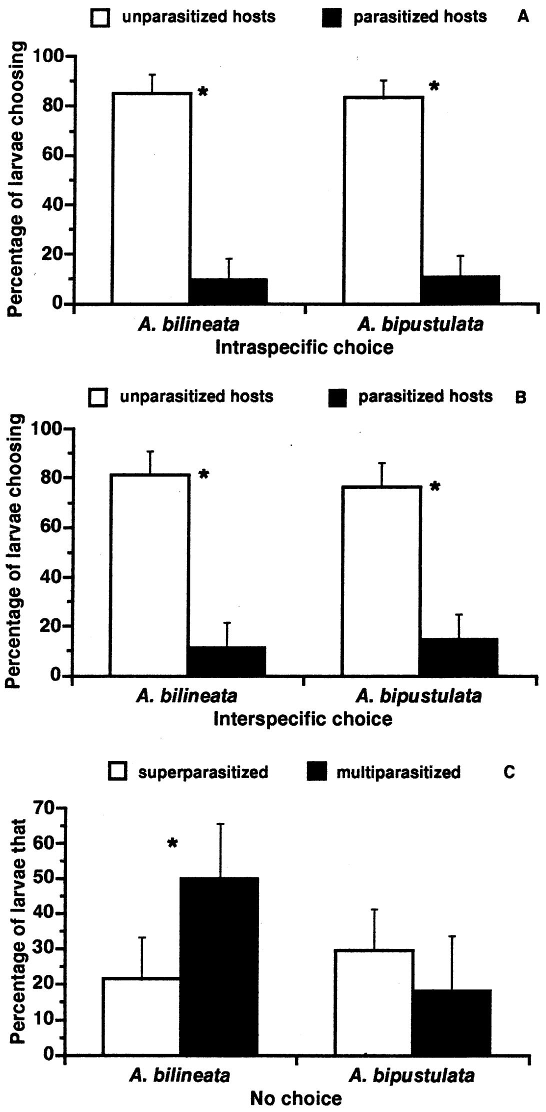

Fig. 6 Percentage of *A. bilineata* and *A. bipustulata* larvae that entered unparasitized cabbage maggot puparia and puparia parasitized by either **A** a conspecific ($n = 64$) or **B** a larva of the other species ($n = 64$) in a choice experiment. An *asterisk* indicates that distributions of larvae differed from random at $P = 0.05$ (chi-square test). **C** Percentage of *A. bilineata* and *A. bipustulata* larvae that entered cabbage maggot puparia already parasitized by a larva of either their own species ($n = 30$) or the other species ($n = 30$) in a no-choice experiment. An *asterisk* indicates that percentages of superparasitism and multiparasitism for the given species differed at $P = 0.05$ (chi-square test); *error bars* correspond to the 95% confidence limits for percentage

larvae probably use chemical cues to find hosts. The presence of holes may increase the emanation of semiochemicals or CO_2 from cabbage maggot puparia, which could explain the observed preference. Moreover, the majority of the larvae entered the pierced puparia by the available entrance holes. As *A. bilineata* larvae explore the puparium surface before piercing the cuticle (Fuldner 1960), they may use visual and/or tactile cues to find the entrance hole. The use of a preexisting hole may save the time and energy required to penetrate puparia where no larva is present, as the process of gnawing the puparium cuticle lasts several hours (Colhoun 1953; Fuldner 1960). The first *A. bilineata* larva to enter a puparium usually gnaws its entrance hole at the abdominal-central extremity of the puparium's dorsal surface (Fuldner 1960; Royer et al. 1998) and stays in the vicinity of its hole, feeding on the cabbage maggot pupa (Fuldner 1960). The few *A. bilineata* larvae that superparasitized when offered unparasitized and parasitized hosts always pierced the puparium on the ventral surface. Such behavior would be adaptive as it would enable the superparasitizing larva to feed, and therefore replenish energy resources, before confronting the first occupier. Colhoun (1953) and Fuldner (1960) also pointed out that the frequency of entrance holes on the ventral surface tended to increase when puparia were parasitized by several larvae. The fact that *A. bilineata* larvae entered by the available hole therefore indicates that these larvae determined that these puparia did not contain conspecifics, and that visual and/or tactile cues are secondary in the recognition of parasitized hosts.

The detection of a conspecific inside the puparium may be based on olfactory cues, as *A. bilineata* larvae possess well-developed antennae that bear several trichoid and basiconic sensillae (L. Royer, unpublished work). However, salivary gland secretions of *A. bilineata* larvae were not the semiochemical source, since larvae preferred unparasitized puparia pierced by conspecifics to intact puparia. These results also rule out the possibility that *A. bilineata* larvae deposit a marking substance on the puparium surface during their exploration. Inside puparia, *A. bilineata* larvae feed on the cabbage maggot pupa by piercing the cuticle to absorb haemolymph (Fuldner 1960) which usually does not exude outside the puparium. The odour of the haemolymph (van Lenteren 1981) is one possible semiochemical source in this system, although injuries heal rapidly (Fuldner 1960). Feeding activity may also change the physiological condition of cabbage maggot pupae, changes that may be detected by parasitoids (van Lenteren 1981). Moreover, *A. bilineata* larvae close the entrance hole with excrement (Fuldner 1960). Daily observations of 16 larvae indicated that they began to fill the entrance hole soon after penetration and required 72–96 h to complete the plug under our experimental conditions (L. Royer, unpublished work). Although no entrance hole plug was completed during choice tests between unparasitized hosts and hosts parasitized by conspecifics, most were begun. This entrance hole plug may function as an external marker. Therefore, host discrimination in *A. bilineata* larvae may be based on the detection of semiochemicals originating from the *A. bilineata* larva itself and its activity. However, further studies are needed to determine the exact sources of these semiochemicals.

The life expectancy of *A. bilineata* was shorter in the presence than in the absence of hosts. *A. bilineata* larvae live on their energy reserves until they penetrate cabbage maggot puparia (Fuldner 1960). The observed decrease in survival of *A. bilineata* larvae in the presence of a host may be due to an increase in the larva's searching activity, leading to a more rapid depletion of energy reserves. In hymenopteran parasitoid females, host-associated odor can elicit intensive searching activity (Noldus et al. 1991; Frenoy et al. 1992; Hemerik et al. 1993), although the effect on longevity was not reported.

The daily parasitism success decreased as *A. bilineata* larvae became older, 4-day-old individuals parasitizing only 51% of the available puparia. The survival curve in the presence of hosts indicated that only 27.6% of the larvae died from the 4th to the 5th days after eclosion. Although the mortality may be higher in soil than in polyethylene capsules, we believe that the low parasitism success of 4-day-old individuals was not only explained by the mortality of *A. bilineata* larvae. Fuldner (1960) observed that *A. bilineata* larvae decrease locomotion activity 36 h after eclosion. As the volume of soil (3 cm^3) to explore was large compared to the parasitoid size (body length: 1 mm; L. Royer, personal observations), slow-moving larvae may not find the puparium during the allocated 24 h period. By its possible effect on the larval responsiveness to host semiochemicals, the senescence of olfactory receptors may also play an indirect role in the diminution of locomotion activity. The parasitism success of old larvae was also low during the 24-h choice experiment, whereas it was comparable to those of younger larvae when the treatment lasted 72 h. Therefore, the diminution in the parasitism success of older larvae may result from the combined effect of mortality and decreased locomotion in old larvae.

Models based on data obtained in hymenopteran parasitoids predict that parasitoids with short life expectancy should superparasitize more frequently than those with long life expectancy (Roitberg et al. 1992). Our results were in agreement with these models only when hosts were scarce (host/larva ratio = 0.1). When hosts were abundant (host/larva ratio = 2), the age of *A. bilineata* larvae did not significantly affect host discrimination, suggesting that old larvae continue to search for unparasitized puparia despite a low life expectancy. However, when hosts were limited (host/larva ratio = 0.1), a significant increase in superparasitism was noted as the larvae of *A. bilineata* aged (0 days old, 2.1 larvae/host; 2 days old, 3.1 larvae/host).

The presence of conspecifics did not affect superparasitism in *A. bilineata* larvae when the ratio of host per larva was greater than or equal to one. However, the parasitism success of newly emerged larvae in the experiments where several larvae were searching simultaneously was rather low compared with results in other experiments. Behavioral interactions between searching parasitoids were shown to reduce the searching efficiency of *Venturia canescens* (Grav.) females (Ridout 1981; Hughes et al. 1994), a phenomenon referred to as mutual interference (Free et al. 1977). The encounter of two foraging *A. bilineata* larvae usually results in the death of one larva (Fuldner 1960), but the high parasitism levels after 48 and 72 h suggested a low mortality and therefore probably a low level of mutual interference. However, the number of unparasitized hosts in the patch gradually decreased as *A. bilineata* larvae found and entered unparasitized hosts, and this decreasing proportion of unparasitized hosts would increase searching time. The gradual increase in patch exploitation, called pseudo-interference (Free et al. 1977), may explain why only 49% of the puparia were parasitized in the 24-h treatment, where several larvae simultaneously searched on a patch containing previously unparasitized puparia, while similar experiments with only one pupa and one larva resulted in almost 80% of parasitism after 24 h. Therefore, as long as host availability was relatively high the presence of competitors did not affect the degree of superparasitism avoidance in *A. bilineata* larvae, as it does in Hymenoptera females (Visser et al. 1990, 1992; Michaud and Mackauer 1995; Visser 1995), although the larva's searching efficiency may decrease as the patch exploitation increases.

Superparasitism significantly increased as the ratio of host per larva decreased, suggesting that the availability of hosts plays an important role in the decision to superparasitize. When the availability of hosts continued to decrease, the larvae more readily accepted parasitized hosts. *A. bilineata* larvae are short-lived and the probability of finding another host patch is probably so low that patch leaving is not an alternative, as it is for hymenopteran parasitoids (Nelson and Roitberg 1995). Even at low host density, *A. bilineata* larvae continued to discriminate, and most larvae died before entering a parasitized puparium. For example, only 12% of the larvae entered a puparium in the treatment of 0.025 hosts per larva lasting 72 h, a period of time that corresponds to a large part of the larva's total searching time. But the observed increase in superparasitism as the larvae of *A. bilineata* aged when hosts were scarce (host/larva ratio = 0.1) suggests that the short life expectancy may lower the threshold at which larvae start to superparasitize.

Both *Aleochara* species studied avoided multiparasitism when given the choice between unparasitized and parasitized hosts, thus showing interspecific host discrimination, a behavior rarely observed (Bakker et al. 1985). This also suggests that the semiochemical used to discriminate between parasitized and unparasitized hosts may not be species-specific. In contrast to *A. bipustulata*, *A. bilineata* larvae entered puparia parasitized by *A. bipustulata* larvae more frequently than puparia parasitized by conspecifics when a single parasitized puparium was available for a 96-h period. Interspecific host discrimination has been found mostly in systems where one of the species had a marked competitive advantage (McBrien and Mackauer 1991; Scholz and Höller 1992). Our results do not show whether the observed high multiparasitism indicated a competitive advantage of *A. bilineata*. This possibility should be explored in choice experiments. Van Baaren et al. (1994) found that *Anaphes victus* Huber preferred to oviposit in hosts parasitized by their own species rather than by *A. listronoti* Huber in choice experiments, reflecting the slight competitive advantage of *A. listronoti*. Multiparasitism with another *D. radicum* parasitoid, *Trybliographa rapae* (Westw.) (Hymenoptera: Eucolidae), is also avoided by *Aleochara bilineata* larvae, but only when their probability of winning the competition is low (Reader and Jones 1990). We believe that the different rearing methods for *A. bilineata* adults had no effect on the conclusions of the study, since results on intraspecific discrimination in multiparasitism experiments were comparable to those obtained earlier.

Feener and Brown (1997) suggested that host discrimination could be less advantageous for host-seeking larvae than female parasitoids, because host encounter rates for such larvae are probably low. Our results indicate that *A. bilineata* and *A. bipustulata* larvae can discriminate, an adaptive behavior when host encounter rate is high because of the contagious distribution of the host, as in *D. radicum* in crucifer fields (Mukerji and Harcourt 1970). Our results also suggest that host-seeking *A. bilineata* larvae used semiochemicals that may increase the probability of encountering hosts. This is the first demonstration of intraspecific host discrimination behavior in a host-seeking parasitoid larva, but we expect this behavior to be widespread when suitable conditions are met.

Host-seeking parasitoid larvae face different ecological constraints from ovipositing parasitoid females, but in some respects they are functionally similar. In both cases, selection acts to maximize genetic representation in future generations. For a host-seeking larva, the first step in achieving genetic representation is its survival until adulthood by selecting a single suitable host in the available searching time. While host discrimination is adaptive to maximize the lifetime fitness of an individual, a switch to superparasitism is also adaptive when unparasitized hosts are scarce and the second larva has a reasonable chance of winning the contest. We have also demonstrated an increase in superparasitism as host availability decreases. Superparasitism may be a larva's best option when the probability of winning the competition is higher than the probability of finding an unparasitized host before dying.

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