CONSPECIFIC AND HETEROSPECIFIC INTERACTIONS IN TWO SPECIES OF APHID PARASITOIDS, *APHIDIUS ERVI* AND *APHELINUS ASYCHIS* (HYMENOPTERA: APHIDIIDAE, APHELINIDAE)

by

Baorong Barry Bai
B.Sc., Northwestern Agricultural University, 1982
M.Sc., University of California, Riverside, 1986

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(HYMENOPTERA: APHIDIIDAE, APHELINIDAE)

Examining Committee:

Chairman: Dr. L.J. Albright, Professor

Dr. M. Mackauer, Professor,
Senior Supervisor,
Dept. of Biological Sciences, SFU

Dr. B. Roitberg, Associate Professor,
Dept. Biological Sciences, SFU

Dr. L. Dill, Professor,
Dept. Biological Sciences, SFU

Dr. B.D. Frazer, Research Scientist,
Agriculture Canada, Vancouver, B.C.
Public Examiner

Dr. S. Smith, Assistant Professor,
Faculty of Forestry, University of Toronto
External Examiner

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Title of Thesis/Project/Extended Essay

Conspecific and heterospecific interactions in two species of

aphid parasitoids, *Aphidius ervi* and *Aphelinus asychis*

(Hymenoptera: Aphidiidae, Aphelinidae)

Author:

(signature)

Baorong Barry Bai

(name)

Dec. 19, 1991

(date)

ABSTRACT

Competitive interactions in two solitary species of endoparasitoids, *Aphidius ervi* Haliday and *Aphelinus asychis* Walker (Hymenoptera: Aphidiidae, Aphelinidae), were studied in the laboratory. Both species are parasitoids of the pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae). Females of *A. ervi* invest little in each egg, and have more eggs available in their ovaries, than do females of *A. asychis*. Such a difference in reproductive biology is expected to make *A. ervi* less and *A. asychis* more selective in their host choice, which may vary with host quality. I tested this hypothesis by providing females of each species with four kinds of hosts: unparasitized, conspecific-parasitized, heterospecific-parasitized, and self-parasitized.

When unparasitized and parasitized hosts were available, both species preferred to oviposit in unparasitized hosts. This pattern of host discrimination is adaptive because oviposition in a parasitized host results in superparasitism and larval competition. When provided with parasitized hosts only, *A. ervi* did not reject newly parasitized hosts but rejected aphids parasitized ≥ 24 h earlier. *Aphelinus asychis* rejected all parasitized hosts, independent of the age of the egg or larva inside. The difference in oviposition decisions by these two species reflects their respective reproductive strategies. *Aphidius ervi* is time- but not egg-limited and tends to be relatively non-selective about hosts. By contrast, *A. asychis* is egg-limited and tends to be selective in oviposition. Both species used the ovipositor to detect internal cues when discriminating between hosts. *Aphelinus asychis* did not discriminate between self- and conspecific-parasitized aphids. However, patterns of oviposition or host feeding in this species were influenced by host density.

When superparasitism occurred, the first hatched "oldest" larva usually won conspecific contests. In heterospecific competition, *A. ervi* was superior to *A. asychis*. *Aphidius ervi* eliminated competitors by physical combat in the early first instar and

by physiological suppression in later stages; *A. asychis* used physiological suppression in all larval stages. In *A. ervi*, superparasitism did not affect the survivor's development time, but it had a positive effect on adult body size, probably due to the increased growth potential of superparasitized aphids.

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CHAPTER I

GENERAL INTRODUCTION

Competition is a common phenomenon among insect parasitoids (DeBach and Sundby 1963; Force 1974, 1985; Price 1980; Luck and Podoler 1985; Mackauer and Kambhampati 1986; Price *et al.* 1986; Steinberg *et al.* 1987). Parasitic insects that share the same host species as their common food resource [usually called a 'parasitoid guild' (Ehler 1979; Vinson 1990)] often compete either directly or indirectly for food, living space, and other resources at the adult stage and, more severely, in the larval stages. Interactions within a parasitoid guild can involve members of the same species (conspecifics) or members of different species (heterospecifics). Various mechanisms have evolved that govern the interactions and coexistence of members in parasitoid guilds. Some mechanisms involve adult females avoiding oviposition into already parasitized hosts through host discrimination (van Lenteren 1981). This avoidance eliminates or reduces any possibilities of larval competition for hosts. Alternatively, females may inject a toxin into previously parasitized hosts at the time of oviposition, thereby killing the immatures already present in the hosts, to achieve the same 'avoidance' effect (Mackauer 1990). Other mechanisms involve immature stages, for example, larvae may directly engage in physical combat, or the development of one larva changes the host's physiological or biochemical conditions so that the host becomes unsuitable for the development of other larvae (physiological suppression).

Insect parasitoids as a group have received considerable attention from entomologists and biologists due to their economic and ecological importance. Throughout the history of biological control, many species have been introduced into a new environment for pest control (Clausen *et al.* 1977). In almost all the successful biological control programs, introduced parasitoids interacted among themselves or with indigenous species during colonization. Once established, they became part of the natural enemy complex of the target pest (Dowden 1962; Clausen *et al.* 1977). Due to their short generation time and easy laboratory cultivation, insect parasitoids and their hosts also provide a useful system for evolutionary and ecological studies, such as clutch size and oviposition decisions (Iwasa *et al.* 1984; Godfray 1987; Mangel and Roitberg 1989), population regulation (May and Hassel 1981), and trophic and food web interactions (Price 1984). Studies of evolutionary biology in insect parasitoids have led to a better understanding in a number of behavioral ecology fields, including host selection (Roitberg 1991) and reproductive strategies (Price 1975; Blackburn 1991b).

Parasitoids of the genera *Aphidius* (Hymenoptera: Aphidiidae) and *Aphelinus* (Hymenoptera: Aphelinidae) are two important primary parasitoid groups that specifically attack aphids (Mackauer and Finlayson 1967; Stary 1988). Some of the common species in these two genera have been used or introduced as biological control agents for aphid pests in North America (Hagen and van den Bosch 1968). As in other protelean parasites (Askew 1971), these parasitoids have a distinct life style in different life-stages. The adult wasps are free living; females select and oviposit in a host and therefore decide where their offspring will develop. The immature stages are parasitic but not involved in host choice. Thus, host selection and host exploitation are separated into different life stages in these animals. In solitary species, only one individual normally completes its development to the adult stage in each host. Supernumerary larvae, if there are any, are eliminated either by some form of physiological suppression or by direct physical combat among the larvae. This is in contrast to gregarious species, where a variable number of larvae may develop in a single host (e.g., Sato *et al.* 1986; LeMasurier 1987).

Because parasitoid immature stages are confined in or on a host which represents the complete nutritional and physiological environment during larval development, host choice made by an ovipositing female can have a profound effect on a larva's survival and growth. A high quality host, such as a healthy host with an abundance of resources, ensures the successful development and survival of the larva; a low quality host, for example, a host of small size or one already being exploited by other parasitoids, reduces the larva's chances of survival or growth. Therefore, competitive interactions in these species involve not only larval stages in which individuals may directly fight for possession of the host, but also adult stages where females determine in which hosts they lay eggs, and thus whether the progeny must compete for resources.

The main objective of this thesis was to study conspecific and heterospecific interactions in two solitary species of aphid parasitoids, and to provide evolutionary explanations for any differences in their host selection behavior. Such studies were intended to elucidate not only how parasitoids interact, but also why they interact in certain ways. The how (first) part investigates mechanisms of parasitoid interactions at either the adult or the immature stages. The why (second) part explains the possible reasons underlying certain behavior patterns in the parasitoids' host selection, and thus can provide a tool for general predictions which may have broader implications. Because host selection by adult females may have important fitness consequences for the offspring, especially in solitary parasitoid species, I first examined the interactions at this stage, i.e., how females interacted (indirectly) with others when ovipositing. Some specific questions addressed were (1) could a female parasitoid discriminate between healthy, unparasitized hosts and those that were parasitized either by herself, or a conspecific, or a member of a different species, and so avoid oviposition into already exploited hosts, thus eliminating the chance of larval competition? (2) What factors, such as the identity of competing species, time or age advantage of the competitors, influenced a female's host selection decision? (3) Would a female's oviposition decision change with the probability of her offspring's survival and her reproductive strategy? A reproductive strategy here refers to a set of rules that a parasitoid adopts regarding reproduction. In this thesis, I examined particularly three such rules (factors), i.e., the time and resources invested in each egg, the ovary capacity, and the ability to recover resources invested in eggs by oosorption.

For studies of interactions among immature stages, I determined the mechanisms and consequences of larval competition, both conspecific and heterospecific, if more than one egg was laid in a host. Questions raised in this part of the study included (1) what factors influence the outcome of larval competition--the sequence of attack, time intervals between ovipositions, or the species involved? (2) What mechanisms are used by each species to eliminate competitors, either conspecifics or heterospecifics? Do these mechanisms change with the development of the larvae or with the species involved? The last part of the thesis addressed the question of why these two parasitoid species had different host selection behaviors through studies of their reproductive biologies.

The thesis contains nine chapters, with the first and the last serving as a general introduction and a general conclusion. Chapter II describes general methods and parasitoid biology, which serves as background information for the rest of the thesis. Chapter III addresses one of the important aspects in *Aphelinus* biology, i.e., host feeding and oviposition patterns as influenced by different host densities. This chapter also establishes a foundation for basic techniques such as parasitoid handling, oviposition determination and host dissection, which are useful for the understanding and conduction of other experiments. Chapters IV and V examine aspects of conspecific interactions in each of the two species and address questions of whether the two species discriminate between different host types (unparasitized, self-parasitized, conspecific-parasitized), what mechanisms each species uses in host discrimination and larval competition and, furthermore, which individual wins the competition. Chapter VI deals with heterospecific interactions and addresses similar questions by examining two species interactions. Chapter VII determines the effects of superparasitism on fitness components, such as developmental rate and adult size, in *A. ervi*. This is an extension of Chapters IV and V and further examines the consequences of superparasitism in terms of its effect on the survivor. The last main chapter (Chapter VIII) analyzes the differences in the wasps' oviposition decisions by examining reproductive strategies employed by the two species, and thereby provides an evolutionary explanation for different host selection behaviors.

CHAPTER II

GENERAL METHODS AND PARASITOID BIOLOGY

Members of the genera *Aphidius* and *Aphelinus* have similar life cycles despite the fact that the two groups belong to different hymenopteran families. Like many other hymenopterans, both groups are bisexual and arrhenotokous, i.e., fertilized eggs develop into females and unfertilized eggs into males. The two species, *Aphidius ervi* Haliday and *Aphelinus asychis* Walker (=*semiflavus* auctt. nec Howard), used in the present study, are part of a larger parasitoid complex on pea aphids in North America. This complex includes both indigenous and introduced species (Mackauer and Stary 1967; Gonzalez *et al.* 1978; Aeschlimann 1981; Mackauer and Kambhampati 1986). Although they attack all stages of pea aphids including adults, these species prefer to parasitize hosts in their young, e.g., second to third, nymphal instars (Gerling *et al.* 1990, McBrien 1991). The female wasp inserts her ovipositor into a host and lays a single egg in the aphid hemocoele. Durations of ovipositions are significantly different between the two species (see Chapters IV and VIII). Eggs of both species hatch in three to three and a half days at 21°C and immatures grow by feeding on the host's internal contents. Parasitized aphids provide nutrients and a living environment for the parasitoid, but are not killed until the later stages. Pupation takes place inside the aphid, which becomes a mummy after its entire body contents have been consumed, leaving only the integument intact. After pupation, the parasitoid cuts an emergence hole in the mummy and leaves to begin the free-living adult stage. Besides causing mortality by oviposition, *A. asychis* females can also cause aphid death by direct host-feeding, which is believed to provide protein sources for parasitoid egg maturation (Flanders 1942, 1950). Generation time is temperature dependent. At 21°C, developmental time from egg to adult is approximately two weeks for both species. All the immature stages as well as adults of *A. ervi* can be easily distinguished from those of *A. asychis* based on their morphology (Mackauer and Finlayson 1967). This makes studies of heterospecific competition easier, because identification of competitors can be certain even in early stages (see Chapter VI).

A laboratory culture of *A. ervi* was established from mummified pea aphids, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), collected from an alfalfa field near Kamloops, British Columbia. This culture was replenished with newly collected field materials in 1989. A colony of *A. asychis* was started with specimens, obtained from R. D. Eikenbary, that had been introduced originally from Europe for the biological control of the greenbug, *Schizaphis graminum* Rondani, (Jackson and Eikenbary 1971). Both parasitoid species were reared on pea aphids feeding on broad beans, *Vicia faba* L. cv. 'Broad Windsor'. A synchronous stock colony of *A. ervi* was prepared, by exposing female wasps to third instar nymphs of pea aphids for 4 to 6 h, once each week or every other week depending on experimental demands. Stock colonies of *A. asychis* were maintained on mixed aged pea aphids in screened Plexiglass cages. The parasitoids used in experiments were provided with honey water as a food source upon emergence. Unless otherwise stated, all stock colonies and experimental materials were reared at 21±2°C, 55±10% RH, and continuous light (hereafter called laboratory conditions).

Colonies of pea aphids were maintained on broad beans. In greenhouses, beans can be easily potted in 'garden mix' soil. The aphid colony, under laboratory conditions, consisted almost entirely of apterous (wingless) females which reproduce parthenogenetically, viz., females reproduce by giving birth to young (viviparous). The aphid goes through four nymphal instars before molting to the adult stage. To obtain synchronous colonies of pea aphids for both parasitoid maintainence and experiments, a cohort of adult aphids was transferred to a pot of bean plants and confined there for one day. Any offspring produced were removed at the end of the day and put on fresh host plants. These offspring were thereafter maintained in the laboratory until use.

CHAPTER III

PATTERNS OF HOST FEEDING AND OVIPOSITION IN *APHELINUS ASYCHIS* AT DIFFERENT APHID DENSITIES

Introduction

Host density may have an important effect on population processes and, by implication, on the ability of a hymenopterous parasitoid to control its host (Hassell 1978; Stiling 1988; Walde & Murdoch 1988). Much of the research has focused on density-dependent changes in parasitoid fecundity and searching behavior. However, in many species of hymenopterous parasitoids, females cause significant additional mortality by feeding on their hosts (e. g., Flanders 1935, 1953; DeBach, 1943; Bartlett 1964; Cate *et al.* 1974, 1977; Sandlan 1979; van Lenteren *et al.* 1980; Arakawa 1982; Viggiani 1984; Walter 1988). The influence of host density on parasitoid oviposition and feeding decisions is less well understood. Jervis & Kidd (1986) recently examined oviposition and feeding patterns in parasitic wasps with regard to host availability and energy requirements. They suggested that these patterns are influenced by the parasitoid's adaptive response to host scarcity, such as regulation of oogenesis and oosorption.

In this chapter I examine oviposition and feeding decisions by the parasitoid *Aphelinus asychis* at different densities of its host, the pea aphid, under controlled laboratory conditions. As the first chapter of the thesis, this will also familiarize the reader with the parasitoid in terms of its basic biology, recognition, handling and dissection techniques.

When foraging for hosts, females either feed on or deposit an egg into a suitable aphid. *Aphelinus* females are synovigenic (maturing eggs continously through adult life) and are capable of adjusting their age-specific fecundity schedule in accordance with host density. Variations in the egg maturation rate and resorption of mature eggs when hosts are in short supply (Chapter VIII) may result in a shift of available eggs from an earlier to a later reproductive age (Mackauer, 1982). I show that a female's choice between feeding and egg deposition is dependent on the duration of and the time since her last feeding, factors that determine her physiological status. Host density affects the number of eggs laid but not in any obvious way the pattern of host-feeding, except at very low aphid numbers. I discuss the results with reference to theories about reproductive strategies among the parasitic Hymenoptera. I also tested a fast method for parasitism confirmation at the beginning of the experiments.

Materials and Methods

Parasitoid biology

Aphelinus asychis is a protelean parasitoid of aphids, about 1 mm in length. Females attack a broad range of aphid species including the pea aphid (Wilbert 1964). The larva develops as a solitary endoparasite. Pupation takes place inside the dead host, which is transformed into a bluish-black mummy. *Aphelinus* females feed on host fluids that exude from wounds made with the ovipositor, often causing immediate death of the host (Boyle & Barrows 1978). Feeding attacks are considerably longer than ovipositional attacks (Hartley 1922; Wilbert 1964; Hamilton 1973; Boyle & Barrows 1978; Collins *et al.* 1981). The oviposition success of *A. asychis* females is influenced by aphid size and instar-specific defense reactions (Gerling *et al.* 1990). I used second-instar pea aphids in all experiments, as this instar is readily attacked by *A. asychis*. All experimental wasps were 2 to 3 days old. To ensure that females were mated and had experience in handling hosts, I caged them together with males and aphids prior to the tests.

Host dissection

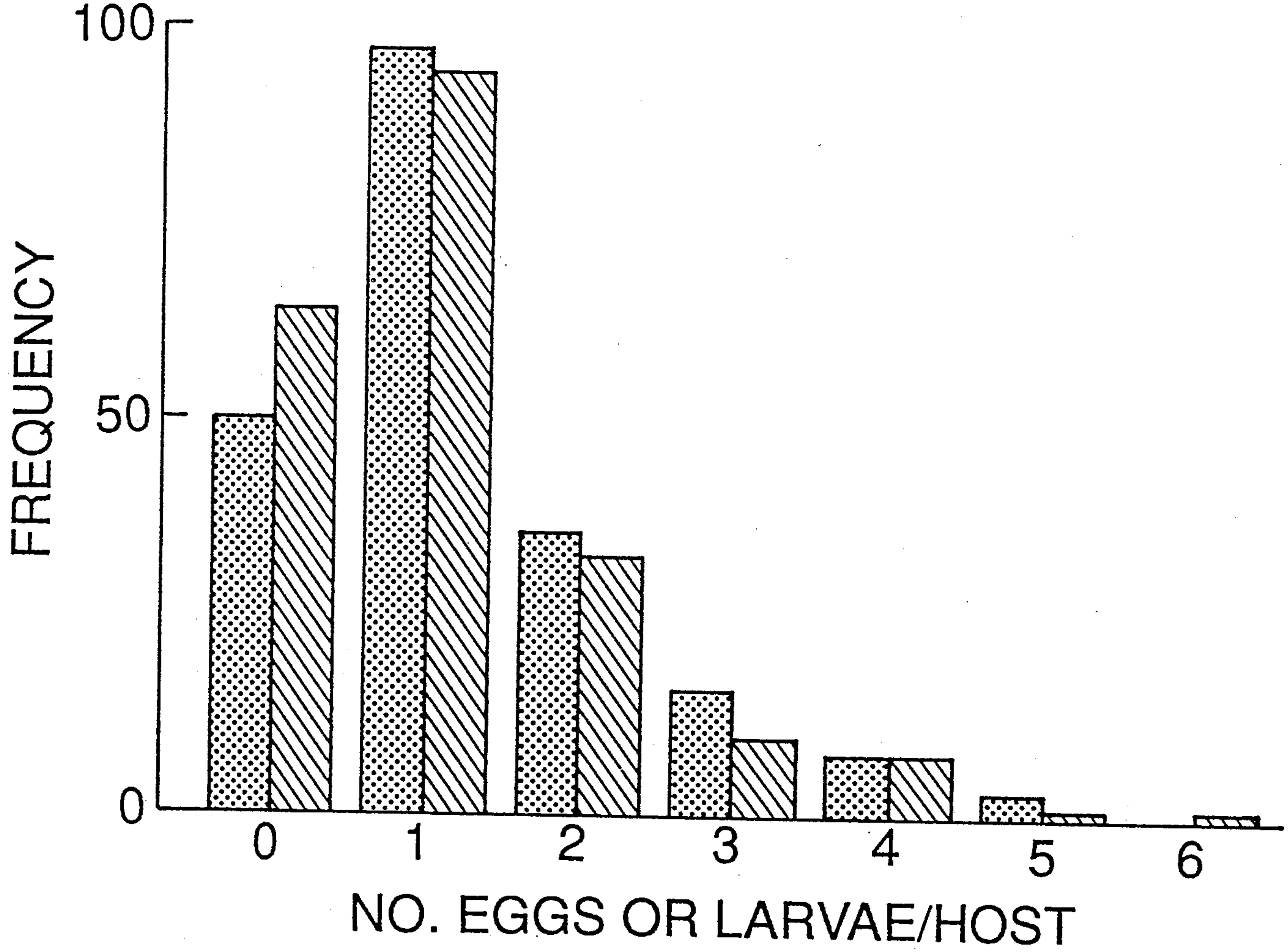
In a preliminary experiment, I determined if parasitism could be detected reliably by the dissection of aphids immediately after they had been stung. I caged six *A. asychis* females with 20 pea aphids in an 8.5 by 3.0 cm screened minicage (Mackauer & Bisdee 1965) that contained a bean stalk. After 24 h, the wasps were removed and the aphids were divided into two groups of approximately equal size. Aphids in the first group were dissected immediately in distilled water under a stereomicroscope; aphids in the second group were returned to the cage and dissected after 3 days when the egg had hatched into the first instar larva. I counted the number of parasitoid eggs and larvae found in each aphid.

Dissected aphids contained up to 6 parasitoid eggs (Fig. 3.1). 76.2% (N = 210) of the aphids dissected within 24 h after an ovipositional attack were parasitized, as compared with 69.7% (N = 211) of those that were dissected 3 to 4 days after an attack. The two frequency distributions of the numbers of parasitoid immatures found in dissected aphids did not differ statistically ($\chi^2 = 7.19$; df = 5; $P = 0.21$), indicating that eggs could be detected immediately after they had been deposited. For this reason I dissected all aphids right after an experiment was completed to reduce the amount of work involved in insect rearing. This also applies to those dissections in Chapter IV.

Effect of aphid density

To evaluate whether the parasitoid's oviposition and host-feeding rates varied with the number of pea aphids available, I set up eight screened minicages, each of which contained 1, 3, 6, 9, 12, 15, 20, and 40 aphids feeding on broad bean. I introduced

Figure 3.1. Frequencies of *A. asychis* eggs or larvae found in dissected pea aphids. The stippled bars indicate eggs found in aphids (N = 210) dissected within 24 h after parasitoid attack and the shaded bars larvae found in aphids (N = 211) dissected after 3-4 days.



FREQUENCY
100
50
0
0
1
2
3
4
5
6
NO. EGGS OR LARVAE/HOST

one 2- to 3-day-old *A. asychis* female into each cage. After 24 h, the female was removed and the dead aphids in each cage were counted. Aphids killed by parasitoid feeding can be recognized on the basis of their shrivelled appearance and pale-yellowish colour. As aphids killed by host-feeding were not replaced, the number of live aphids available in each cage declined during the test, usually by one. Surviving aphids were dissected for parasitoid eggs. A total of 21 *A. asychis* females were tested at each aphid density.

Pattern of host-feeding

Oviposition and feeding behavior may be influenced by a female's nutritional status. About 120 *A. asychis* females from the same emergence cohort were caged for 24 h with a large number of aphids for feeding and oviposition. They were then divided into four groups which were transferred to empty glass vials (4.8 by 1.5 cm) and starved for 12, 18, 21, and 24 h respectively. At the end of the starvation period, each female (then 2 to 3 days old) was placed in a 5.0 by 1.5 cm petri dish together with 40 to 60 aphids. Parasitoid behavior was observed under a stereomicroscope. I recorded the sequence of oviposition and host-feeding for about 30 females in each treatment group. All attacked aphids were dissected to determine if they had in fact been parasitized.

Length of host-feeding

I set up two kinds of experiments. In the first experiment, I tested the proposition that *A. asychis* females required one second-instar pea aphid for feeding each day (which was indicated by a preliminary experiment). Females, starved for 24 h, were divided into two groups. Those in the first group were permitted to feed on one aphid each without being disturbed, whereas those in the second group were forced to stop feeding after 3 min (a natural, undisturbed feeding normally took more than 10 min, see Results section below). I used a fine camel's hair brush to separate the wasp gently from the aphid on which she was feeding. Females in both groups were then starved again for 18 h before they were placed individually in a petri dish and provided with aphids. The decision to feed on or oviposit in the first aphid encountered was recorded. I predicted that a female's decision whether to feed or oviposit would differ between the two treatment groups. On the assumption that wasps required one (and only one) full feeding during each 24 h period, I expected that those in the first group would oviposit in the first aphid they encountered while those in the second group would feed on it.

In the second experiment, I evaluated whether the time *A. asychis* females spent on host-feeding was influenced by a previous feeding experience. I set up three treatment groups. Females in group 1 were starved for 24 h; those in group 2 were starved for 24 h, after which they were allowed to host-feed for 3 min followed by another 18 h of starvation; and females in group 3 were starved for 24 h, after which they were allowed to complete one full feeding followed by 18 h of starvation. After the various treatments, females were provided individually with 40 to 60 pea aphids in a petri dish. The wasps were observed under a stereomicroscope, and the length of any host-feeding was measured with a stopwatch.

Statistical analysis

I used the BMDPAR program for derivative-free nonlinear regression (Dixon 1983) to fit curvilinear regression equations to the individual data of the numbers of eggs laid, of aphids parasitized, and of aphids killed by feeding at each density.

Results

Effect of aphid density

The relationship between host density and the numbers of aphids that were parasitized and were killed by host-feeding is shown in Fig. 3.2 A. The number of eggs laid by 2- to 3-day-old *A. asychis* females during a 24 h period increased with the number of hosts available (Fig. 3.2 B). Under the given conditions, the density-dependent oviposition rate was satisfactorily described (residual SS = 0.379; F = 784.6; df = 1; $P < 0.001$) by a curvilinear regression equation of the form $y = x / (0.0553x + 2.3975)$, where x is the number of aphids available per day and y is the number of eggs laid per female.

The mean number of eggs laid per host available showed a dome-shaped relationship with the host density (Fig. 3.2 C). As the number of eggs laid was proportional ($y = 1.173\,x$; $r = 0.981$) to the number of aphids attacked (Fig. 3.3), superparasitism increased at low host densities even when unparasitized aphids were available (Fig. 3.2 D).

Host-feeding

On average, each wasp fed on and killed one (variation from 0.7 to 1.2) second-instar pea aphid during the 24 h observation periods. The feeding rate did not vary significantly at densities between six and forty aphids per day (Brown-Forsythe test for ANOVA not assuming equal variances; F = 2.12; df = 5, 95; P = 0.07). A curvilinear regression equation (Fig. 3.2 A) fitted to the numbers of aphids killed by individual females, $y = x / (0.8316x + 2.7771)$ [residual SS = 0.1068; F = 37.3; df = 1, 6; $P < 0.001$], reached 90% of its maximum asymptotic value at a density $x = 30.1$ and 95% at $x = 63.5$.

Figure 3.2. Relationship between host density and oviposition or host-feeding by *A. asychis*. Second-instar pea aphids were caged with one 2- to 3-day-old wasp (N = 21) for 24 h. (A) Numbers of aphids that were parasitized (solid circles), and were killed by host-feeding (open circles); (B) numbers of eggs laid per female; (C) numbers of eggs laid per host available; (D) numbers of aphids superparasitized per female. Dots indicate means (± 1 SEM) at each density.

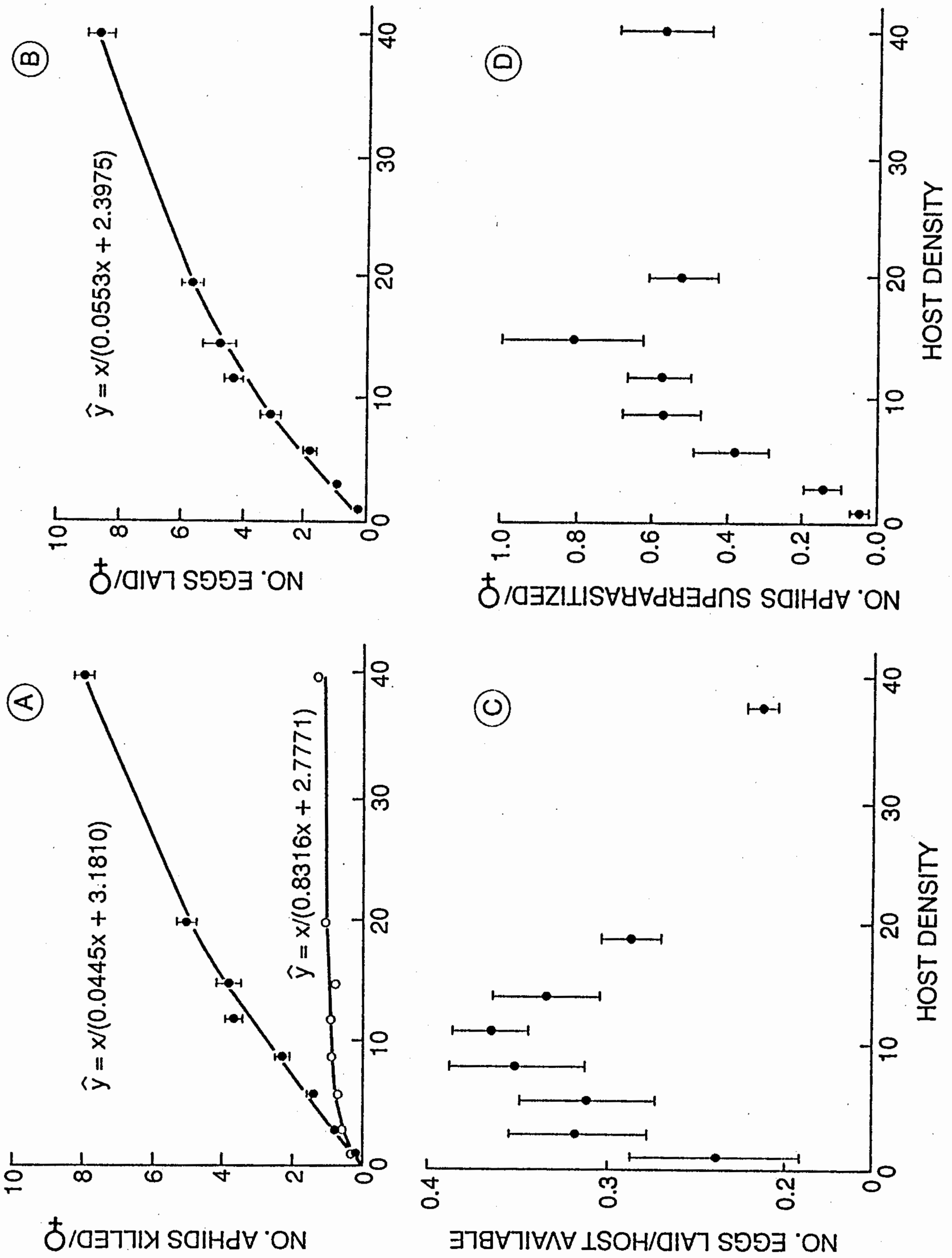
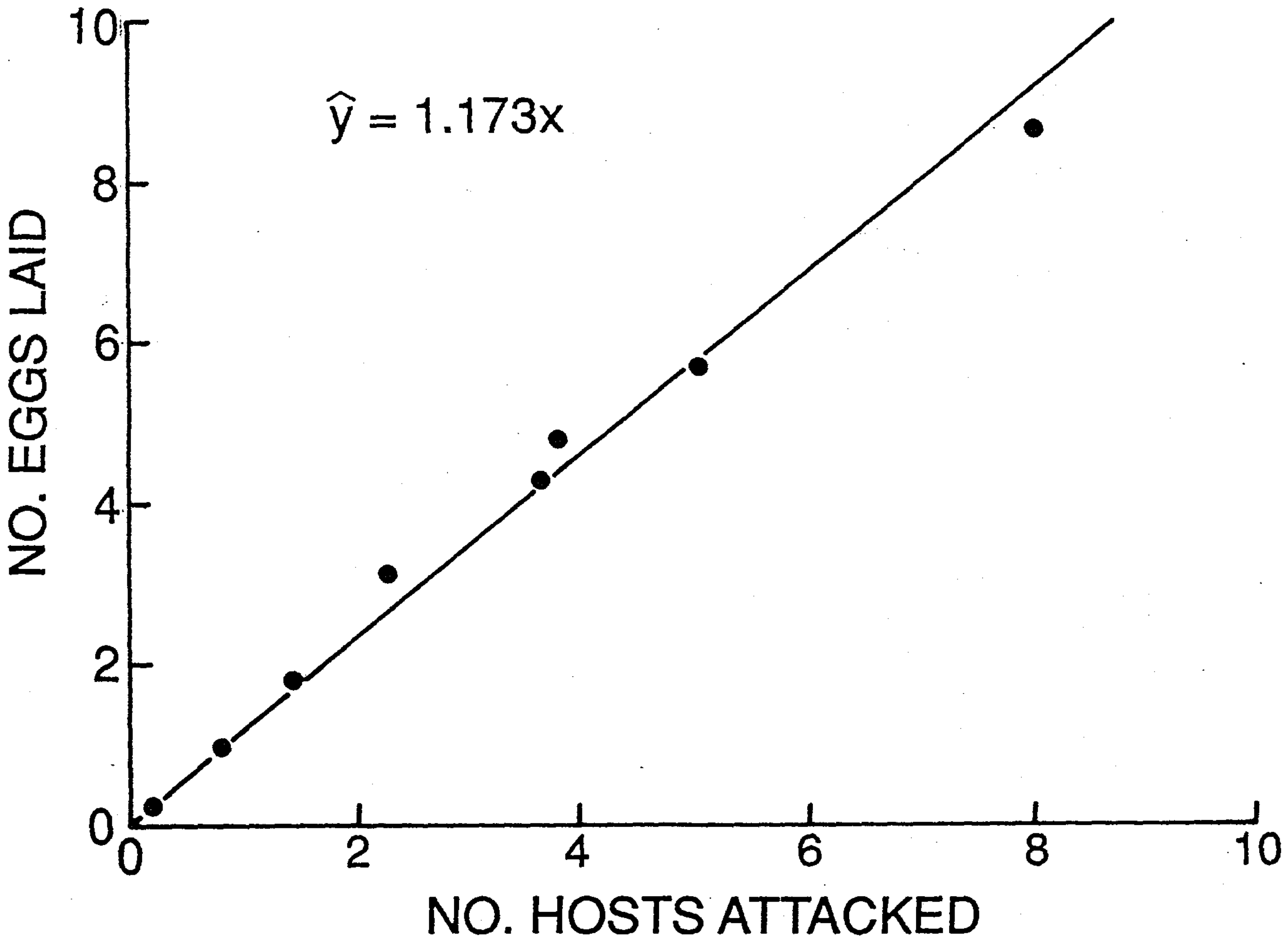
A
B
C
D
NO. APHIDS KILLED/♀
NO. EGGS LAID/♀
NO. EGGS LAID/HOST AVAILABLE
NO. APHIDS SUPERPARASITIZED/♀
HOST DENSITY
HOST DENSITY
ŷ = x/(0.0445x + 3.1810)
ŷ = x/(0.8316x + 2.7771)
ŷ = x/(0.0553x + 2.3975)
0
2
4
6
8
10
0.0
0.2
0.4
0.6
0.8
1.0
0.2
0.3
0.4
0
10
20
30
40

Figure 3.3. Regression of (y) the number of eggs laid by *A. asychis* on the number of hosts attacked (x). Dots indicate means for each treatment (=density) group.


$\hat{y} = 1.173x$
NO. EGGS LAID
0
2
4
6
8
10
NO. HOSTS ATTACKED
0
2
4
6
8
10

Host-feeding by *A. asychis* females lasted between 4.0 min and 41.6 min, with a median time of 13.5 min ($x = 14.4$ min; $N = 77$). Feeding times (Table 3.1) did not vary with starvation treatment (1-way ANOVA; $F = 0.33$; df = 2, 74; $P = 0.72$). However, the length of feeding, whether to satiation or terminated after 3 min, influenced a wasp's subsequent oviposition behavior. Of fully-fed wasps, 88% ($N = 32$) oviposited in the first aphid they encountered after 18 h of starvation as compared to only 26% ($N = 31$) of those that had only a partial meal. Similarly, when given a choice, females that had not fed on an aphid for ≤ 18 h usually laid one or several eggs before feeding again. By contrast, when aphids were withheld for ≥ 21 h , wasps usually fed on the first host they encountered (Fig. 3.4). None of the aphids on which wasps had fed contained any parasitoid eggs when dissected.

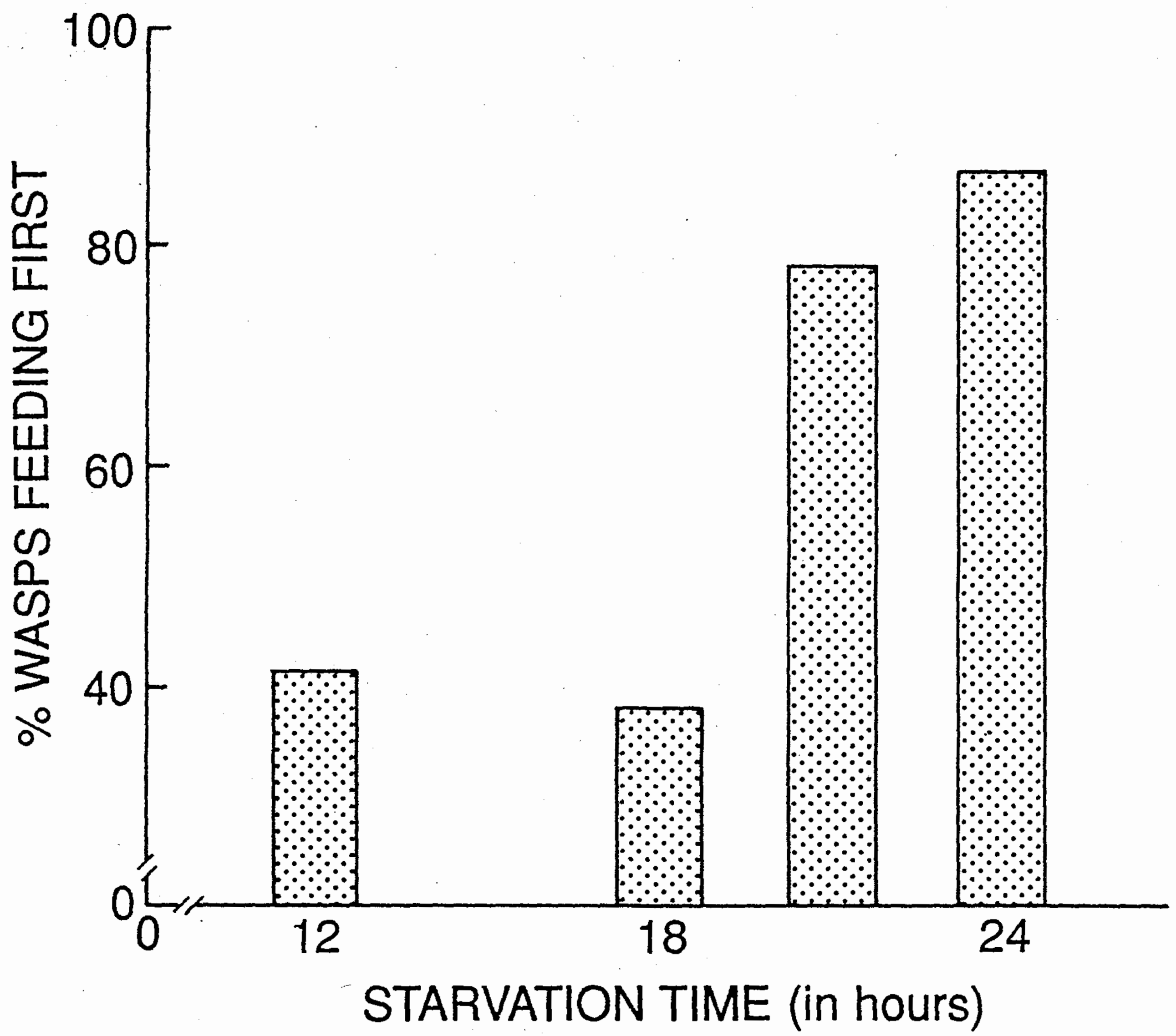
Discussion

Although *Aphelinus* eggs are smaller and less conspicuous than their larvae, they can be readily identified and counted under a dissecting microscope (Hartley 1922). The orange-colored egg is elongate ovate, and slightly bent in the middle; the shape is much like a banana. When a freshly parasitized aphid is dissected, the parasitoid egg usually flows out of the host hemolymph and is then clearly visible. When confirming parasitization, I found that immediate dissection of aphids to detect *Aphelinus* eggs was as accurate as dissection after ≥ 3 days to find *Aphelinus* larvae. This is not the case for some other species of aphid parasitoid. For example, in *Aphidius ervi* and *Aphidius smithi* Sharma & Subba Rao (Hymenoptera: Aphidiidae), the eggs are very small and their color does not contrast well with that of the host's hemolymph and tissues, making detection of newly-laid eggs almost impossible. One has to wait for at least 2 days until the egg becomes large enough to be seen before

Table 3.1. Length of host-feeding by females of *A. asychis* after different periods of starvation.

Treatment	Feeding time (min)	
	N	x ± 1 SEM
Hosts withheld for 24 h	34	14.1 ± 1.3
Hosts withheld for 24 h, then 3 min of feeding and 18 h of starvation	30	14.8 ± 1.5
Hosts withheld for 24 h, then full feeding and 18 h of starvation	13	16.3 ± 2.5

Figure 3.4. Percentage of *A. asychis* that host-fed before oviposition after various periods of starvation (in hours).


100
80
60
40
0
0
12
18
24
% WASPS FEEDING FIRST
STARVATION TIME (in hours)

parasitization of aphids can be confirmed. The advantage of immediate dissection over dissection ≥ 3 days after parasitization is that data are obtained faster and more efficiently.

Among the four types of host-feeding behavior distinguished by Jervis & Kidd (1986), *A. asychis* conforms to the destructive non-concurrent type. Aphids intended for feeding are first paralysed and usually die, even when feeding is interrupted. Hartley (1922) reported that females of *A. semiflavus* Howard fed on parasitized aphids, a behavior that would result in the loss of offspring. However, Wilbert (1964), working with *A. asychis*, found such behavior uncommon. My observations agree with those of Boyle & Barrows (1978), who considered oviposition and host feeding mutually exclusive events. Such behavior is apparently adaptive, because if a female puts an egg in a host that she is going to feed on, her progeny will be wasted. Host-feeding usually precedes oviposition (Flanders 1942), especially in newly eclosed females (Lagace 1964; Michel 1967; Cate *et al.* 1973) and in females that have been starved or deprived of hosts for some time (Esmaili & Wilde 1972; Collins *et al.* 1981).

A wasp's hunger level and the number of mature eggs present in her ovaries may influence feeding and oviposition decisions (Sandlan 1979; Jervis & Kidd 1986). In *A. asychis*, female behavior is also influenced by the number of available hosts. The rate of oviposition per host over a 24-h period showed a dome-shaped relationship to host density, being density-dependent at low aphid numbers and inverse density-dependent at high aphid numbers (Fig. 3.2 C). Similar responses to host density have been demonstrated in many other parasitic insects (Morrison & Lewis 1984; Lessells 1985). It is interesting that superparasitism by *A. asychis* increased with increased host availability before it declined (Fig. 3.2 D). I have no conclusive evidence to explain this response, which is at variance with observations on other insect parasitoids (van Alphen 1988). It is possible that, in *A. asychis*, searching and oviposition are depressed at very low host densities or, alternatively, that they are stimulated at moderately high encounter rate, changes that could result in more eggs being laid than the number of hosts available, especially by females with a high egg load. Also, an aphid's defensive behavior, such as kicking and walking away (Gerling *et al.* 1990), could be diminished after an attack, a condition that would make such aphids vulnerable to repeated attacks.

My results indicate that host-feeding in *A. asychis* did not increase with increased host density; females responded to increasing host density with an increasing tendency to oviposit rather than to feed (Fig. 3.2 A). Under the given conditions, a single second-instar pea aphid per day apparently was sufficient to satisfy a wasp's nutritional requirements. Occasionally, two or even three wasps were seen feeding simultaneously on the same aphid. However, feeding requirements probably vary in relation to a wasp's body size, age, activity level, and perhaps other factors including aphid size. For example, *A. asychis* females fed on about one-and-a-half aphids per day when reared on greenbugs, which are smaller than pea aphids (Cate *et al.* 1973). This would explain why, in *A. thomsoni* Graham [= *flavus* Thomson], the number of aphids used for feeding was correlated with the number of eggs laid (Hamilton 1973). Having fed to ensure continued oogenesis, *A. asychis* females used any other aphids encountered as hosts for oviposition. Thus, the proportion of aphids killed by feeding among those attacked declined with increasing host density.

The pattern of host-feeding observed in *A. asychis* agrees with Jervis & Kidd's (1986) model for synovigenic species with anhydropic eggs. Flanders (1942, 1962) and Dowell (1978) distinguished two reproductive strategies among the parasitic Hymenoptera. In hydropic species, eggs absorb host fluids and increase greatly in size during incubation; females in general are relatively short-lived, may carry a large number of mature eggs in their ovaries, and do not host-feed. By contrast, anhydropic females, which are expected to be long-lived, produce eggs that contain all the necessary nutrients for embryonic development; eggs tend to be relatively large, and females have only few mature eggs in their ovaries at any one time. In the absence of suitable hosts, anhydropic eggs may be resorbed in a slow cycle of egg maturation and oosorption (Flanders 1962; Dowell 1978). However, Walter (1988) suggested that oosorption might be more a consequence of starvation than of a lack of hosts. Both reproductive strategies are represented among aphid parasitoids. Whereas Aphidiidae, which do not feed on their hosts and do not resorb eggs (Chapter VIII, also see Mackauer & Chow [1986] and Stary [1988] for recent reviews), conform to the hydropic pattern, species of *Aphelinus* and related genera agree with the anhydropic pattern. Under optimal conditions, *A. asychis* females lived an average of 47 days and produced a mean of 879 eggs at 21°C (Force & Messenger 1964a, 1965). However, their ovarial capacity is limited to about 20 - 25 mature eggs (Chapter VIII). This number closely approximates the average daily number of aphids parasitized by caged females (Force & Messenger 1965; Cate *et al.* 1973). It is interesting that females of *Aphelinus abdominalis* (Dalman), which were not observed feeding on their hosts but fed instead on aphid honeydew and water droplets, had a maximum oviposition rate of only seven eggs per day (Wahab 1985).

My data suggest that host-feeding by *A. asychis* is determined largely by a female's nutritional status. Fully-fed wasps tended to oviposit in available aphids while partially-fed or starved wasps normally fed first before they laid any eggs. For a given host size or quality, the rate of host-feeding (but not the rate of oviposition) is density-independent, except at very low encounter rates when feeding has priority over egg deposition. A reproductive strategy that incorporates host-feeding, but only at levels necessary to satisfy basic nutritional and presumably reproductive requirements, enables *A. asychis* females to allocate more time and energy to oviposition. This strategy corresponds with the destructive non-concurrent host-feeding pattern of synovigenic species among the parasitic Hymenoptera (Jervis & Kidd 1986).

CHAPTER IV

CONSPECIFIC INTERACTIONS: I. HOST DISCRIMINATION IN *APHELINUS ASYCHIS*

Introduction

When searching, female parasitoids do not always oviposit in the hosts they encounter. Whereas some hosts successfully defend themselves against an attack, others are rejected because they are unsuitable for parasitoid development. As stated in Chapter I, in solitary species usually only one larva develops in each host. If a wasp deposits one or more eggs in an already parasitized host (a behaviour that causes superparasitism), supernumeraries die or are eliminated by competition in the egg or, more commonly, in the first larval instar stage (Mackauer 1990). Many species of hymenopteran parasitoids are known to discriminate between unparasitized and parasitized hosts and thus to avoid superparasitism, except when unparasitized hosts are scarce or not available (e.g. Salt 1937, 1961; Jackson 1966; Rogers 1975; van Lenteren 1976, 1981; Klomp *et al.* 1980; van Alphen and Nell 1982; Chow and Mackauer 1984, 1986). Host discrimination can occur in response either to an external or an internal marker. The former include epideictic pheromones (Salt 1937; Corbet 1972) and physical marks made on the parasitized host (Takasu and Hirose 1988) or the host patch (Price 1970; Sugimoto *et al.* 1986); parasitism-related changes in the host's haemolymph (Fisher 1971; Beckage and Templeton 1986; Thompson 1986; Vinson 1990) can serve as internal markers. Discrimination may be influenced by a wasp's previous experience with (unparasitized) hosts. However, the supposition that discrimination is a learned response (van Lenteren and Bakker 1975; van Lenteren

1981; Klomp *et al.* 1980) has recently been challenged (van Alphen *et al.* 1987; van Alphen 1988; Volkl and Mackauer 1990).

Various authors have considered conditions when it may be adaptive for a solitary parasitoid to deposit an egg in an already parasitized host (e.g., van Alphen and Nell 1982; Charnov and Skinner 1984, 1985; Iwasa *et al.* 1984; Waage and Godfray 1985; van Alphen and Vet 1986). Conspecific superparasitism (i.e., a wasp lays one or more eggs in a host already parasitized by a conspecific female) may increase a female's fitness if unparasitized hosts are scarce and if the younger larva from the second female has a greater-than-zero chance of winning a contest with an older competitor (van Alphen and Nell 1982; van Alphen *et al.* 1987; van Dijken and Waage 1987; Hubbard *et al.* 1987). By contrast, self superparasitism (i.e. a wasp lays one or more eggs in a host that she herself has previously parasitized) normally decreases a female's fitness because only one of her offspring will survive. A possible exception was noted by Cloutier (1984), who suggested that self superparasitism could be advantageous at low encounter rates of unparasitized hosts. Under conditions in which conspecific superparasitism is very likely, self superparasitism could increase the probability that one of the female's own offspring will survive larval competition.

In this chapter, I evaluate the first part of conspecific interactions, i.e., host discrimination, by using *Aphelinus asychis* as experimental animals. Anecdotal observations suggest that *Aphelinus* females rarely superparasitize (e.g. Hartley 1922; Finney *et al.* 1960; Force and Messenger 1965; Mackauer 1982; Wahab 1985), but the modalities of and the proximal mechanism(s) involved in host discrimination are not known. For example, it is not clear if females respond to an external or an internal cue and if learning plays a role in host discrimination. I test the hypothesis that *A. asychis* can discriminate between unparasitized and parasitized aphids, and I predict that females will prefer the former for oviposition. I distinguish between self- and conspecific-parasitized hosts. Using experienced and inexperienced wasps (i.e. wasps with and without previous contact with unparasitized aphids), I test next if discrimination is a learned response. Finally, I show that wasps react to internal cues. I discuss my findings with regard to theories about progeny allocation and reproduction in hymenopteran parasitoids.

Materials and Methods

Oviposition behaviour

Oviposition can last several minutes from the time the ovipositor is inserted into a potential host to the time an egg is deposited and the ovipositor withdrawn. A detailed description of the oviposition behaviour of *A. asychis* is given by Gerling *et al.* (1990). In the first experiment (Expt 1) in this chapter, I examined the usefulness of insertion time as a predictor of oviposition success. Having collected this information, I could obtain parasitized aphids needed in the later experiments just by recording ovipositor insertion duration. I presented wasps individually in a Petri dish with a small number of lightly anaesthetized (with CO_2) aphids. Anaesthetization temporarily eliminated the aphid's defensive reactions to parasitoid attacks (Gerling *et al.* 1990). Thus the relationship between the length of attack and the success of oviposition would not be confounded by host defence. Anesthetized aphids usually remained motionless for ca. 1 h, after which they returned to normal. The insects were observed continuously under a stereomicroscope. Wasps were permitted to attack each aphid once. The interval (in sec) from ovipositor insertion to withdrawal was measured with a stop watch. Any aphid attacked was removed with a fine camel's hair brush and assigned to one of six groups in accordance with insertion time: ≤ 40, 60, 80, 100, 120, and > 120 sec. At the end of each trial (N = 32 wasps), I dissected the aphids to confirm the presence or absence of a parasitoid egg (see Chapter III).

Host discrimination

I evaluated host discrimination by *A. asychis* females using two different procedures. The first test (Expt 2, an indirect method) was designed to determine the degree, if any, to which females would superparasitize when they were confined with a limited number of hosts. The second test (Expt 3, a direct method) was designed to determine, by direct observation, a wasp's response to unparasitized and parasitized aphids provided at the same time.

Both methods examine the same question--host discrimination, but the relative reliability might vary. The indirect method may conceal the ability of the parasitoids to discriminate because of host scarcity at the end of each experimental period. When fewer and fewer unparasitized hosts became available, repeated encounters with parasitized hosts could cause a breakdown of the wasp's restraint to oviposit into parasitized hosts, thus causing superparasitism. Furthermore, parasitoids were caged and thus not able to emigrate as they might have done under natural conditions. These factors could bias the results toward a lack of discrimination. By contrast, the direct method offered the parasitoid a choice. Both parasitized and unparasitized aphids were always available in equal numbers. The parasitoids could also emigrate. Therefore, the results can be considered more reliable and accurate. Both procedures, however, were employed in my studies to reinforce conclusions and to provide complementary information.

In the first set of trials (N = 42), I caged one experienced female in a screened (8.5 by 3 cm) minicage (see Chapter III) together with 15 aphids feeding on a bean shoot (Expt 2 A). The wasp was removed after 24 h, and all surviving aphids were dissected; the parasitoid eggs in each aphid were counted. In a second set of trials (N = 27), I caged six experienced females together with 20 aphids in a screened minicage (Expt 2 B). The data from each set of trials were pooled into two frequency distributions which I compared with expected Poisson frequencies as a rough test of selective behaviour. I expected that, in the absence of discrimination, the percentage of superparasitism would be higher in Expt 2 B than in Expt 2 A, in absolute as well as in relative terms.

To test discrimination directly (Expt 3), I introduced one inexperienced female (N = 235) into a small (4.0 by 0.6 cm) Petri-dish arena that contained four pea aphids: two of the aphids were unparasitized, and two had been parasitized by a conspecific wasp about 1 h before the test. All aphids were lightly anaesthetized, and parasitized aphids were marked by antennal amputation (Mackauer 1972). I recorded the kind of host attacked first (unparasitized versus parasitized) and the length of ovipositor insertion. As soon as an oviposition was finished, the attacked aphid was removed and replaced by the same host type (parasitized or unparasitized) using a camel hair brush. To control for the effects of experience or learning each wasp was used only once, i.e. only one attack was permitted for each wasp, thereafter it was discarded and a new wasp was introduced to start the next observation. Trial runs lasted about 1 h, during which time 10 - 16 females could be tested. I dissected the attacked aphids at the end of each run to determine if an egg had been deposited. The pooled data were classified by the kind of host attacked (unparasitized versus parasitized) and by parasitoid response (accepted versus rejected for oviposition) and arranged in a 2 x 2 contingency table. As each wasp had an equal chance of encountering either kind of aphid, the null hypothesis of no discrimination would be confirmed if wasps accepted equal proportions of both host classes. Alternatively, a statistically significant excess of "unparasitized" aphids among those accepted would signify discrimination. I evaluated the data by χ^2 analysis, using two criteria: first, I used insertion time (> 80 sec) and, second, the presence of a parasitoid egg in dissected aphids, as evidence of host acceptance and successful oviposition.

Self and conspecific superparasitism

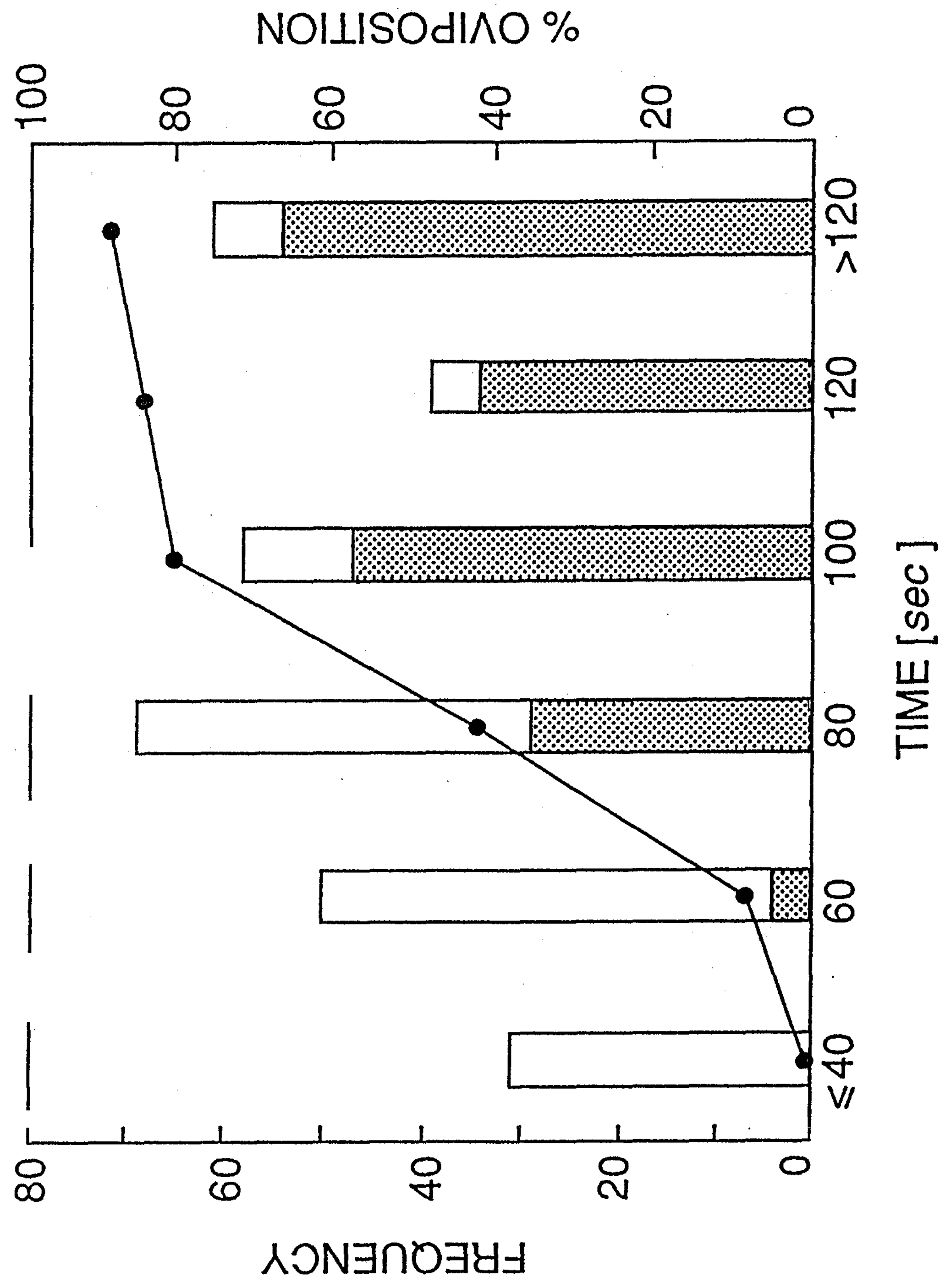
To test if *A. asychis* females would distinguish between aphids containing their own egg and those containing the egg of a conspecific female (Expt 4), I used the same design as for Expt 3. An experienced female (N = 70) was given a choice between two self- and two conspecific- parasitized aphids in a Petri-dish arena. Each wasp was permitted to strike two (rather than only one) different aphids. I recorded the kind(s) of aphid attacked and the length of time the ovipositor was inserted; attacked aphids were dissected and checked for the presence of parasitoid eggs. I predicted that if individuality exists in parasitoid markers, i.e. the hosts parasitized by herself and a conspecific female appear chemically different to the searching female, the searcher should reject more of the former host type than the latter due to the difference in fitness gains.

Results

Host examination and oviposition

A female of *A. asychis*, when encountering a potential host, normally examined and probed it with her ovipositor. The total time the ovipositor remained inserted in the host included probing (i.e., the wasp used her ovipositor to assess presumably the host's suitability) and, if she accepted the host, oviposition (i.e., an egg was passed through the ovipositor and deposited in the host). Figure 4.1 shows the distribution of insertion times that resulted, as determined by dissection, in the acceptance (mean

Figure 4.1. Frequencies of hosts accepted (shaded bars) and rejected for oviposition (open bars) by *Aphelinus asychis* in relation to length of attack. Percentages of hosts containing a parasitoid egg indicated by dots. Hosts were second-instar nymphs of pea aphid.

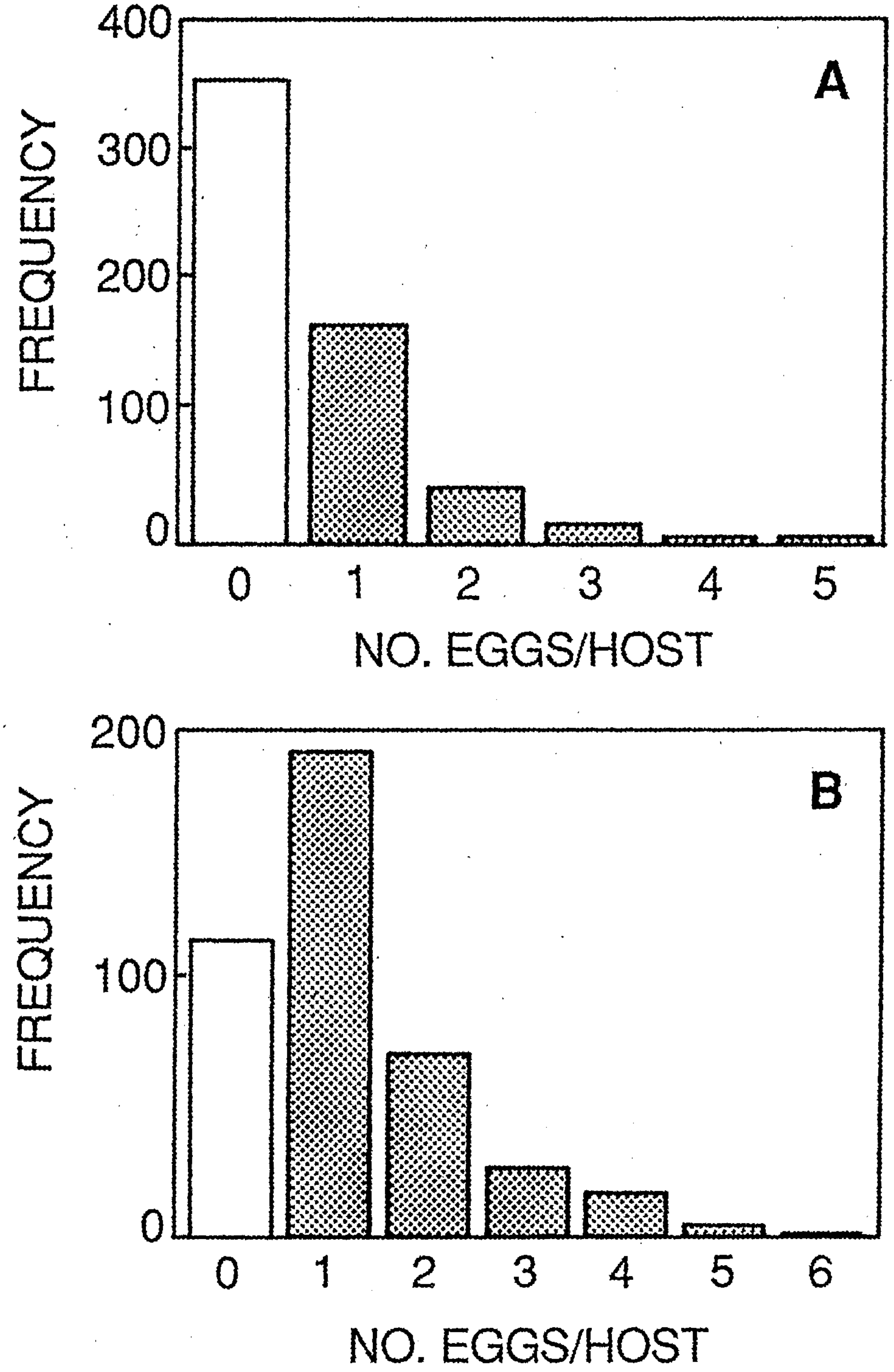

% OVIPOSITION
100
80
60
40
20
0
FREQUENCY
80
60
40
20
0
≤40
60
80
100
120
>120
TIME [sec]

insertion time ± SD = 108.0 ± 35.6 sec; median = 102.4 sec; N = 168) and the rejection of a previously unparasitized aphid (60.5 ± 33.0 sec; median = 57.0 sec; N = 140). The probability of oviposition increased with the duration of ovipositor insertion. Of 308 attacks observed, about 26% lasted $\leq$ 60 sec; only four of those resulted in oviposition. Thirty one attacks that lasted $\leq$ 40 sec resulted in no eggs at all. This suggested that the minimum duration for oviposition by *A. asychis* was from 40 to 60 sec. By contrast, 42% of the attacks that lasted 60 to 80 sec contained one egg; the percentage of successful ovipositions increased to 88% in attacks that lasted > 120 sec. Therefore, in preparing aphids for later experiments, I used a threshold value estimated conservatively as 80 sec, that is, I classified insertions lasting > 80 sec as ovipositions and those lasting $\leq$ 80 sec as attacks that presumably did not result in the deposition of an egg.

Host discrimination

Superparasitism was common among aphids that had been caged for 24 h with one or six females of *A. asychis* (Fig. 4.2). As expected, the proportion of hosts that contained more than one parasitoid egg was higher (27.6%) and more eggs were laid (1.18 eggs laid/aphid available) when six females rather than only one wasp (9.7% superparasitized with 0.51 eggs laid/aphid available) were searching, in spite of the fact that unparasitized aphids were not in short supply in either experiment. Although one of the observed frequency distributions differed significantly from a Poisson distribution with equal mean (Fig. 4.2 B), the other did not (Fig. 4.2 A). It is worth while to note that, in both sets of trials, the frequency classes of repeatedly attacked aphids ($f_i \geq 2$) made greater contributions to the calculated χ^2 values than did the class of singly parasitized aphids. This suggested that after a few ovipositional attacks, aphids were more susceptible to additional ovipositions, probably due to weakened

Figure 4.2. Pooled frequencies of eggs laid by *Aphelinus asychis* in second-instar nymphs of pea aphid: A, one female (N = 42) caged with 15 hosts for 24 h; B, six females (N = 27 x 6) caged with 20 hosts for 24 h. (Fit to expected Poisson distribution with equal mean: (A) x = 0.51; $\chi^2 = 6.425$; df = 3; $P = 0.09$; (B) x = 1.18; $\chi^2 = 23.06$; df = 4; $P < 0.001$).


400
300
200
100
0
FREQUENCY
A
0
1
2
3
4
5
NO. EGGS/HOST
200
100
0
FREQUENCY
B
0
1
2
3
4
5
6
NO. EGGS/HOST

defense.

When inexperienced *A. asychis* females were given a choice between unparasitized and parasitized aphids (Expt 3), they oviposited mainly in unparasitized hosts. Using the length of attack (> 80 sec) as the classification criterion, I found that wasps had "parasitized" 47.0% of the unparasitized aphids but had "superparasitized" only 5.1% of the previously parasitized counterparts (Table 4.1 a). However, dissection showed that 12 of the supposedly parasitized aphids did not contain an *Aphelinus* egg, indicating that the first wasp had attacked them without depositing an egg. I did not include these falsely categorized aphids in the contingency analysis (Table 4.1 b), which confirmed that *A. asychis* was more likely ($P < 0.001$) to oviposit in unparasitized than parasitized hosts. Wasps apparently did not have to learn to discriminate, because all the wasps used in my experiments were inexperienced and only used once.

In Expt 4, I tested the response of *A. asychis* females to self- and conspecific-parasitized aphids. Experienced wasps, when given a choice between these two kinds of hosts, avoided both to the same high degree (Table 4.2 a). On dissecting the stung aphids, I found that some contained no parasitoid eggs and, for that reason could not be classified (see above). A contingency test based on the reduced data set failed to show a statistically significant difference ($P = 0.95$) in the rejection rate between self- and conspecific-parasitized aphids by *A. asychis* (Table 4.2 b). The percent superparasitism was very low for both host categories; 1.85% for self-parasitized hosts and 3.45% for conspecifically parasitized hosts.

Wasps examined with the antennae and stung all the aphids that they encountered, a behaviour suggesting that host acceptance and rejection depended on ovipositor insertion (or detection of internal host cues). The decision to reject a previously parasitized host required much less time (18.5 ± 22.8 sec; median = 9.4 sec; N = 103) (Fig. 4.3) than the acceptance (108.0 ± 35.6 sec; median = 102.4 sec;

Table 4.1. Discrimination by inexperienced females of *Aphelinus asychis* between unparasitized and conspecific-parasitized second-instar nymphs of pea aphid [*]

Criterion	Unparasitized	Parasitized	χ^2
(a) Ovipositor insertion			
≤ 80 sec	62	112	51.57
> 80 sec	55	6	
(b) No. parasitoid eggs/aphid [†]			
0 (rejected)	62	103	54.13
1 (accepted)	55	3	

[*] Each wasp (N = 235) was permitted to select one from a total of two unparasitized and two parasitized hosts (see text for details).

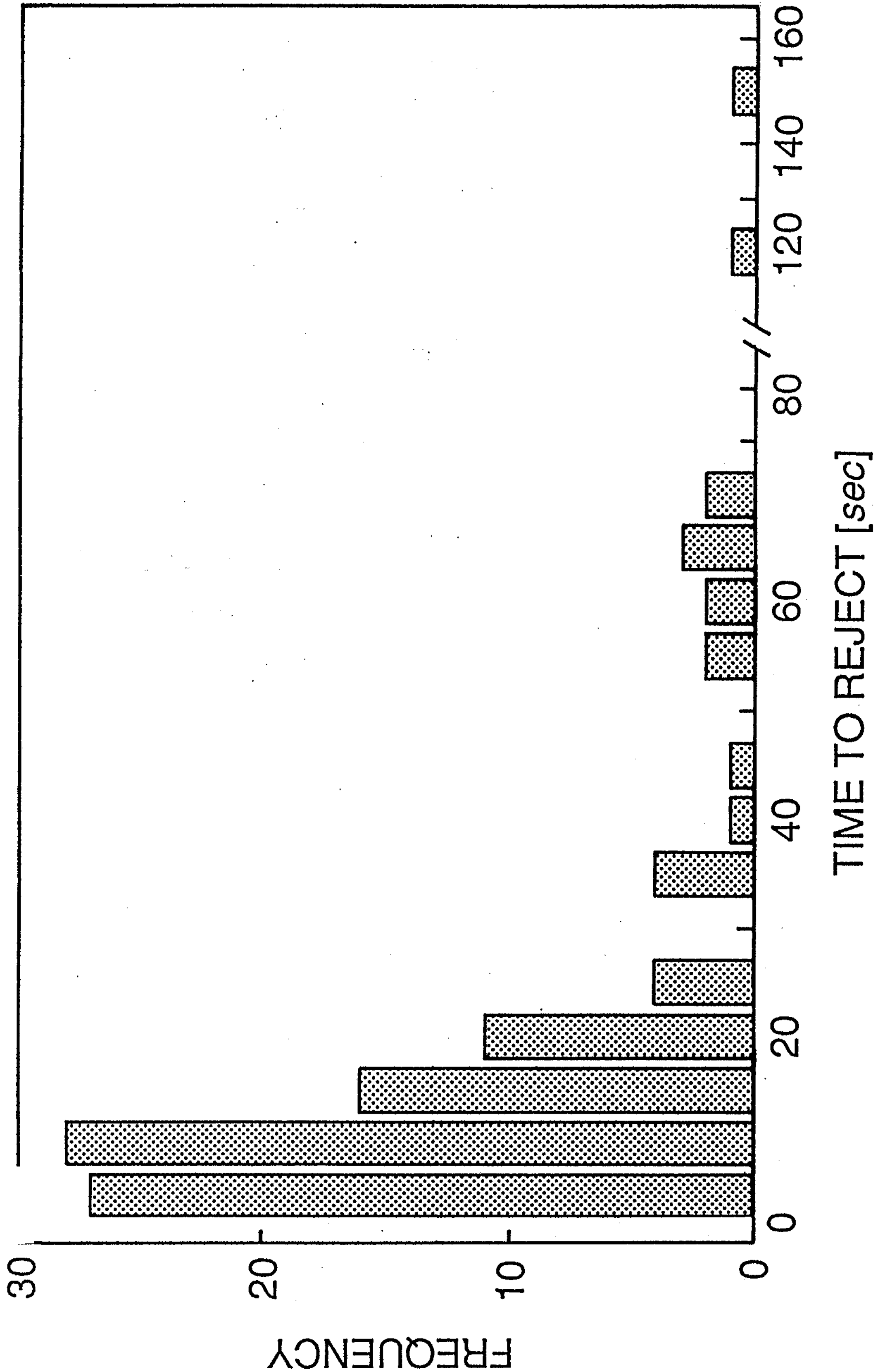
[†] Numbers refer to eggs laid by the second female.

Table 4.2. Discrimination by experienced females of *Aphelinus asychis* between self- and conspecific-parasitized second-instar nymphs of pea aphid [*]

Criterion	Self-parasitized	Conspecific-parasitized	χ^2
(a) Ovipositor insertion			
≤ 80 sec	70	66	0.001
> 80 sec	1	2	
(b) No. parasitoid eggs/aphid			
1 (rejected)	53	56	0.004
2 (accepted)	1	2	

[*] Each wasp (N = 70) was permitted to select two from a total of two self- and two conspecific-parasitized hosts; one aphid was lost during transfers (see text for details).

Figure 4.3. Frequency distribution of rejection times by *Aphelinus asychis* provided with previously parasitized second-instar nymphs of pea aphid.


FREQUENCY
0
10
20
30
TIME TO REJECT [sec]
0
20
40
60
80
120
140
160

N = 168) or the rejection (60.5 ± 33.0 sec; median = 57.0 sec; N = 140) of an unparasitized aphid.

Discussion

When two solitary larvae belonging to the same parasitoid species compete for host resources, the oldest is expected, in general, to survive either by killing in direct combat or by physiologically suppressing a younger competitor (Chapter V; Fisher 1971; Mackauer 1990). Thus, a female can reduce the risk to her offspring and gain in fitness if she avoids depositing eggs in parasitized hosts (Salt 1961; van Lenteren 1981). For host discrimination to be adaptive under these conditions, two implicit assumptions must be valid. One assumption is that the rates of development and growth do not vary appreciably among conspecific larvae, that is, that the chronologically oldest larva will reach first the relatively short "window of interaction" (Chow and Mackauer 1986). The second assumption is that the parasitoid female lives long enough in order to deposit any eggs not laid in future hosts. Both assumptions are highly restrictive and are unlikely to hold very widely. Thus, when unparasitized hosts are not available or scarce, it may be advantageous for a female to superparasitize if the probability of her offspring surviving competition is greater than zero (e.g. van Alphen and Nell 1982; Bakker *et al.* 1985). However, the proposition that superparasitism can be adaptive is thought to apply mainly to conspecific superparasitism, that is, to competition between the offspring of different females. It does not normally apply to self superparasitism, which almost always results in a female wasting offspring, search time or both, regardless of which egg or larva survives and which one dies (van Dijken and Waage 1987; Hubbard *et al.* 1987; for an exception see Cloutier 1984).

Females of *A. asychis* discriminated between unparasitized and parasitized pea aphids and selectively oviposited in the former under all (laboratory) conditions tested. These findings are consistent with most earlier reports that superparasitism is virtually absent among species of *Aphelinus* (e.g. Hartley 1922; Mackauer 1982; Wahab 1985). However, avoidance of superparasitism is often not 100%. To what extent a parasitoid should superparasitize her hosts depends on specific conditions. Hamilton (1973) found up to four eggs in one host when he caged females of *A. thomsoni* Graham (= *flavus* Thomson) with a small number of aphids for 2 to 3 days. Similarly, I observed that *A. asychis* superparasitized a variable proportion of the available hosts if one or several wasps were confined to the same patch for extended periods; both self (Fig. 4.2 A) and conspecific superparasitism (Fig. 4.2 B) occurred under these conditions.

In *A. asychis*, host discrimination is not a learned response, as shown by the fact that inexperienced females selectively rejected parasitized aphids and hence were able to distinguish the latter from their unparasitized counterparts (Table 4.1). Interestingly, superparasitism occurred even though unparasitized aphids were available, which suggests that not all unparasitized aphids were equally acceptable (Gerling *et al.* 1990) or perhaps equally encountered. Also, rejection times indicated subtle differences between rejected hosts. Wasps probed parasitized aphids on average for only 18 sec before withdrawing the ovipositor, but they invested considerably more time (61 sec) to probe any unparasitized aphids that they eventually rejected. In general, wasps stung and probed with the ovipositor all aphids they encountered, a behaviour indicating that external cues either are not involved or play only a minor role in host discrimination. Sensory structures on the ovipositor are probably implicated in the detection of parasitoid eggs and of host quality in general (Wylie 1965; Fisher and Ganesalingam 1970; Fisher 1971).

A wasp's oviposition behaviour is expected to be shaped by natural selection to search efficiently and to minimize possible risks to offspring survival by discriminating between suitable and unsuitable hosts, including previously parasitized hosts (Hughes 1979; Waage 1986). Because superparasitism can reduce offspring survival and, perhaps more important, result in wasted search time, it is usually considered maladaptive. This applies in particular to species in which the time required to handle and reject an unsuitable host is less than the time invested in oviposition as, e. g., *A. asychis* (18 - 61 versus 108 sec). Citing observations on the ichneumonid wasp *Nemeritis canescens* Gravenhorst, Hubbard *et al.* (1987) proposed that females should always reject self-parasitized hosts but accept, under some conditions, those containing the offspring of conspecifics. However, this supposition, which assumes a functional distinction between self and conspecific superparasitism, is not supported by all the experimental evidence available. Although host selection by *Ephedrus californicus* Baker (Volkl and Mackauer 1990) is consistent with the hypothesis, some other species of parasitoids, such as *Asobara tabida* (Nees) (van Alphen and Nell 1982) and *Trichogramma evanescens* Westwood (van Dijken and Waage 1987), apparently do not distinguish between these host classes.

In an effort to test Hubbard *et al.*'s (1987) hypothesis experimentally, I found that females of *A. asychis* did not selectively oviposit, as predicted, in conspecific-parasitized aphids when they had a choice between the latter and self-parasitized hosts (Table 4.2). Such discrimination requires that any epideictic marker or markers must vary among individual females (Hubbard *et al.* 1987; Mackauer 1990). Although I have no evidence to exclude such variation in *A. asychis*, it is relevant that a female inserts her ovipositor and probes a potential host before she accepts or rejects it for oviposition, a behaviour indicative of a response to an internal cue. In contrast to external cues or patch marks (Price 1970; Sugimoto *et al.* 1986) which generally are made by the female during or after she has laid an egg and thus could be expected to vary, internal cues may originate either with the parasitoid or with the host. In the latter case, they are unlikely to show variation related to differences between individual females but rather may reflect host changes of a general nature that are associated with parasitism, such as differences in haemolymph composition (e.g., Beckage and Templeton 1986; Thompson 1986; Vinson 1990). Such internal "biochemical" cues could be highly effective as indicators of the host's general suitability (i.e. enabling a wasp to discriminate between a parasitized and an unparasitized host) but, I suspect, would have limited utility as a means of distinguishing self-parasitized hosts from those parasitized by a conspecific female.

Another possible explanation for the wasps' avoidance of both conspecific- and self-parasitized hosts (even when other hosts were unavailable) is that the conditions under which superparasitism might be adaptive do not in general apply to *A. asychis*. In particular, conspecific superparasitism is thought to be advantageous only if the female carries a relatively large number of mature eggs that would be wasted unless she finds high quality hosts later. However, in species that can resorb mature eggs, resource reallocation (Flanders 1942; Mackauer 1990) may represent a more efficient adaptive tactic than conspecific superparasitism with its inherent risks for offspring survival. Females of *A. asychis* produce about 15 - 20 eggs per day which are resorbed if the females are starved or denied hosts (Chapter VIII). This reproductive strategy (Jervis and Kidd 1986) makes it possible for a wasp to use the resources gained by oosorption either to prolong her effective search time (Flanders 1942) or to produce eggs at a later age (Mackauer 1982). In parasitoid species that are egg- rather than time- limited, such as *A. asychis*, regulation of egg production and oosorption may explain the equal rejection of self- and conspecific-parasitized hosts.

CHAPTER V

CONSPECIFIC INTERACTIONS: II. HOST DISCRIMINATION IN *APHIDIUS ERVI* AND LARVAL COMPETITION IN BOTH *APHIDIUS ERVI* AND *APHELINUS ASYCHIS*

Introduction

The decision made by a parasitoid female whether or not to oviposit in a particular host can have important fitness consequences. In solitary species, the oviposition decision is especially important due to the immature's zero tolerance of other individuals in the same host. To avoid the loss of offspring and search time, a parasitic wasp is expected to vary its host selection behavior so as to maximize offspring survival and potential fitness gains (Charnov and Stephens 1988; Mangel 1989; McBrien and Mackauer 1991). Hosts that provide the highest probability of offspring survival are generally unparasitized hosts, and these are expected to be preferred for oviposition over their parasitized counterparts. In addition, a parasitoid's oviposition decision is influenced by factors such as her egg load and other physiological and environmental conditions, including her probable remaining life expectancy (Mangel and Clark 1988; Volkl and Mackauer 1990), the availability of unparasitized hosts (Hubbard *et al.* 1987; van Dijken and Waage 1987), and the number of females exploiting the same host patch (van Alphen 1988).

Parasitoid larvae have been reported to employ various mechanisms to eliminate competitors in case of superparasitism. These generally include: (1) toxin secretion at egg hatch (Mackauer 1959; Tremblay 1966); (2) combat by early larval instars (Salt 1961); (3) physiological suppression through selective starvation or oxygen deprivation (Fisher 1961b); (4) destructive feeding in late larval stages (Chow and

Mackauer 1985; Hagvar 1988). A species may use one or more such mechanisms to eliminate supernumeraries. The outcome of conspecific competition often depends on the sequence of and the time interval between ovipositions, which determine the relative age of each individual at the time of interaction (Fisher 1961b, 1971; Mackauer 1990).

Studies on parasitoid interactions have focused mainly on the mechanisms and consequences of interactions, i.e. how parasitoids interact with each other in adult and larval stages and which species is superior in the interactions. In contrast, the evolutionary implications of such interactions have received attention mainly by theoreticians and modelers. There are few experiments designed to address the question of why parasitoids interact in the way they do (van Alphen and Vet 1986; but see McBrien and Mackauer 1991). From an evolutionary viewpoint, what is adaptive behavior in one parasitoid species may not be adaptive in another (Roitberg 1991). Species that employ different reproductive strategies may behave differently in their host selection. Such behavior may appear inconsistent when various species are examined, yet different strategies may be optimal in each case.

As part of a project to study conspecific and heterospecific interactions in two distantly related aphid parasitoid species, I investigated competitive interactions among conspecifics of the two species, *Aphidius ervi* and *Aphelinus asychis*. Observations on heterospecific competition between these species are to be reported in Chapter VI. Here I examine host selection by *A. ervi* given the choice of different host types and the mechanisms used by both species to eliminate conspecific competitors. I discuss each species' host selection behavior in the context of its reproductive strategy.

Materials and Methods

The colonies of parasitoids and aphids were maintained as described in Chapter II. I used second instar nymphs of the pea aphid in all experiments. For *A. asychis*, the oviposition can be determined with relative certainty by recording the duration of ovipositor insertion (Chapter IV). However, in *A. ervi*, an oviposition lasts only a fraction of a second. There are no perceptible time or behavioral differences between an oviposition and a rejection. Thus, parasitism by this species can be confirmed only by host dissection. The morphological differences between the two species in eggs and larvae can be found in Chapter VI.

Host selection by *Aphidius ervi*

Unparasitized versus parasitized hosts. In Expt 1, I tested the hypothesis that parasitoids selectively attacked and oviposited in unparasitized hosts when provided with a choice. I introduced a female wasp into a Petri dish (1.0 cm height by 3.5 cm diameter) that contained five unparasitized and five conspecific parasitized pea aphids. The parasitized aphids were prepared between 30 min and 1 h before the trial by allowing a female to attack each unparasitized aphid once, and were marked by amputation of the distal third of one antenna. Because a wasp did not always lay an egg into a host she attacked, some of the parasitized hosts used were in fact pseudoparasitized, i.e., they were struck by a parasitoid but contained no eggs (Jones 1985; Jones *et al.* 1986). The percent parasitism or pseudoparasitism among attacked hosts was estimated through a subsample (control) set aside from the supposedly parasitized aphid group. Of 147 aphids dissected from 10 subsamples, percent parasitism averaged 93.2% (SEM = 1.8, N = 10). Because the error margin was fairly small, I assumed that the proportion of aphids actually parasitized after the first oviposition strike was constant and equaled 93.2%. This figure was used later in analyses of the dissection data (see below and Fig. 5.1) to determine how many aphids were actually parasitized (and pseudoparasitized) by the first wasp and then either rejected or oviposited in by the second wasp, i.e., the testing female.

A host in the Petri dish arena was immediately removed after it was struck by the searching wasp and was replaced by one of the same host type (unparasitized or parasitized) using a fine camel's hair brush. Each wasp was allowed to search for 30 min or until she wandered off the arena repeatedly or stopped searching for 10 min, whichever came first. All struck aphids were reared for 4 days, then dissected to check the number of parasitoid larvae in each. For each parasitoid female, I recorded the number of attacks on unparasitized and parasitized hosts. The latter also included pseudoparasitized ones whose number could be estimated according to the control (see previous paragraph). I also calculated for each type of host the corresponding percent oviposition given attack. Because a preliminary analysis indicated homogeneity in the females' host selection behavior, I pooled the data for all females (N = 10) in final analyses.

Parasitized and pseudoparasitized hosts only. In Expt 2, I tested whether a female's decision to oviposit into a parasitized host was influenced by the expected survival of the offspring (which I tested in Expt 3). I offered each female in a Petri dish aphids that were attacked once, and therefore potentially parasitized, by a conspecific either 0, 24, 48, or 72 h earlier. Each aphid in the arena was allowed to be struck once, then immediately removed. All the removed aphids were reared for 4-5 days, and then dissected to count the number of progeny in each. Two progeny in a host signified ovipositions by both parasitoids. No progeny indicated that the host was pseudoparasitized by the first and the second wasp; one progeny meant that either the first or the second wasp had laid an egg. In such situations I determined from which wasp the progeny came by examining the size difference between the immatures if the time interval between the two ovipositions was ≥ 24 h. Because superparasitism, or double attacks, did not obviously influence the development of a parasitoid larva, a minimum difference of 24 h generally resulted in a fairly reliable variation in size. When the time interval was 0 h, it was impossible to judge from which parental wasp the immature came (Table 5.1, first line). The number of ovipositions made by the first wasp was therefore estimated from the control sample in which each unparasitized aphid was struck once (Table 5.1, control). The total number of attacks on both parasitized and pseudoparasitized aphids, multiplied by the percent oviposition (from the control), yielded an estimate of the number of ovipositions by the first wasp.

Conspecific larval competition in *Aphidius ervi* and *Aphelinus asychis*

In Expt 3, I examined competition between, and survival of, conspecific larvae by dissecting aphids that had been superparasitized at different intervals after the initial attack ($T_{sup} = 0, 24, 48, 72 \pm 1$ h). A female of either *A. ervi* or *A. asychis* was introduced into a Petri dish containing about 30 aphids previously attacked once by conspecifics. I observed each female for 40 min and removed any aphids struck by the searching wasp. The removed aphids were reared on bean plants for several days before they were dissected. Any live or dead parasitoid larvae found in dissected aphids were identified, and their size and other evidence of competition noted. To increase the total number of potentially superparasitized aphids, I replicated each trial 7-11 times for *A. asychis* and 4 times for *A. ervi*, and then pooled the data over all replicates for each species.

Results

Host selection by *Aphidius ervi*

When provided with unparasitized, pseudoparasitized, and parasitized aphids in an arena (Expt 1), female *A. ervi* attacked the three types of hosts in proportion to their relative abundance (Fig. 5.1 A). The observed number of attacks on each host type did not differ from that expected assuming that females did not discriminate among these hosts (G test: $G=0.536$; df=2; $P=0.97$). Note that the number and proportion of pseudoparasitized aphids available to a searching wasp were estimated from controls. Wasps attacked each host readily as it was encountered, indicating a lack of external host discrimination. Probability of oviposition given attack differed (Fig. 5.1 B) among the three host types (RxC test of independence using G test: $G=27.0$; df=2; $P=0.001$), with unparasitized hosts being most preferred and pseudoparasitized ones least preferred. This signifies an internal recognition of different host types by parasitoid females.

When female *A. ervi* were provided with parasitized and pseudoparasitized aphids only (Expt 2), I expected a wasp to oviposit in hosts where her offspring survivorship would be high, such as pseudoparasitized or newly parasitized hosts, and to reject those aphids in which her offspring survivorship would be low, e.g., aphids parasitized ≥ 24 h earlier. My results show that a wasp's probability of oviposition into pseudoparasitized hosts was always higher than that into parasitized ones of the same time intervals (G tests; $P<0.05$) (Table 5.1). A wasp's decision to oviposit into a parasitized host was determined by the time interval between the first and the second attacks. Short intervals (0 h) between two attacks resulted in no oviposition restraint by the second female when her oviposition probability was compared with that of the first attacking female, i.e., control ($G=2.071$; df=1; $P>0.05$), but longer intervals (≥ 24 h)

Figure 5.1. Number of hosts attacked (A) and percent oviposition (B) by *Aphidius ervi* searching in an arena containing unparasitized (Up), pseudoparasitized (Psp) and parasitized (Pp) pea aphids which were in the proportion of Up:Psp:Pp = 50%:3.4%:46.6% as determined from controls (see text for details).

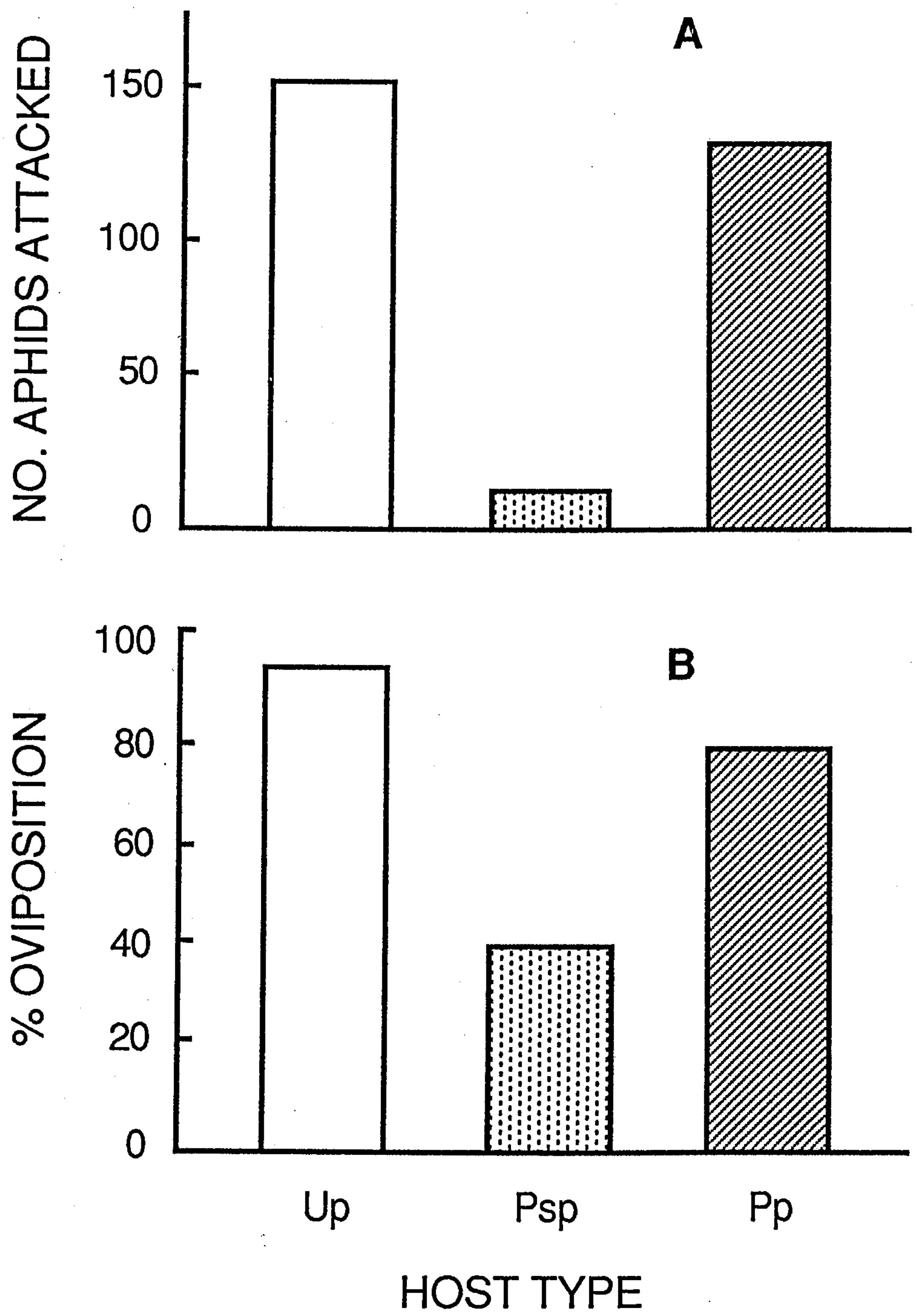

A
NO. APHIDS ATTACKED
150
100
50
0
B
% OVIPOSITION
100
80
60
40
20
0
Up
Psp
Pp
HOST TYPE

Table 5.1. Oviposition (%) into conspecific parasitized and pseudoparasitized pea aphids by *Aphidius ervi* at various time intervals following the first oviposition.

T_{sup}[1] (h)	Parasitized pea aphids		Pseudoparasitized pea aphids	
	No. attacks	Percent[2] oviposition	No. attacks	Percent[2] oviposition
0	138	72.5 ab	37	97.3 a
24	213	12.2 c	28	67.9 b
48	199	10.6 c	52	76.9 ab
72	169	14.2 c	28	60.7 b
Control	Unparasitized pea aphids			
	342	78.7 ab		

1. T_{sup}: time of superparasitization (= the interval between the first and second ovipositions).
2. Subsets sharing the same letter(s) are not significantly different ($\alpha = 0.05$) by simultaneous test procedure for contingency tables (Sokal and Rohlf 1981).

made the second wasp reject almost 90% of the hosts (Table 5.1, $G = 195.043$, df=3, $P < 0.001$).

Larval competition in *Aphidius ervi* and *Aphelinus asychis*

In superparasitized aphids, the bigger larva was from the first egg laid, except at $T_{sup} = 0$ h when I could not determine if it was actually from the first wasp due to its similarity in size to the competitor. For *A. ervi,* the bigger larva generally had the advantage in conspecific competition. It won up to 80% of the contests compared with 0 - 30% of those won by the smaller larva (Table 5.2). The winner eliminated its competitor soon after the second egg had hatched by means of either physical combat or physiological suppression. During dissection I occasionally found one *A. ervi* larva with its mandibles embedded in the body of the other; but mostly I saw one healthy and one dying or dead larva that were not directly engaged in physical combat in a dissected host. Most dead larvae were translucent or opaque in color, and lacked evidence of actual combat such as wound marks or gross deformities, suggesting that physiological suppression was the cause of death. Examination of larvae showed that competitors were never suppressed or eliminated during the egg stage. At the time of dissection, the size of the winning larva in superparastized aphids was usually 1.5 to 2.0 fold smaller (estimated by eye) than that of non-competing larvae of the same age in singly parasitized aphids.

In *A. asychis*, the outcome of conspecific competition depended entirely on the relative size of the interacting larvae. The bigger larva always won the competition (Table 5.2). When two eggs were laid right after each other (0 h interval), both eggs generally hatched and then one larva was eliminated in the early first instar. I found that in cases where both larvae were still alive at the time of dissection, one was always much bigger (3- to 5- fold by eye estimate) than the other, a fact indicating that the latter's development was suppressed. When two eggs were laid ≥ 24 h apart, the most

Table 5.2. Outcome of conspecific competition among larvae of *Aphidius ervi* and *Aphelinus asychis* in superparasitized pea aphids.

T_{sup}[1] (h)	Day of[2] dissection	N[1]	Outcome (%)[3]		
			the first larva won	the second larva won	uncertain (both alive)
Aphidius ervi					
0	4	100	96.0[4] a		4.0
24	5	26	80.8 ab	19.2	--
48	6	21	61.9 b	28.6	9.5
72	7	24	79.2 ab	--	20.8
Aphelinus asychis					
0	4	81	65.4[4] b		34.6
24	5	104	72.1 b	--	27.9
48	6	67	91.0 a	--	9.0
72	6	65	93.9 a	--	6.1

1. T_{sup}: time of superparasitization (= the interval between the first and second ovipositions). N: sample size.
2. Days after the first parasitoid oviposition.
3. In each species, subsets sharing the same letter(s) are not significantly different ($\alpha = 0.05$) by simultaneous test procedure for contingency tables (Sokal and Rohlf 1981).
4. At 0 h interval, it is impossible to judge if the winner is from the first or second wasp because of similarity in size. The % shown here merely indicates one of the larva won.

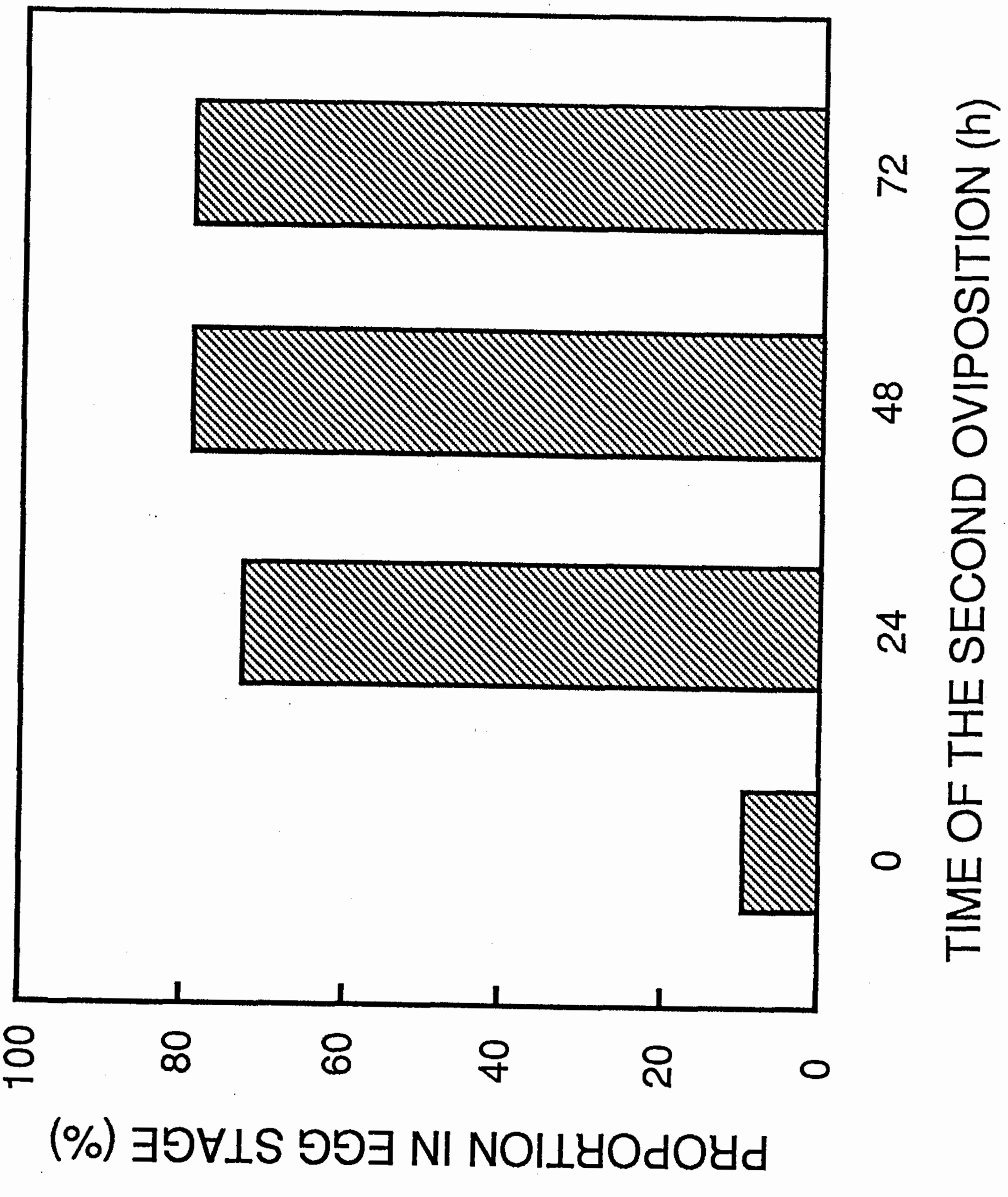
recently laid egg did not hatch (Fig. 5.2). This indicated that factors, or changes in host internal condition associated with the first egg hatching suppressed the second egg. In all the larva-larva or larva-egg interactions, I did not find any evidence of physical combat such as actual biting among competing larvae, wound marks or gross deformities on the competitors. This suggests that physiological suppression was the mechanism of conspecific larval competition in *A. asychis*.

Discussion

A wasp's decision to oviposit or not is influenced, among other factors, by the condition of the host (van Alphen and Visser 1990; Bakker *et al.* 1990). Host conditions such as species, size, and whether or not it has been parasitized by another wasp, determine the overall host quality, which in turn determines the fitness consequences for the females that oviposit into such hosts. In my experiments I tested if parasitoids responded to changes in host condition by offering the females choices of differently parasitized or unparasitized hosts. I expected parasitoids to behave adaptively, i.e., to choose hosts which maximize offspring survival. Because parasitized hosts are generally of lower quality than unparasitized ones (Waage and Godfray 1985), I anticipated that females would choose the latter when both host types were available. In cases where the host resource is heavily exploited, e.g., all the hosts are already parasitized, wasps should only oviposit into those hosts where their offspring have a good chance to survive, or should not oviposit at all.

My results show that female *A. ervi* indeed behaved as expected. When given the choice among unparasitized, pseudoparasitized, and parasitized aphids, the wasps selectively oviposited into unparasitized ones although they did not discriminate among these host types (Fig. 5.1) with regard to attacks (probes), which is the first step in the

Figure 5.2. Proportion of the second *Aphelinus asychis* immatures that were still in the egg by the time of dissection; the second egg was laid at various time intervals after the first egg deposition.


PROPORTION IN EGG STAGE (%)
0
20
40
60
80
100
0
24
48
72
TIME OF THE SECOND OVIPOSITION (h)

process of oviposition. When there was no choice of unparasitized hosts and only parasitized and pseudoparasitized aphids were available, the wasps preferably oviposited into pseudoparasitized hosts (Table 5.1). Except at 0 h interval between two ovipositions, females showed very strong reluctance to oviposit in parasitized aphids. This was evidence that the high survival probability of the second egg at 0 h interval and the low probability of its survival at ≥ 24 h intervals (Table 5.2) apparently influenced the second wasp's oviposition decision.

The fact that female *A. ervi* readily laid eggs into recently (0 h) parasitized aphids but rejected hosts parasitized ≥ 24 h earlier suggests that females could somehow evaluate either the age of the first egg, or the change in host condition associated with the first parasitism, and make their oviposition decisions accordingly. The decision was made only after the ovipositor was inserted. Host physiological changes after parasitization have been reported for some parasitoid-host associations (for a review on the topic, see Vinson 1990).

My results from the choice test (Expt 1) also suggest that internal cues were more important than external cues for host discrimination. I did not find any evidence that female *A. ervi* depended on external cues to discriminate between hosts at 0 h interval (Fig. 5.1). However, this does not allow me to conclude that external cues were absent. The cues may take some time to develop (Cloutier *et al.* 1984) or may be present but ignored by the second wasp (Visser *et al.* 1990). The latter is likely the case for *A. ervi* considering the nature of the external cues (generally marking pheromones). Short-lived marking pheromones are believed to have evolved for the benefit of the markers (Roitberg and Mangel 1989). I have no evidence to indicate whether or not *A. ervi* possess such characters. Other studies (McBrien and Mackauer 1990, 1991) suggest that *A. ervi* females did leave some factors on hosts after oviposition. Although the exact nature of the factors was unknown, a related species *Aphidius smithi* was shown to avoid attacking hosts that were parasitized by *A. ervi* due to the external cues left on the hosts by the first ovipositing wasp.

The oviposition response by *A. ervi* to recently (0 h interval) parasitized aphids (shown in this work) was different from that by *A. asychis* to the same host condition (Chapter IV). Such difference in oviposition decision was likely due to their different reproductive strategies. I showed in Chapter IV that *A. asychis* females not only preferred unparasitized hosts over parasitized ones when the former were available, but also had very strong oviposition restraints when provided with only parasitized and pseudoparasitized hosts. Of 112 parasitized aphids attacked, only 3 (or 2.7%) were accepted by females for oviposition. For *A. asychis*, oviposition takes about 80 sec, resulting in a relatively long handling time per host. The eggs are relatively large and their numbers are small (about 15 to 20 eggs per female ovary), and females can resorb eggs if they are not laid (Chapter VIII). Consequently, each egg represents a large reproductive investment in this species. A wasp sustains a potentially higher fitness cost if it oviposits into a host where the egg has to compete with conspecifics, even in cases (e.g., 0 h interval) where the egg has a finite chance of survival. If the egg loses the competition, the parental female has lost much in terms of search and handling time and fitness. Due to the expense of handling hosts in this species, and the large investment per egg, a risk-sensitive strategy is more adaptive. *Aphidius ervi*, on the other hand, takes only a fraction of a second to lay an egg. The eggs are relatively small in size and very abundant in the ovaries (generally >100 eggs, see Chapter VIII). Females of this species cannot resorb eggs that are not laid. These considerations suggest that each egg of this species is relatively inexpensive and represents a small fraction of total fitness. Therefore, the wasp does not lose much in terms of search time and fitness if the egg loses in competition. In fact, it may be the best strategy for a female to superparasitize when there are no unparasitized hosts and when the egg has any chance of surviving (as the data indicate in Table 5.1, 0 h interval), simply because it is worth the risk under such conditions. Here, I see the totally different responses of different parasitoid species to the same host conditions (for details of *A. asychis* discrimination at 0 h interval, see Chapter IV), yet both are adaptive for the particular species.

It is generally believed that, except when $T_{sup}=0$ h, the first laid egg or the older larva usually wins conspecific larval competition (Mackauer 1990). Dissection of superparasitized aphids showed that in *A. asychis*, this was always the case. But in *A. ervi*, sometimes the second laid egg won (Table 5.2). In both species, however, when two eggs were laid one soon after the other (0 h interval), both eggs hatched and developed normally until one was killed or suppressed. There was no substantial size difference between the two larvae immediately before interactions, suggesting that neither of the larvae had a competitive advantage. When there is no major difference in larval age (e.g., $T_{sup}=0$ h), individual variation in developmental time creates uncertainty. A chronologically younger larva may grow faster and become bigger than its older competitor by the time of interaction. Thus in $T_{sup}=0$ results, I did not know if the slightly bigger larva was always from the first wasp. My data in Table 5.2 (line 1 for each species) indicate only that one of the larvae won. It is clear that to gain significant advantage in competition there must be a substantial age difference ($\geq$ 24 h) between competitors.

CHAPTER VI

HETEROSPECIFIC INTERACTIONS BETWEEN *APHIDIUS ERVI* AND *APHELINUS ASYCHIS*

Introduction

Many species of aphids serve as hosts to unusually rich and diverse guilds of insect parasitoids, including members of the hymenopteran families Aphidiidae and Aphelinidae and of the dipteran family Cecidomyiidae (Mackauer and Chow 1986). Consequently, it is to be expected that these parasitoids compete for hosts, directly and indirectly, when their preferred aphid species or stages are scarce. Despite the economic importance of the genera *Aphdius* and *Aphelinus* as controlling agents for some aphid pests, competitive interactions between the two aphid parasitoid groups are poorly understood and have been reported in only a few studies (Hartley 1922; Force and Messenger 1965). These earlier reports suggest that *Aphelinus* larvae in general did not survive when competing with aphidiids in superparasitized (= multiparasitized) aphids. However, these observations were largely anecdotal and did not provide insight into the dynamic aspects of, or the mechanisms involved in, heterospecific interactions. I was interested in particular in two features of such interactions, namely host discrimination and larval competition between members of unrelated species of parasitoids.

Host discrimination is a common phenomenon among parasitic Hymenoptera (Chapters IV-V; for recent reviews see Mackauer 1990; van Alphen and Visser 1990). Either external or internal cues, or both, may cause a searching wasp to reject a previously parasitized host. In general, external cues are believed to be species-specific due to their parasitoid origin (e.g. Turlings *et al.* 1985; Bakker *et al.* 1985; van Alphen and Visser 1990) although recent evidence supports the assumption that pheromone-like external markers vary among conspecific females (Volkl and Mackauer 1990). In contrast, internal cues, such as changes in host quality (physiological and biochemical) associated with parasitism, are unlikely to show such specificity (Chow and Mackauer 1986; Strand 1986; Hofsvang 1988; Mackauer 1990). Accordingly, the ability to discriminate between unparasitized hosts and hosts parasitized by a different species is thought to be rare (Turlings *et al.* 1985; Bakker *et al.* 1985; van Alphen and Visser 1990) or, if it has evolved, to depend on the degree of relatedness between the competing parasitoids (Vet *et al.* 1984).

The pea aphid and its large guild of aphidiid and aphelinid parasitoids provide an ideal system to test hypotheses about heterospecific host discrimination and larval competition. In this study, I used two sympatric parasitoid species, *Aphidius ervi* and *Aphelinus asychis*. Although both species discriminate between unparasitized aphids and conspecific parasitized ones (Chapters IV-V; Force and Messenger 1965), females may superparasitize when encountering few suitable hosts. Recently, McBrien and Mackauer (1990) showed that *A. ervi* discriminates between aphids parasitized by conspecific females and those parasitized by females of the related *Aphidius smithi*. In this chapter I investigate the mechanisms of host discrimination and larval competition between members of different hymenopteran families. First, I show that females of *A. ervi* and *A. asychis* discriminate between unparasitized pea aphids and aphids parasitized by the other species. Second, I show that avoidance of oviposition is based on internal cues. Third, I describe larval competition between these parasitoids and show that *A. ervi* is the superior competitor independent of age differences between the larvae. And finally, I discuss heterospecific host discrimination as an adaptive behavior.

Materials and Methods

Parasitoids and hosts.

Wasps emerging from mummified aphids were kept in wax paper cups and fed daily with honey water. I used 2- to 4-day-old, inexperienced parasitoid females and second-instar nymphs of the pea aphid in all experiments; each female was used only once. Immatures of *A. ervi* and *A. asychis* can be readily distinguished. Eggs of *A. asychis* are banana-shaped; they are relatively large and can be detected in dissected aphids immediately after oviposition (Chapter III). The first-instar larva is spindle-shaped, while later instars are more or less pear-shaped (Hartley, 1922). Eggs of *A. ervi* are roundish and quite small; eggs less than 24 h old are difficult to find in dissected aphids. The first instar is elongate, hymenopteriform, and has large mandibles. The time from oviposition to hatching of the first instar is about 3.5 days in both species under the given conditions.

Host discrimination

I followed the procedures of the previous two chapters (IV-V) and McBrien and Mackauer (1990), who provided additional details.

In Expt 1, I tested the hypothesis that females of either or both parasitoid species discriminate between unparasitized pea aphids and aphids already parasitized by the other species. The experiment was designed to provide insight into the mechanisms by which wasps can distinguish between different host classes. A wasp of species B (= searching wasp) was placed singly in a plastic Petri dish (5.5 cm diameter by 1.0 cm high) containing five unparasitized aphids and five aphids that had been attacked and potentially parasitized $\leq$ 1 h earlier by species A (A = *A. ervi* and B = *A. asychis*, and vice versa). As discussed in Chapter V, some of the presumably parasitized hosts will in fact be "pseudoparasitized". In *A. asychis*, I again used the time from the ovipositor's insertion to its withdrawal from an aphid as the criterion for judging oviposition, with withdrawals after ≥ 80 s signifying success (Chapter IV). No similar criterion is available to determine successful oviposition by *A. ervi*, which requires << 1 sec to do so. Unparasitized aphids were marked by amputation of the distal third of one antenna (Mackauer 1972). This marking method has no influence on the aphid's behavior or its probability of being encountered by a female parasitoid.

For species B = *A. asychis*, I anaesthetized aphids lightly with CO_2 to minimize any possible bias of host defenses on parasitoid oviposition decisions (Gerling *et al.* 1990). However, for B = *A. ervi*, I permitted aphids to move freely in the arena; oviposition success is little affected by host movement because of this species' rapid attack behavior. Observation times were standardized, with each wasp being observed for about 1 h. Any stung aphid was removed immediately and replaced by one of the same kind. I reared all removed aphids on bean plants for 3 - 4 days and then dissected them to verify oviposition. Lack of oviposition restraint (= superparasitism) by species B was indicated if an aphid contained two parasitoid eggs or larvae, one from each species. I tested the data separately for each species but pooled over all females (*A. ervi*: N = 48; *A. asychis*: N = 61), using the G-test with Williams' correction for 2 x 2 tables (Sokal and Rohlf 1981).

I considered two hypotheses with regard to the proximal mechanisms involved in host discrimination. I expected that the wasps would attack equal proportions of each host class if discrimination did not depend on any external cues. Next I compared the conditional probabilities of oviposition given attack; I predicted that the probabilities of egg deposition (as determined by host dissection) were the same for both host classes if wasps did not use any internal cues. Implicit in both hypotheses is the assumption that parasitoid females did not distinguish between parasitized and pseudoparasitized aphids; this assumption was tested separately (see below).

Larval competition

In Expt 2, I evaluated competition between parasitoid larvae by dissecting aphids that contained two individuals, one from each species, of different ages. The age difference between competing larvae was $T_{sup} = 0, 24, 48, 72 \pm 1$ h, which was controlled by permitting a wasp to oviposit in a host parasitized by the other species after different intervals. A female of species B was introduced into a Petri dish containing 20 aphids previously stung and potentially parasitized by species A (A = *A. ervi*, B = *A. asychis*, and vice versa); each female was observed for a constant period (about 1 h). I removed any aphid struck by the searching wasp and replaced it with one of the same kind. These aphids were reared on bean plants for several days before they were dissected. I recorded the number of parasitoid eggs or larvae in each dissected host, and identified which immatures were alive and which were dead along with the possible causes of the death. Dead larvae of both *A. ervi* and *A. asychis* were easy to recognize in dissected aphids. They were usually opaque, and motionless after being prodded repeatedly by a dissecting needle. I replicated each trial 8 - 11 times to increase the sample size (N) for superparasitized aphids. Data were pooled over all replicates in analyses (see above).

Except analyzing larval competition, I also used the data from Expt 2 to determine if a parasitoid female could distinguish between aphids that were parasitized and those that were pseudoparasitized by a different species after various time intervals. I expected that, if unparasitized hosts were not available, wasps would selectively oviposit in pseudoparasitized rather than parasitized aphids. To test this hypothesis, I assigned all dissected aphids in a sample to one of four classes:

unparasitized by both A and B (n_0), parasitized by A but not B (n_A), parasitized by B but not A (= pseudoparasitized by A) (n_B), and parasitized by both A and B (n_{AB}), with $N = n_0 + n_A + n_B + n_{AB}$. Note that the independent oviposition rate (in unparasitized aphids) of parasitoid A can be estimated directly, $p_A = (n_A + n_{AB}) / N$. By analogy, the observed oviposition rate of B is $p_B = (n_B + n_{AB}) / N$, which in the absence of restraint (i.e. oviposition rate of B is independent of the oviposition rate of A) equals the expected oviposition rate, E. The formula is $E(B) = (p_{AB} / p_A)$.

Results

Host discrimination

In choice tests (Expt 1) that included the same numbers of unparasitized aphids and of aphids that had been stung and potentially parasitized (T_{sup} = 0-1 h) by the other species, wasps attacked both host classes equally [Fig. 6.1, *A. ervi* (N = 48), unparasitized versus "parasitized": mean attacks ± SD = 6.35 ± 1.95 versus 6.42 ± 1.05; H_o = both host classes are equally likely to be attacked, pooled data, G_{adj} = 0.015, df = 1, P = 0.90. -- *A. asychis* (N = 61): 4.95 ± 1.01 versus 5.38 ± 1.39; G_{adj} = 0.911, df = 1, P = 0.34]. This finding indicates that searching females either did not recognize or ignored any external cues left by the other species. However, a wasp may still reject a potential host on the basis of internal cues, that is, after probing it with her ovipositor. Dissection of attacked hosts showed that both *A. ervi* (G_{adj} = 7.981, df = 1, P = 0.005) and *A. asychis* (G_{adj} = 9.640, df = 1, P = 0.002) were more likely to oviposit in unparasitized aphids than in their heterospecifically parasitized counterparts (Fig. 6.2). Interestingly, the two parasitoid species responded differently to aphids that had only been stung (but not parasitized) by the first wasp. Whereas *A. ervi* females (Fig. 6.2 top) treated such pseudoparasitized aphids as if they had in fact been parasitized by

Figure 6.1. Numbers of unparasitized (uP; open) and heterospecifically parasitized (hP; shaded) pea aphids attacked by female wasps of *A. ervi* or *A. asychis*, ≤ 1 h after the hP aphids had been attacked by the other species, when both host types were present in equal numbers.

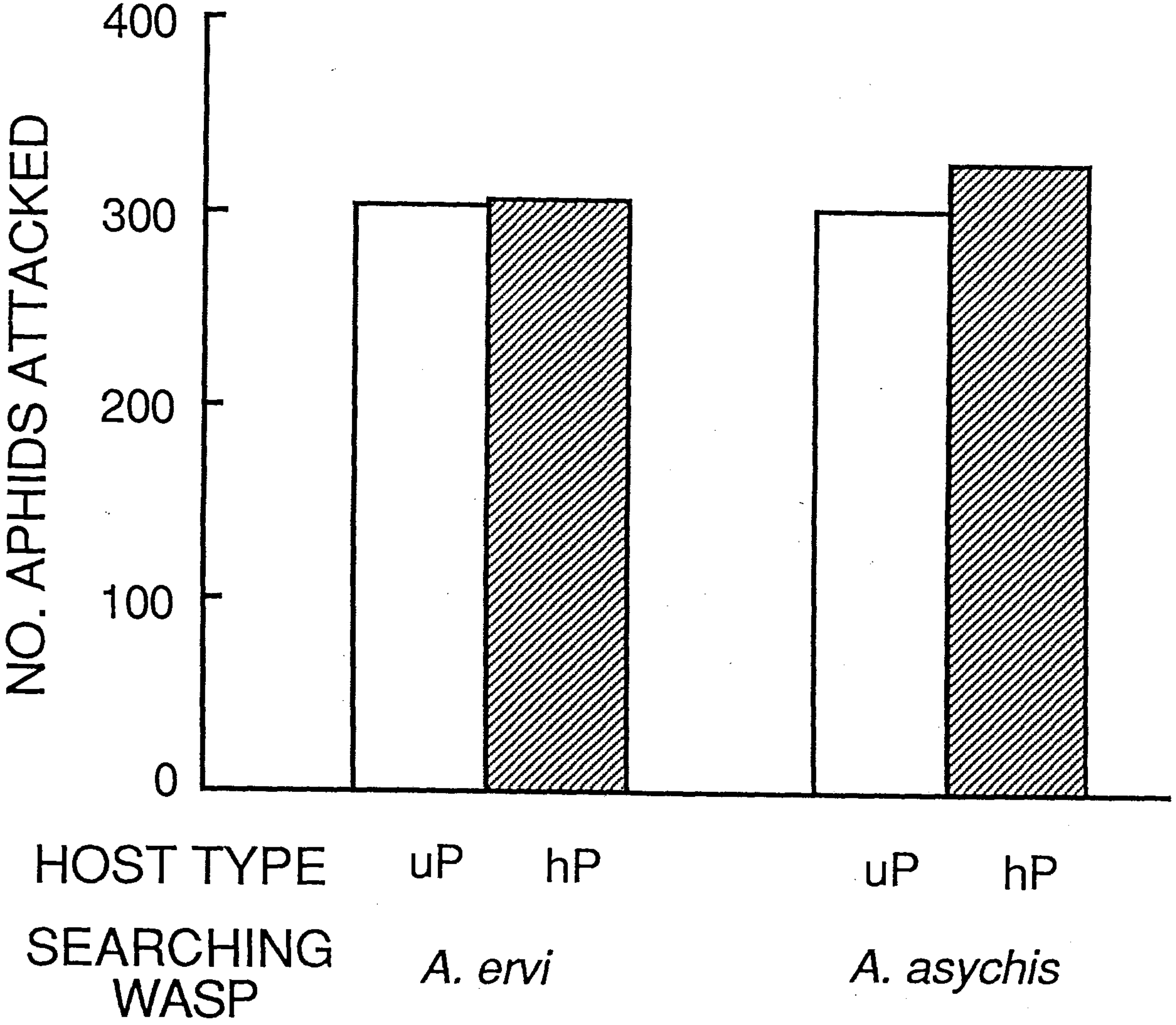
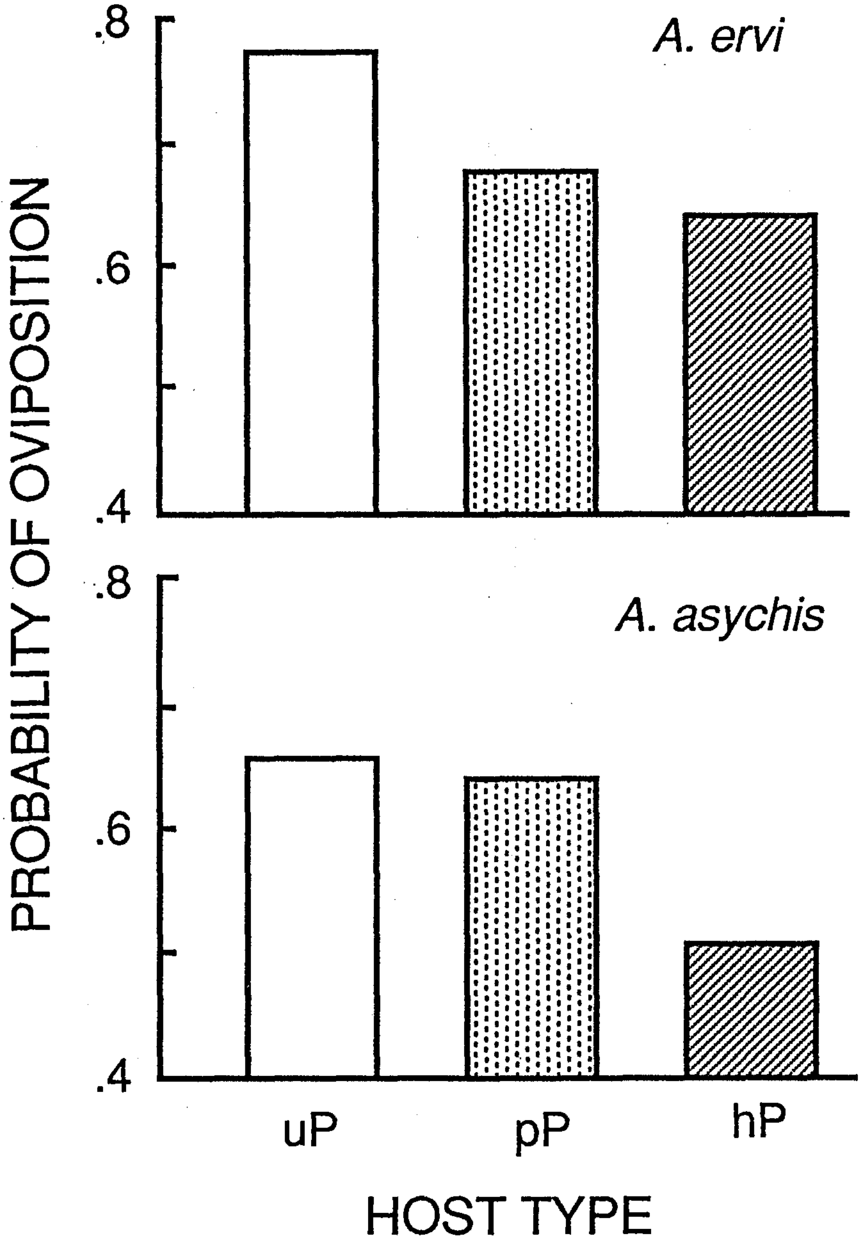

NO. APHIDS ATTACKED
400
300
200
100
0
HOST TYPE
uP
hP
uP
hP
SEARCHING WASP
A. ervi
A. asychis

Figure 6.2. Conditional probabilities of oviposition resulting from attack by *A. ervi* and *A. asychis* when provided with a choice of three types of pea aphids. uP (open) = unparasitized; pP (stippled) = pseudoparasitized; hP (shaded) = heterospecifically parasitized $\leq$ 1 h before trial.


A. ervi
A. asychis
PROBABILITY OF OVIPOSITION
.8
.6
.4
.8
.6
.4
uP
pP
hP
HOST TYPE

A. asychis (G_{adj} = 0.246, df = 1, P = 0.62), *A. asychis* females (Fig. 6.2 bottom) treated aphids pseudoparasitized by *A. ervi* like unparasitized aphids (G_{adj} = 0.050, df = 1, P = 0.82).

Superparasitism and larval competition

When unparasitized aphids were not available (Expt 2), searching females readily oviposited in aphids that had been parasitized or pseudoparasitized by the other species, independent of the length of the interval between attacks (Table 6.1). It should be noted that, in no-choice trials, the observed percentages of superparasitism (p_{AB}) were solely determined by the independent oviposition rates of *A. ervi* and *A. asychis*; the latter did not significantly differ from the parasitization rates of unparasitized aphids in choice tests (*A. ervi* = 77.6%, *A. asychis* = 65.7%).

One of the two competing larvae was usually eliminated within two, or rarely three, days after the second-laid egg had hatched to a first instar. *Aphidius ervi* tended to survive in superparasitized aphids at all intervals tested, except when *A. asychis* had a 7-day developmental advantage (Table 6.2). For T_{sup} = 168 h, *A. asychis* pupated before or soon after an *A. ervi* egg had hatched. Although some dissected aphids contained two live larvae, in the majority of such cases the *A. ervi* larva appeared healthy, while the *A. asychis* larva was abnormally small, a condition indicating that its growth was suppressed and the larva would eventually die. In two aphids, both competitors were dead from unknown causes (Table 6.2).

Examination of dead larvae in dissected aphids showed that *A. ervi* killed a potential competitor either by physical combat or possibly physiological suppression. Physical combat was restricted to a short period (≤ 24 h), a "fighting window", after the first instar hatched. During embryonic development, *A. ervi* did not affect *A. asychis* or vice versa. Both eggs hatched normally; thereafter interactions started and were usually

Table 6.1. Proportions of pea aphids parasitized by either or both of *Aphidius ervi* and *Aphelinus asychis* in no-choice trials.

T_{sup}[1] (h)	N[1]	p_0[1]	p_{AB}[1]	p_A[1]	p_B[1]	p_B/E(B)[1]
Oviposition sequence: A[2] = *A. ervi*; B[2] = *A. asychis*						
0	213	0.038	0.451	0.789	0.624	1.093
24	194	0.041	0.433	0.799	0.593	1.094
48	216	0.102	0.509	0.773	0.681	1.033
72	223	0.090	0.386	0.740	0.556	1.067
Oviposition sequence: A[2] = *A. asychis*; B[2] = *A. ervi*						
0	147	0.041	0.558	0.626	0.891	1.000
24	188	0.043	0.676	0.824	0.809	0.987
48	135	0.007	0.593	0.830	0.756	1.058
72	236	0.042	0.581	0.801	0.737	1.017

1. T_{sup} = time of superparasitism, i.e., interval between the first and second attack (in h); N = sample size; P = proportions of aphids escaping parasitism (p_0), parasitized by A (p_A), parasitized by B (p_B) and parasitized by both A and B (p_{AB}); E(B) = expected parasitism by B. Note that p_A and p_B include p_{AB} (see Materials and Methods for details).

2. Species A, B = first- and second-attacking parasitoid females in a sequence.

Table 6.2. Outcome of heterospecific larval competition between *Aphidius ervi* and *Aphelinus asychis* in superparasitized pea aphids.

T_{sup}[1] (h)	Age of larva A at host dissection (days)	N[1]	Outcome of larval competition (%)[2]			
			A. ervi survived	*A. asychis* survived	Both larvae	
					Live	Dead
Oviposition sequence: A[3] = *A. ervi*; B[3] = *A. asychis*						
0	5	96	86.5a,b	5.2	7.3	1.0
24	5	84	94.0a	3.6	2.4	-
48	8	110	92.7a	-	7.3	-
72	9	86	75.6b	5.8	18.6	-
Oviposition sequence: A[3] = *A. asychis*; B[3] = *A. ervi*						
0	5	82	85.4a,b	1.2	13.4	-
24	6	127	82.7b	-	16.5	0.8
48	7	80	86.3a,b	8.8	5.0	-
72	8	137	93.4a	-	6.6	-
168	10	12	-	100.0	-	-

1. T_{sup} = time of superparasitism (in h); N = sample size.
2. Subsets sharing the same letter(s) are not significantly different ($\alpha = 0.05$) by simultaneous test procedure for contingency tables (Sokal and Rohlf, 1981); only cases in which either species A or species B survived are included.
3. Species A, B = first- and second-attacking parasitoid females in a sequence.

finished within 1 to 3 days after the latest egg hatched. I observed two dissected aphids in which a first instar larva of *A. ervi* had its mandibles tightly embedded in the side of an early first instar larva of *A. asychis*. It was very hard to separate the two larvae with dissecting needles. Many dead first instar *A. asychis* larvae were also grossly deformed. After being bitten, their body contents sometimes exuded and were deposited around the wound forming a lump. Although older *A. ervi* larvae obviously had killed newly hatched *A. asychis*, I found no evidence on dead larvae of actual combat, such as wound marks or gross deformities, suggesting that physiological suppression was the cause of death. In a few cases, *A. asychis* survived by killing a competing *A. ervi* larva, probably by physiological means rather than physical combat, for which I found no evidence.

Discussion

A wasp's acceptance or rejection of an already parasitized host for oviposition is influenced by various factors. These factors can generally be divided into two groups: (1) those that are related to the female herself, such as her experience, her egg load and physiology, (2) those that are related to the host (environment), such as choices (good versus bad hosts) available, and the number of other wasps on the patch. Due to the difference in quality between unparasitized hosts and parasitized ones, wasps are expected to avoid superparasitism, and accept high quality hosts (generally unparasitized ones), under most conditions. However, it is not always clear how wasps distinguish between parasitized and unparasitized hosts. Although many species use external cues to identify parasitized hosts (Rabb and Bradley 1970; Price 1970; Bosque and Rabinovich 1979; Takasu and Hirose 1988), other species of parasitoids recognize internal cues (for a review, see Vinson 1976; Mackauer 1990). In the present study, I do not know the exact nature of the internal cues used by *A. ervi* and *A. asychis*. Other studies suggest that the presence of eggs or larvae (Fisher 1971), chemicals released by an adult in oviposition (Wylie 1970, 1972; Polaszek 1986; Stoltz 1986), and changes of host conditions due to the immature parasitoid (Thompson 1986; Vinson 1990) could all serve as internal cues to the second wasp.

My data show that females of *A. ervi* and *A. asychis* discriminated between unparasitized pea aphids and those stung or parasitized by the other species. Females attacked both host types equally as they were encountered, a fact indicating that they either did not recognize or ignored any external marks. It is worth noting that *A. ervi* uses a pheromone or pheromone-like substance to mark parasitized hosts (McBrien and Mackauer 1990) although I have no evidence in either this or the previous chapter to indicate this. Similar results on avoidance of conspecific superparasitism were reported for other species of aphidiid parasitoids, including *Ephedrus californicus* Baker (Chow and Mackauer 1986; Volkl and Mackauer 1990) and *E. cerasicola* Stary (Hofsvang and Hagvar 1986; Hofsvang 1988). By contrast, *A. asychis* must probe the host with the ovipositor to distinguish between parasitized and unparasitized aphids, a behavior indicating that oviposition decisions are influenced by internal rather than external cues (Chapters IV-V). The incidence of superparasitism varied with the availability of unparasitized hosts, which were generally preferred by both species. These laboratory results agree with observations in greenhouses (Hartley 1922) and in the field (Force and Messenger 1965) that *Aphelinus* females rarely oviposit in aphids already parasitized by aphidiids.

The differential cost in lost opportunity (= searching) time of making the wrong decision is reflected in the response of *A. ervi* and *A. asychis* to pseudoparasitized pea aphids. Whereas *A. ervi* females tended to reject pseudoparasitized aphids as if they were in fact parasitized, *A. asychis* accepted pseudoparasitized aphids as if they were unparasitized (Fig. 6.2). Because *A. asychis* requires on average 108 sec to attack and deposit an egg in, and about 61 sec to reject, an unparasitized aphid (Chapters IV, VIII), the opportunity cost of wrongly rejecting a suitable host is much higher for *A. asychis* than for *A. ervi*, which needs << 1 sec for attack and oviposition or rejection.

The dissection of superparasitized aphids showed that *A. asychis* larvae normally did not survive when competing with those of *A. ervi* for host resources, regardless of the oviposition sequence and the time between ovipositions (Table 6.2). *Aphelinus asychis* was likely to survive only if it could complete larval development (T_{sup} = 168 h) before *A. ervi* had hatched to a first instar (Table 6.2). To eliminate a possible competitor, *A. ervi* larvae used both physical attack and probably physiological suppression.

Aggressive behavior was restricted to a short period during the early first-instar stage. During dissection I found some cases of a living early first instar *A. ervi* (at the fighting window) together with a dead late first instar *A. asychis*. There were no wound marks or any other signs of physical combat on the dead *A. asychis*. I concluded that the death of *A. asychis* was most likely due to some form (unknown) of physiological suppression, but could not completely rule out the possibility of biting from *A. ervi*. Because there was plenty of food present and enough space for both parasitoids at the time of *A. asychis*' death, it is unlikely that selective starvation or lack of oxygen, two means of physiological suppression possibly used by *A. ervi*, were the causes for *A. asychis*' death. I also found no evidence that *A. asychis* eggs failed to hatch in hosts containing a 1-day older competitor, as was reported by Hartley (1922) for *Aphelinus semiflavus* Howard competing with *Aphidius matricariae* Haliday. As ambient conditions, especially differential effects of temperature on development, can influence and even reverse the outcome of larval competition (Force and Messenger 1965), Hartley's (1922) observations should not be rejected.

The mechanisms used by both *A. ervi* and *A. asychis* in heterospecific competition did not differ from those employed in conspecific larval competition, i.e., physical combat and physiological suppression by *A. ervi*; physiological suppression by *A. asychis* (Chapter V). The observed pattern suggests that the competitive mechanisms employed by these parasitoids are species-specific and relatively fixed, regardless of whether the competitors are conspecifc or heterospecifc.

Heterospecific host discrimination can be adaptive. Although I agree with Vet *et al.* (1984) that discrimination should be expected mainly among closely related species, such as sibling species, it does not follow that avoidance of superparasitism is necessarily rare among unrelated species (van Strien-van Liempt and van Alphen 1981; Bakker *et al.* 1985; Turlings *et al.* 1985). Competition normally incurs a cost, even for the surviving individual. This cost can be, e. g., reduced adult size and hence reduced fecundity (Force and Messenger 1965) or increased development time (McBrien and Mackauer 1990). Consequently, as long as "high quality" unparasitized hosts are available, solitary species of wasps should avoid oviposition in already parasitized hosts. However, when only parasitized hosts are available, oviposition decisions should vary with, among other factors, a wasp's previous oviposition experience and the availability and value of mature eggs in relation to the probability of her offspring's survival (Chapters V, VIII; Mackauer and Chow 1990; McBrien and Mackauer 1991).

Parasitoid oviposition decisions are influenced by a variety of signals and host cues that, singly or in combination, cause a particular host to be accepted or rejected. Some signals, such as external marking pheromones, are probably highly specific indicators of previous parasitism. Other cues are more general in nature, indicating low or altered host quality (Mackauer 1990). Although physiological changes in parasitized hosts are more likely to manifest themselves during the late phases of the interaction (Cloutier and Mackauer 1979; Cloutier 1986; Beckage and Templeton 1986; Hawlitzky and Boulay 1986; Thompson 1986), this fact by itself does not preclude the possibility that similar, but more subtle, changes occur shortly after oviposition when they would be more difficult to detect experimentally. These changes could include general host responses to, e.g., stress induced by stinging and oviposition with or without the injection of a venom or other material (Fisher 1971; Beard 1972; Stoltz 1986; Strand 1986; Mackauer and Chow 1990). It was reported that stinging by aphelinid (Wilbert 1964; Boyle and Barrows 1978) and some aphidiid wasps can cause short-term paralysis (Calvert and van den Bosch 1972) and otherwise affect aphid behavior (Gardner *et al.* 1984; Mackauer and Chow 1990). I suggest, and my data support, that host discrimination and avoidance of heterospecific superparasitism in *A. ervi* and *A. asychis* are based on the recognition of internal, and probably general, cues rather than on the wasps' ability to recognize external, and probably specific, oviposition marks made by a different species. It is not clear whether these internal cues originate with the parasitoid or the host. However, a response to a subtle change in host quality (rather than to some parasitoid-derived product) would seem a more likely explanation, especially in the case of unrelated parasitoid species, such as *A. ervi* and *A. asychis*.

CHAPTER VII

EFFECT OF SUPERPARASITISM ON SELECTED FITNESS COMPONENTS IN *APHIDIUS ERVI*

Introduction

Superparasitism refers to the phenomenon in which one or several parasitoid wasps deposit more eggs in or on a host than can successfully develop as larvae (Mackauer 1990). Recently, the traditional view of superparasitism as being maladaptive has been challenged by various researchers (Charnov and Skinner 1984; van Alphen and Visser 1990; van der Hoeven and Hemerik 1990; Visser *et al.* 1990). They have demonstrated, mostly through mathematic modelling, that superparasitism can be adaptive under certain circumstances. While this is true, and superparasitism can indeed be an evolutionarily stable strategy under certain situations as shown by van der Hoeven and Hemerik (1990), other aspects, e.g., the costs or disadvantages of superparasitism, have rarely been studied or have largely been ignored. The most obvious cost of superparasitism is that if the second egg (or additional clutch) allocated to a host loses the competition, the parental female loses one offspring and wastes opportunity time. For conspecifics, the probability of the second egg winning the competition is relatively small, normally ≤ 0.5, even when the time interval between the first and second oviposition is very short (Chapter V; Mackauer 1990), and this probability decreases with increases in time after the first oviposition. In other words, the second egg or clutch will generally be outcompeted by the first. Even if the second egg does manage to win the competition and survive, the competition itself or elimination of the competitor must incur some cost to the survivor. Such a cost may be evident in reduced body size and increased development time or reduced growth rate

(Vinson and Sroka 1978; Wylie 1983; Lawrence 1988). These changes in life history parameters may affect parasitoid fecundity, longevity and even offspring sex ratio and other parameters that contribute to total fitness (e.g., Salt 1941; Liu 1985).

The implicit assumption in models of superparasitism as an adaptive strategy, that superparasitism by solitary wasps has no fitness consequences for the surviving larva, has yet to be tested formally and is in fact at variance with several anecdotal observations. For example, Wylie (1983) reported that larvae of the solitary braconid parasitoid *Microctonus vittatae* Muesebeck required longer to complete development in superparasitized than in single-parasitized chrysomelid beetles. Observations of McBrien & Mackauer (1990) indicated an early delay in the development of *Aphidius smithi* when developing in the presence of an older competing larva of *Aphidius ervi*. Both parasitoids are koinobionts (Askew & Shaw 1986). In contrast to idiobionts, which develop in non-growing or paralyzed hosts, the host of a koinobiont continues to feed, grow, and metamorphose during the initial phases of parasitism. In koinobiont-host associations, host quality is not a linear function of host size but varies with future host growth, which is dependent on the host stage at parasitization (Sequeira & Mackauer, 1991a). The cost, if any, of superparasitism in terms of reduced offspring fitness thus will be determined by the ability of the surviving larva to compensate for reduced growth during embryonic and early larval development (when it must compete with other larvae for host resources) by increased growth during late larval development.

Here I tested this hypothesis by using *A. ervi* and the pea aphid as my experimental system. Unlike gregarious parasitoids, in which there is no clear separation between superparasitism and crowding (but no superparasitism, according to definition), solitary species such as *A. ervi*, and its host, provide a workable system for testing hypotheses because the degree of superparasitism can be experimentally controlled. This chapter extends those studies presented in previous chapters, and asks further questions on the consequences of superparasitism in the survivors. In this chapter, I compare the rate of development and adult size between (male) parasitoids that developed in single-parasitized and in superparasitized hosts. I show that the total development time from oviposition to adult eclosion was the same in both groups. However, wasps that developed in superparasitized aphids had greater dry mass than their counterparts from single-parasitized hosts. I discuss these findings with regard to the evolutionary consequenses of superparasitism.

Materials and Methods

Parasitoid preparation

I set up a new parasitoid generation by placing 10-15 mated *A. ervi* females in a wax paper cup which contained about 100 third-instar nymphs of pea aphids. After 3-4 h, the aphids were transferred to fresh bean plants and left to develop until those that had been parasitized formed mummies containing parasitoid pupae. The mummies were removed from the plants and placed singly in gelatin capsules (size 00; Parke-Davies Canada Ltd., Scarborough, Ontario). Eclosed wasps were sorted by sex. Females were provided with water-diluted honey as food and were used for experiments when 3-5 days old, while males were returned to the stock colony or were discarded. In all experiments, I used 3- to 4-day-old (second to early third) nymphal instars of the pea aphid.

Experimental design

In three different trials, I compared development time (DT) from oviposition to adult eclosion and dry mass (DM) between parasitoids that had developed in single-parasitized aphids and those that had developed in superparasitized aphids. The three trials differed in the rearing temperature, which varied between 24 ± 1°C (trial 1), 21 ± 1°C (trial 2), and 20 ± 1°C (trial 3). The experimental design gave information as to whether parasitoid DT and DM were affected by superparasitism and different temperatures.

To obtain single-parasitized aphids, I introduced an unmated female wasp into a Petri dish (6 cm in diameter) that contained about 100 aphids. Because unmated females lay only unfertilized eggs, all offspring were male, a fact that allowed me to avoid possible bias resulting from differential development and survival between male and female larvae. The wasp was permitted to strike with her ovipositor any aphid she encountered; these aphids were removed immediately. After about 20-30 oviposition attempts, the female was replaced by another. For an estimate of the percentage of parasitism, I set aside a subsample of these single-attacked aphids, which were reared on bean plants until mummy formation.

To obtain superparasitized aphids, I returned about half of the single-parasitized aphids to the Petri dish, where they were exposed to a second ovipositional attack by an *A. ervi* female, as described. Because these wasps normally discriminate between unparasitized and parasitized aphids and reject the latter for oviposition (Chapter V), I repeated this procedure once more so that aphids in the "superparasitized" group were stung three times by different females and could contain up to three eggs. The percentage of superparasitism was estimated from a subsample of the aphids set aside as a control; these aphids were dissected after about four days to count the number of parasitoid eggs or larvae in each aphid. The time between the first and the third attack was less than 1.5 h in all trials.

At the end of a trial, the two groups of single- and superparasitized aphids, as well as the respective control samples, were caged on bean plants. The cages were labelled as to treatments and dates, and were kept in a plant growth chamber with controlled temperatures. When the parasitized aphids became mummified, the mummies were removed from the plants and placed singly in gelatin capsulses (size 00). I attached these capsules, arranged in rows, with scotchtape to a square of white cardboard. The cards were placed on the vertical wall inside the growth chamber, and were monitored (through a glass window on the chamber) with a video camera which recorded, for each wasp, the exact time of eclosion and hence DT (Sequeira & Mackauer 1991a). Wasps were left to die in the capsules. They were then dried in an oven at 100°C for two days and individually weighed on a Mettler UM3 electronic microbalance (sensitivity, 0.001 mg) to obtain the DM.

Statistical analysis

I compared the means of DT and of DM between wasps from single- and superparasitized hosts by Kolmogorov-Smirnov 2-sample tests, separately for each trial, and by ANOVA for differences between trials within treatments. For all tests, I used programs in the SPSS_x (Release 3.0 for IBM MTS) collection of statistical procedures. I used the *G*-test with Williams' correction (Sokal & Rohlf 1981, p. 737) for testing the independence of mummy mortality from treatment effects. Correlation between DT and DM was analyzed by the SAS procedure CORR (SAS 1985).

Results

Dissection of control samples showed that parasitism by *A. ervi* was relatively high in all trials. An average of 77.5% (95% C.I., 73.2-81.6%; N = 374) of the aphids that were struck once by a wasp were parasitized (Table 7.1). After three successive attacks, virtually all of the aphids were parasitized (mean = 99.4%; 95% C.I.,

Table 7.1. Percentages of parasitism and superparasitism in control samples of pea aphids which were stung once or three times by unmated females of *Aphidius ervi*. Single-parasitized aphids were reared until they became mummified; superparasitized aphids were dissected to determine the number of parasitoid eggs and larvae in each aphid.[*]

Trial	Single-parasitized		Superparasitized		
	N	% para	N	% super	x_{eggs}
1	114	78.1	51	70.6	2.36 ± 0.54
2	129	81.4	54	87.0	2.49 ± 0.83
3	131	73.3	60	71.7	2.42 ± 0.50

[*] N = sample size; % para = percentage of aphids mummified; % super = percentage of parasitized aphids containing $\geq$ 2 parasitoid eggs/nymph; x_{eggs} = mean (± SEM) number of parasitoid eggs/nymph in superparasitized aphids.

97.7-100%; N = 165) and 76.4% were superparasitized, containing an average of 2.43 (SEM = 0.65) eggs (Table 7.1). Because I was unable to determine the number of parasitoid offspring, if any, in aphids found dead in the cages, these aphids (about 11% overall; no significant differences between aphids that were stung once and three times) were not included in the above totals. In trial 1 (24°C), adult wasps failed to emerge from 29.1% (N = 103) of the mummies formed by "superparasitized" aphids as compared to only 11.2% (N = 89) in the single-parasitized group ($G_{adj} = 9.553$; $P = 0.002$). However, differences in the percentage of non-emergence between the two treatment groups were not significant in trial 2 (superparasitized: 15.4%; N = 130 vs. single-parasitized: 8.6%; N = 105; $G_{adj} = 2.521$; $P = 0.11$) or trial 3 (10.5%; N = 124 vs. 9.4%; N = 96; $G_{adj} = 0.073$; $P = 0.79$).

Parasitoid DT decreased with increased temperature (Fig. 7.1). Superparasitism did not affect DT, which was the same in *A. ervi* males from single- and superparasitized aphids in all three trials (Kolmogorov-Smirnov 2-sample test, trial 1: $D = 0.121, P = 0.64$; trial 2: $D = 0.144, P = 0.24$; trial 3: $D = 0.064, P = 0.99$). However, wasps that developed in superparasitized hosts achieved a significantly higher DM (Fig. 7.2) which exceeded that of their counterparts from single-parasitized aphids by an average of 14% (K-S 2-sample test, trial 1: $D = 0.547, P < 0.001$; trial 2: $D = 0.210, P = 0.02$; trial 3: $D = 0.433, P < 0.001$). Mean DM was highest in trial 2 (1-way ANOVA, single-parasitized aphids: $F_{2,259} = 58.49, P < 0.001$; superparasitized aphids: $F_{2,290} = 13.15, P < 0.001$), a fact indicating that a temperature of about 21°C was most suitable for the growth of parasitoids or hosts, or both.

DM was not significantly correlated with DT in parasitoids from either single- or superparasitized hosts (correlation coefficients for wasps from single-parasitized hosts were 0.17, 0.07 and -0.02 respectively for trials 1, 2, and 3; for those from superparasitized hosts, 0.12, 0.12 and 0.14); larger wasps did not necessarily require

Figure 7.1. Frequency distributions of development time from oviposition to adult eclosion of *Aphidius ervi* males reared in apteriform nymphs of pea aphid which were single-parasitized (open columns) and superparasitized (shaded columns). x = mean; s = standard deviation; n = sample size.

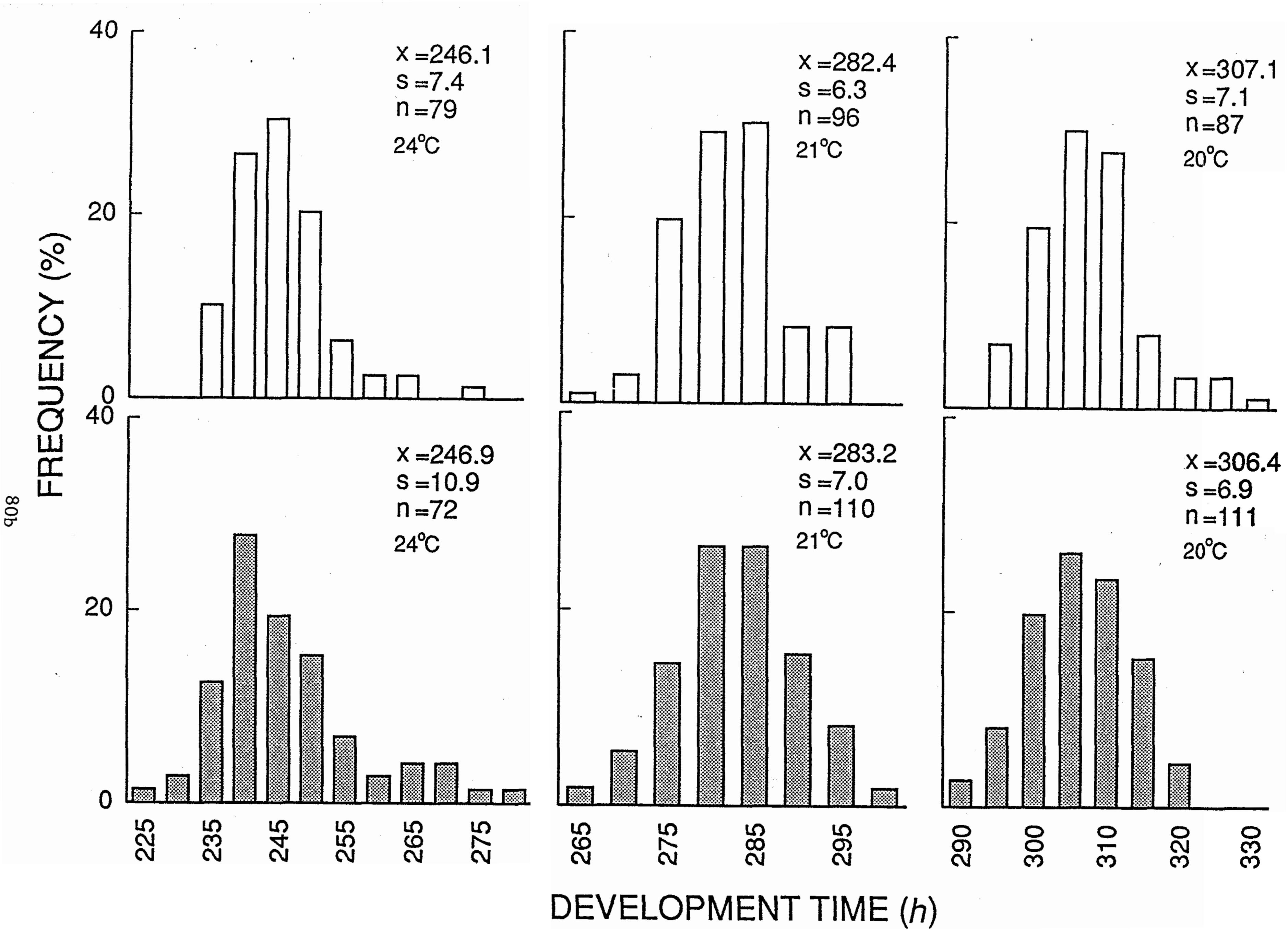
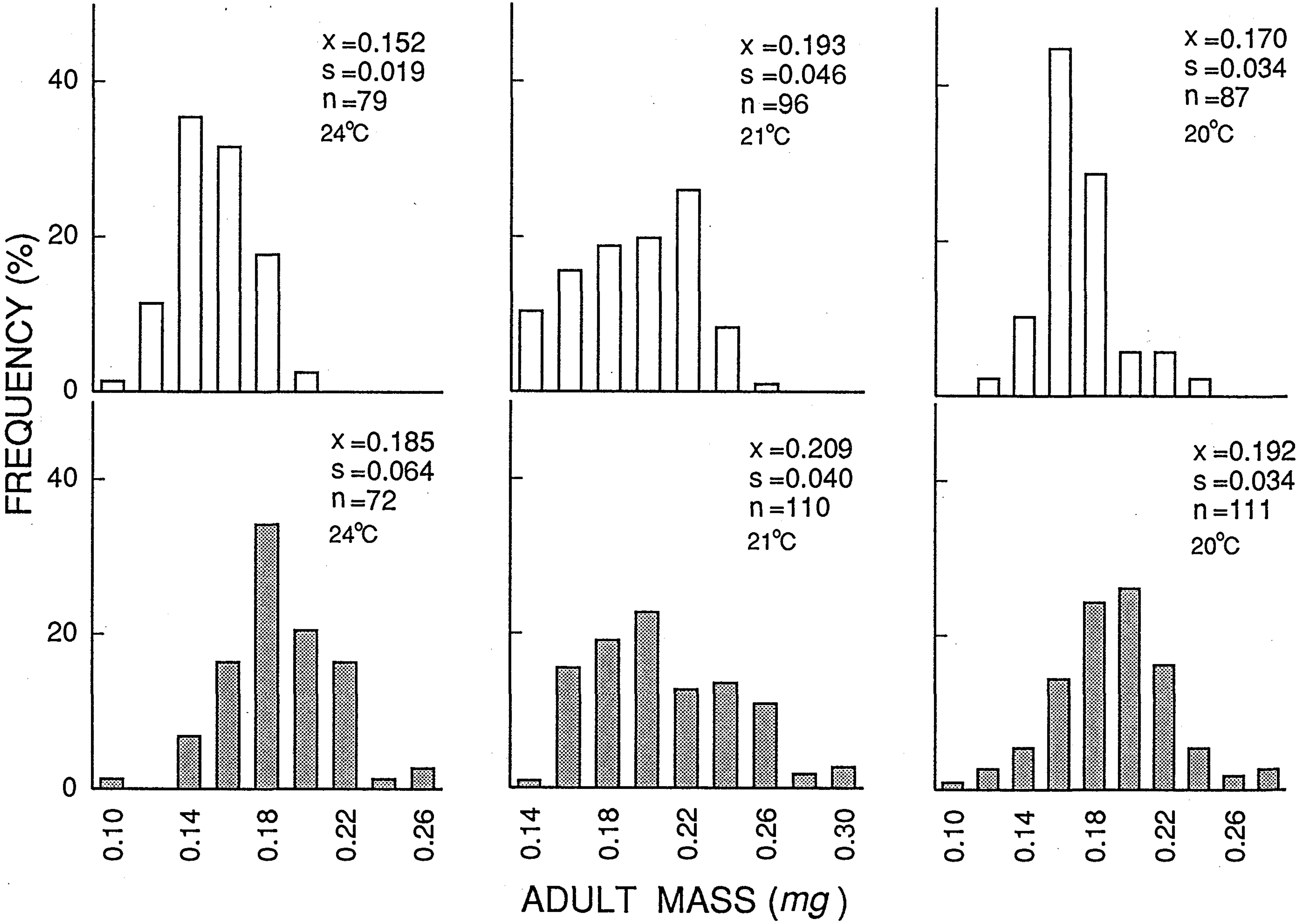

40
20
0
40
20
0
FREQUENCY (%)
x =246.1
s =7.4
n =79
24°C
x =282.4
s =6.3
n =96
21°C
x =307.1
s =7.1
n =87
20°C
x =246.9
s =10.9
n =72
24°C
x =283.2
s =7.0
n =110
21°C
x =306.4
s =6.9
n =111
20°C
225
235
245
255
265
275
265
275
285
295
290
300
310
320
330
DEVELOPMENT TIME (h)

Figure 7.2. Frequency distributions of dry mass of *Aphidius ervi* males reared in apteriform nymphs of pea aphid which were single-parasitized (open columns) and superparasitized (shaded columns). x = mean; s = standard deviation; n = sample size.


x =0.152
s =0.019
n =79
24°C
x =0.193
s =0.046
n =96
21°C
x =0.170
s =0.034
n =87
20°C
x =0.185
s =0.064
n =72
24°C
x =0.209
s =0.040
n =110
21°C
x =0.192
s =0.034
n =111
20°C
FREQUENCY (%)
40
20
0
40
20
0
0.10
0.14
0.18
0.22
0.26
0.14
0.18
0.22
0.26
0.30
0.10
0.14
0.18
0.22
0.26
ADULT MASS (mg)

longer to develop from oviposition to adult eclosion, or vice versa.

Discussion

I designed these experiments to examine the effects of superparasitism on survivors in terms of development rate and adult size--presumed components of fitness. Anecdotal observations on *A. ervi* (McBrien and Mackauer 1990) showed that superparasitism caused a delay or smaller immature size during early larval stages. However, it was not known if this slow development and small body size would persist until adult emergence, or if the survivor could compensate for this early delay by growing faster during later stages, when exponential growth starts (Sequiera and Mackauer 1991a).

My results show that development time and adult mass of *A. ervi* males are affected differently by superparasitism. Superparasitism had no measurable influence on total DT from oviposition to adult eclosion (Fig. 7.1). However, parasitoids achieved a greater DM if they developed in superparasitized rather than in single-parasitized aphids (Fig. 7.2). Because only about 80% of the dissected control aphids that were stung three times by *A. ervi* contained two or more parasitoid larvae, i.e., were in fact superparasitized (Table 7.1), the influence of superparasitism on mean DM gain is probably underestimated. Parasitoid emergence from superparasitized aphids was reduced in trial 1 (at 24°C) but not significantly in trials 2 and 3, a fact suggesting that the rearing temperature may have played a role. These findings on male wasps should hold for females as well. Sequeira and Mackauer (1991b) showed that both sexes of *A. ervi* develop at the same rate and that female DM is 1.1 times the male DM consistently, independent of host instar. My results do not agree, or agree only partially, with observations by Wylie (1983) on *M. vittatae* and Vinson & Sroka

(1978) on *Cardiochiles nigriceps* Viereck, who both reported that parasitoid DT was increased in superparasitized hosts. Also, the percentage of *C. nigriceps* emerging decreased with increased superparasitism, from 92% in hosts stung once to 21% in hosts stung ≥ 5 times. Vinson & Sroka (1978) explained the reduced emergence as a result of wounds the larvae inflicted on each other during contests.

In solitary parasitoids, such as *A. ervi*, larvae compete for possession of the host. Competition usually occurs during the early first instar, with the first-hatched "oldest" larva likely to win in any contest (Chapter V; Mackauer 1990). Because larval growth and development vary non-linearly with host quality in an instar-specific manner, any limitation on host resources will be reflected in reduced parasitoid growth (Sequeira & Mackauer 1991a). Such limitation may result from, e.g., increased parasitoid demands (by several competing larvae) or inadequate host nutrition (Mackauer & Kambhampati 1984; Kouame & Mackauer 1991). However, once all potential competitors have been eliminated, which usually occurs within a relatively short period, 1-3 days after the eggs hatch (Chapters V, VI; Fisher 1961b; McBrien & Mackauer 1990), the surviving *A. ervi* larva can compensate for any early delay during the remainder of its development by accelerating its growth rate. The fourth larval instar, 2-3 days before pupation, is the period in which *A. ervi* generally achieves ca. 50-70% of its final larval body size (Sequeira and Mackauer 1991a). Surviving parasitoids may ingest more food, and easily offset the early developmental reduction in this late larval stage. Thus, in terms of total time required for completing development, survivors in superparasitized hosts can compensate and still emerge at the same time as their counterparts from singly parasitized hosts.

More important perhaps, parasitoids which developed in superparasitized pea aphids had a significantly higher DM than their counterparts, a result suggesting differences in host quality. Parasitism may influence an insect's food consumption which either may increase (Slansky 1978, 1986) or, more commonly, decrease (Thompson 1985). Pea aphids parasitized by *A. smithi* ingested more food, but assimilated it less efficiently, than unparasitized controls (Cloutier & Mackauer 1979). Interestingly, incorporation efficiency was highest in superparasitized aphids, which also grew at a higher rate than their single-parasitized counterparts (Cloutier and Mackauer 1980). As a consequence, the surviving parasitoid larva in superparasitized aphids may benefit from the increased growth potential of its host. Such additional host resources can be allocated to different parasitoid fitness components, including adult size and development rate. It is worth noting that, in *A. ervi*, female DM is correlated with fecundity although the relationship is not linear throughout its range, with fecundity reaching an upper threshold in large wasps (Sequeira & Mackauer 1991b).

Some alternative explanations exist for the greater DM of parasitoid adults emerging from superparasitized hosts. For example, the largest larva (or the larva with the highest growth potential) may be selected in a superparasitized host. Aphid physiological constraints on parasitoid growth, if there are any, may be better overcome by two parasitoid larvae than by one. Materials injected into a host by wasps at the time of oviposition could inhibit host physiological defenses against parasitoid eggs (Vinson 1977). The multiple injections associated with superparasitism may mean less physiological constraints for parasitoid (survivor) growth in superparasitized hosts. Teratocytes, the giant cells derived from serosal membranes of parasitoid embryos and released into hosts upon hatching (Vinson 1970), are more abundant in superparasitized hosts than in singly parasitized ones. If these cells have a nutritional function for the parasitoids, as proposed by other researchers (Strand *et al.* 1988; Dahlman 1990), the survivors in superparasitized hosts will certainly be in a better nutritional position even if the aphids do not consume more food.

Any costs of superparasitism in terms of reduced offspring survival and growth are expected to differ between solitary and gregarious species and, also, between koinobionts and idiobionts. Whereas hosts parasitized by koinobionts may vary their food consumption in response to the parasitoid's nutritional and energetic demands, total food resources are fixed in idiobiontic interactions, such as egg or pupal parasitoids, where hosts represent a fixed amount of resources and do not grow (M. Mackauer & R. Sequeira unpublished). Thus, any effects of superparasitism on the growth and development of the surviving larva(e) should, in theory, be more evident in idiobionts than in koinobionts. Compared to those in singly parasitized hosts, survivors in superparasitized, non-growing hosts have relatively fewer resources available, simply because some of the resources have been consumed by the competitors before their death. Therefore, superparasitism would be expected to produce smaller rather than larger parasitoids. The situation may of course be different if, after parasitization, the hosts continue to feed and grow. However, the notion that the effects of superparasitism should be more evident in idiobionts than in koinobionts may not hold in practice for the following reason. In some solitary koinobionts, such as *A. ervi*, compensatory feeding during the late larval stages can mask any deleterious effects during early development when it will be more difficult to detect by experiment. In gregarious koinobionts (and probably also in gregarious idiobionts), any consequences of superparasitism on adult fitness components may be obscured by resource competition and variable larval survival, resulting from overcrowding. Typically, adult size and survival decline with the initial number of parasitoid eggs (Skinner 1985; Bai *et al.* 1991), often differentially between males and females (Wylie 1965, 1976; Beckage & Riddiford 1978, 1983). Note that gregarious larvae do not engage in physical combat; supernumeraries are eliminated by starvation when host resources become inadequate for the support of all the parasitoid immatures. Also, the potential for resource wastage under superparasitism would seem high in gregarious species because the survivors do not eat the starved larvae (Salt 1961).

My results suggest that, in *A. ervi*, superparasitism has no costs but may result in a benefit to the surviving parasitoid offspring. However, any benefits in terms of increased offspring DM must be balanced against by any costs in reduced offspring survival. These costs differ between self superparasitism and conspecific or heterospecific superparasitism (Mackauer 1990; van Alphen and Visser 1990). Generally, the probability of the second larva winning a conspecific contest decreases with the age advantage of the first larva (Chapter V; Mackauer 1990). A gain of 14% in adult DM (Fig. 7.2) is unlikely to compensate, or compensate completely, for a 50% or greater reduction in egg survival, especially because fecundity and longevity do not increase with size above a threshold value (Sequeira and Mackauer 1991a, b). Therefore, it may not be always adaptive for a female to oviposit in an already parasitized host. Superparasitism can be functional only under certain conditions (van Alphen and Visser 1990).

My results relate to several models of superparasitism as an evolutionary stable strategy (ESS) (van der Hoeven & Hemerik 1990; Visser *et al.* 1990). These models ignore the possibility that, in solitary species of wasps, superparasitism may have fitness consequences for the surviving larva. I have shown that this assumption need not be valid. Any costs (or benefits) of superparasitism in terms of offspring fitness will be association-specific (M. Mackauer & R. Sequeira unpublished). Whereas in the pea aphid-*A. ervi* system, parasitoid offspring gained in adult DM (without a correlated increase in DT) if they developed in superparasitized aphids, I suspect that superparasitism will have negative consequences in other, and perhaps many, parasitoid species (Vinson & Sroka 1978; Wylie 1983). These consequences must be considered in evolutionary models of superparasitism. In particular, studies will be needed of those host-parasitoid associations in which the host's food consumption and growth is reduced or inhibited by superparasitism (Lawrence 1988).

CHAPTER VIII

REPRODUCTIVE STRATEGIES OF *APHIDIUS ERVI* AND *APHELINUS ASYCHIS*

Introduction

Strategies of reproduction differ among species of insect parasitoids (Price 1973, 1975). Parasitic hymenopterans show a 'slow to fast' continuum in life history traits that are related to reproduction (Blackburn 1991a, b). Some species produce many eggs, but invest relatively little in each, as shown by the minute size of each egg; others produce few but relatively large eggs (Hinton 1981). Species of the first group have been classified as r-strategists (r-selection) (Force 1975; Pianka 1984); these species normally lay many eggs in different hosts, only some of which will survive. Species of the second group, comparable to K-strategists (K-selection), usually lay few eggs which suffer relatively low mortality. Different reproductive strategies influence parasitoid oviposition or host selection behaviors (Iwasa *et al.* 1984; Charnov and Stephens 1988; Mangel 1989). Some species are highly selective with regard to the host in which they lay their eggs; others may be less selective.

The oviposition decisions of two aphid parasitoids, *Aphidius ervi* and *Aphelinus asychis*, showed different patterns when tested under the same conditions (Table 8.1; Chapters IV, V). *Aphidius ervi* accepted freshly parasitized hosts and rejected those parasitized ≥ 24 h, whereas *A. asychis* rejected all parasitized hosts, no matter when these were parasitized. The question thus arises why these two species have such different oviposition behaviors with regard to parasitized hosts. In this chapter, I present reasons that may underlie these different host selection patterns. I discuss my findings in a broader sense to include other parasitoids with similar reproductive

Table 8.1. Comparison of host discrimination behavior between *Aphidius ervi* and *Aphelinus asychis* under different host conditions

Host condition	*Aphidius ervi*	*Aphelinus asychis*
Unparasitized hosts available (choice)	discriminates internally	strongly discriminates internally
Unparasitized hosts not available (no choice)	no oviposition restraint at $T_{sup}=0$ h high oviposition restraint at $T_{sup} \geq 24$ h	high oviposition restraint at all T_{sup}

Note: The information in this table is abstracted from Chapters IV and V. T_{sup} = time interval between the first and second oviposition, in hours.

strategies, and show that both behaviors are adaptive.

Basic Concepts

When foraging for hosts, parasitoids go through a series of steps (Salt 1935; van Alphen and Vet 1986). Upon encountering a potential host, a female has to decide whether to accept it and, if so, how many eggs to lay in the host. This chapter deals with the first decision. The second decision relates to clutch size, which was discussed thoroughly by other authors (Waage and Ng 1984; Waage and Godfray 1985; Skinner 1985; Godfray 1987). Whether a parasitoid oviposits in an encountered host depends on a number of factors. The two most important factors are probably (1) host quality, including host status (i.e., if the host has already been parasitized) and host species, size, age and (2) parasitoid oviposition pressure, including a female's egg load, her investment in each egg, and her physiological state. Generally speaking, the probability of a parasitoid ovipositing in a host increases with host quality and oviposition pressure. The two factors may or may not have equal influence on a wasp's oviposition decisions.

In this chapter, I will focus on the status of hosts. I consider this important because allocating additional offspring to an already parasitized host means that the larvae must compete for food. In gregarious parasitoids, this decision may result in reduced offspring size, or high larval mortality, or both. In solitary species, only one individual survives and all others die. Therefore, the status of a host can have significant fitness consequences for an ovipositing female. For parasitoid oviposition pressure, I consider (a) the number of mature eggs in the ovaries (thus, egg pressure), (b) the size of eggs relative to that of adults (therefore, the investment in each egg) and (c) the ability of a parasitoid to recover egg resources, i.e., oosorption in case suitable hosts are unavailable. The general rule is that parasitoids should oviposit in high quality hosts at all times. In case high quality hosts are scarce or not available, for example when most or all the hosts in a patch have already been parasitized, a female may accept parasitized hosts if she has a large egg load, or if an egg does not represent a large investment, or if she cannot recover the egg resources. The probability of the egg surviving competition also affects the parasitoid's decision. High survival is expected to balance the parasitoid's decision towards oviposition, whereas low survival tends towards rejection.

Experiments

Number of eggs in ovaries and size of mature eggs

For both *A. ervi* and *A. asychis*, I determined the age-specific egg loads by dissecting different-aged females, starting with newly emerged wasps up to those 10 days old. Ovaries of a female were separated from her abdomen and placed in a drop of water on a microscope slide. Eggs inside the ovaries were squeezed out by placing a cover slide over the drop and then gently pressing. Eggs from a single female were counted at an appropriate magnification. *A. ervi* eggs required the greater magnification because of their small size, whereas *A. asychis* eggs could be counted reliably under a dissecting microscope with lower magnification (Chapter III).

The maximum length and width of each egg were measured with a micrometer unit mounted in an eyepiece of the microscope. Eggs of *A. asychis* are elongate and banana-shaped. I assumed that they approximated a cylinder, and calculated their volume from the formula $V = \pi R^2 h$, where $\pi = 3.1415926$, R and h are the radius (=1/2 width) and height (=length), respectively. *A. ervi* eggs are ellipsoidal. I calculated their volume by the formula, $V = 4/3\pi ab^2$, where a and b are the long radius (= 1/2 length) and short radius (= 1/2 width) respectively.

Duration of oviposition and adult dry mass

The time invested by a female in depositing an egg was measured as the duration of one oviposition. Using a stop watch I timed each oviposition from ovipositor insertion until withdrawal. Every struck aphid was dissected to confirm oviposition. Only those that contained parasitoid eggs upon dissection were included in the calculation.

The size of adult females of each species was measured as dry mass (micrograms). Females of about 2 - 4 days old were oven-dried at 100°C for 24 h, and then weighed on a Mettler UM3 electronic microbalance. In *A. ervi*, the weight was obtained for individual females. In *A. asychis*, females were weighed in groups of 10 because of their small individual size.

From the data of egg and female size I could calculate the relative resources invested in an individual egg by each species. A higher ratio of egg size/adult size meant that the species had invested relatively more resources in each egg. A lower ratio indicated the opposite. Such an investment ratio can be used as an index to indicate how valuable each egg is to a female parasitoid.

Oosorption and oogenesis

To determine if a species could resorb eggs under adverse conditions, I deprived females of honey and hosts for a period of 48 - 72 h; they had access only to water during this period. The ovarian status of females was monitored every 12 h by dissection. The mature eggs and those that had been resorbed, if any, were counted. Preliminary experiments showed that after 72 h under such conditions almost all females of both species would die. Therefore, between 48 and 72 h, food was provided again to determine if females could oogenate. For *A. ervi*, honey was supplied as food; for *A. asychis*, only hosts (but not honey) were provided. The ovarian status of each species was monitored every 12 h as before, and the mature eggs, produced through oogenesis, were counted and recorded.

Results

Time and resource investment into each egg

The relative resources and time invested into each egg by female *A. ervi* were much smaller than those by *A. asychis* (Table 8.2). *A. ervi* took a fraction of a second to lay a single egg whereas *A. asychis* needed 108 seconds on average to do the same. The *A. ervi* eggs were about one fifth the size of *A. asychis* eggs, whereas adult females of *A. ervi* were almost 6 times larger than those of *A. asychis*. These differences in egg and adult size resulted in a much smaller relative investment per egg in *A. ervi*, and a much greater one in *A. asychis*.

Egg load

Both *A. ervi* and *A. asychis* are synovigenic, with a few mature eggs in their ovaries at emergence (Fig. 8.1). *A. ervi* achieved the highest egg load around day 4-6 with about 290-320 mature eggs. *A. asychis* reached maximum ovary capacity of about 25 mature eggs at day 3 and stayed at a slightly lower level thereafter. At any time during the first 10 days, female *A. ervi* had at least 10 times as many eggs as female *A. asychis*. This fact, in combination with the time invested in oviposition, indicates that *A. ervi* is likely to be time-limited and *A. asychis* likely to be egg-limited.

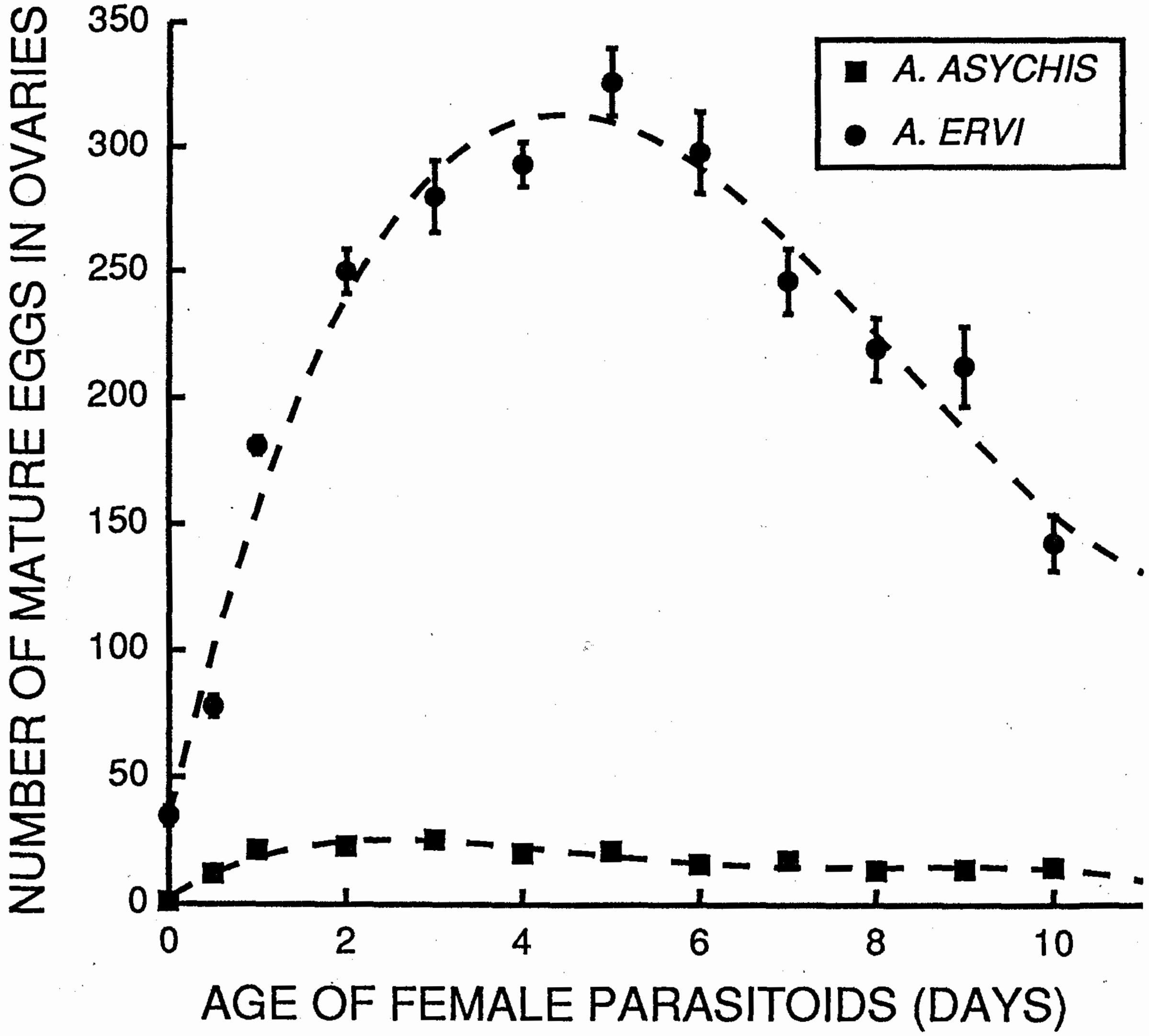
Table 8.2. Comparison of fitness parameters between two species of aphid parasitoids, *Aphidius ervi* and *Aphelinus asychis*

	Aphidius ervi		*Aphelinus asychis*	
	N[†]	Mean ± SEM[†]	N[†]	Mean ± SEM[†]
Female egg load[‡]	133	207.3 ± 8.1	120	16.6 ± 0.7
Duration of oviposition (sec)	110	<<1	168	108.0 ± 2.7
Size of eggs (10^{-3} mm^3)	57	0.256 ± 0.016	30	1.224 ± 0.029
Dry mass (mg) of females	50	0.317 ± 0.009	120	0.054 ± 0.001
Relative investment in each egg (10^{-3} mm^3/mg)	---	0.808	---	22.667

[†] N = sample size; SEM= standard error of mean.

[‡] Ovarian eggs were counted in females of both species every day for the first 10 days after eclosion, then averaged.

Figure 8.1. Comparison of egg loads in *Aphidius ervi* and *Aphelinus asychis* at specific ages.

NUMBER OF MATURE EGGS IN OVARIES
350
300
250
200
150
100
50
0
0
2
4
6
8
10
AGE OF FEMALE PARASITOIDS (DAYS)
■ A. ASYCHIS
● A. ERVI

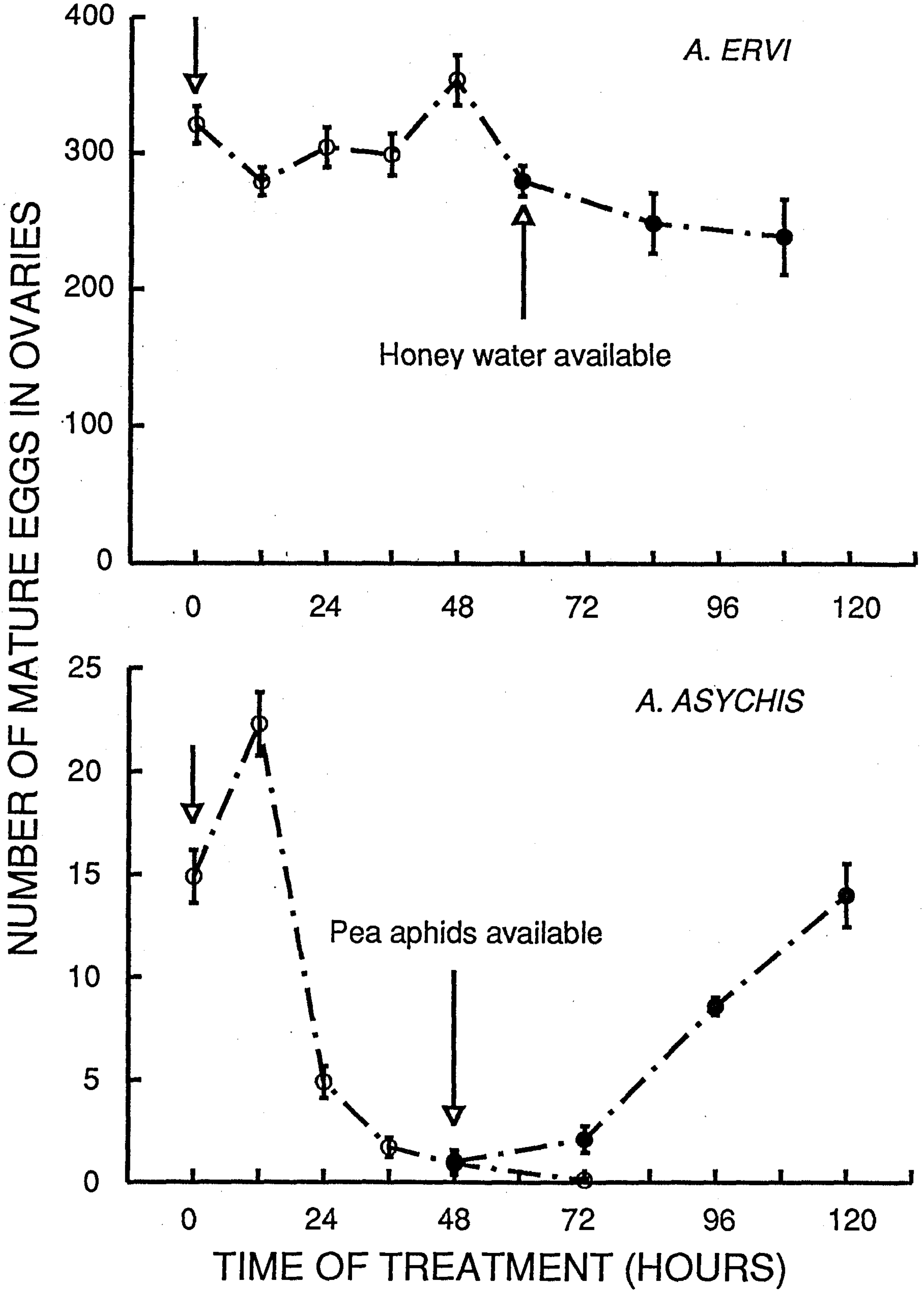
Oosorption and oogenesis

A. ervi could not recover resources invested in eggs through oosorption whereas *A. asychis* could (Fig. 8.2). In *A. ervi*, the number of mature eggs in the ovaries remained more or less the same during the 60 h of starvation (Fig. 8.2 top). Although the wasps suffered a 49% mortality (N=78) by 60 h, those that survived had about 280 mature eggs left in their ovaries. If starvation continued, 90% of the wasps would die by 72 h. Dissection of those wasps that died during 60 - 72 h showed that newly dead females still had on average 250 eggs in their ovaries (N=10, SD=25.7, range 210-280). After diluted honey was provided at 60 h, the wasps no longer died from starvation. However, the egg load remained constant thereafter.

In *A. asychis*, oosorption started between 12 and 24 h after starvation (Fig. 8.2 bottom). The initial increase of egg numbers at 12 h after starvation was resulted from the fact that females were still generating eggs and oosorption had not yet started. Because there were no hosts available, in which these eggs could be laid, the eggs were retained in the ovaries. By 24 h, two thirds of the mature eggs were absorbed, and parasitoids suffered no mortality (N=31) due to starvation. By 48 h almost all the eggs, except occasionally 1-3 eggs positioned at the ovary base close to the oviduct, had been absorbed; parasitoids suffered a 50% (N=59) mortality. Under the microscope, absorbed eggs appeared transparent and slightly shriveled; they were deprived of contents with only the chorion intact. If starvation continued, 71% of the wasps (N=14) would die by 72 h, by which time no eggs were left in the ovaries (Fig. 8.2 bottom).

When provided with hosts at 48 h, female *A. asychis* re-produced eggs gradually. It took 72 h of oogenesis for the wasps to reach the same egg load as that before the starvation.

Figure 8.2. Effect of starvation (open circles) and feeding (closed circles) on oogenesis and oosorption cycles of female *Aphelinus ervi* and *Aphelinus asychis*. Arrows at 0 h indicate the start of starvation treatments. The short line from 48 h to the baseline at 72 h (bottom graph), shows the effect of continued starvation.


A. ERVI
Honey water available
A. ASYCHIS
Pea aphids available
NUMBER OF MATURE EGGS IN OVARIES
TIME OF TREATMENT (HOURS)
400
300
200
100
0
25
20
15
10
5
0
24
48
72
96
120

Discussion

The reproductive biologies of insect parasitoids influence host selection and oviposition decisions (Price 1975, 1980; Blackburn 1991b). Egg-limited parasitoids are expected to maximize fitness by maximizing progeny production per egg (Charnov and Stephens 1988); by contrast, time-limited parasitoids should maximize progeny production per unit of time (Iwasa *et al.* 1984). The classification of parasitoids according to egg or time limitations is analogous to the K/r selection theory (MacArthur and Wilson 1967; Pianka 1984). K-selected organisms generally produce few offspring but invest much in each; their offspring tend to suffer low mortality. Thus, females achieve a high fitness return per progeny. These organisms can be compared with egg-limited parasitoids. Organisms that are r-selected, normally produce many offspring but invest relatively little in each; their offspring generally suffer high mortality. Therefore, females produce many progeny to compensate for the high mortality. As a result, females maximize progeny production per unit of time.

My results indicate that *A. ervi* and *A. asychis* have different reproductive strategies. The former is time-limited and the latter is egg-limited. Species of *Aphidius* are generally short lived; adult females have a mean longevity of about 8 to 13 days and lay an average of 40 to 100 eggs per day (Mackauer 1983; Kambhampati 1987; Sequeira 1991). Most of the eggs are laid during the first 8 days (Sequeira 1991). By contrast, *Aphelinus* species are longer-lived, having a longevity of 20 - 25 days and sometimes even 46 days under laboratory conditions (Hartley 1922; Force and Messenger 1964 a, b), and also are less fecund in terms of daily egg production. Females usually lay a maximum of 20 (mean 10 - 15) eggs per day (Mackauer 1982; Chapter III).

Reproductive biologies probably affect oviposition decisions. In *A. ervi*, females invest very little in time and resources in each individual egg; resources invested in eggs cannot be recovered if the eggs are not laid. Therefore, females do not always discriminate against inferior hosts, such as those that are newly parasitized (Table 8.1). Although laying eggs in such hosts means that the progeny must compete with the ones already inside, and thus have a low chance of survival, it still pays a parasitoid to superparasitize if the egg laid has a high probability of surviving. The reason for this is that parasitoids do not lose much in resources and time if the egg is outcompeted. However, they gain one offspring if the egg survives. The willingness of parasitoids to oviposit in such inferior hosts depends, among other factors, on the probability of egg survival. Generally speaking, the higher the prospect of survival, the greater the probability of oviposition. If the expected survival is zero, i.e., the fitness return becomes nil, then females should not oviposit at all (total restraint). This trend was shown in my earlier experiments on *A. ervi* (Chapter V) where a high survival of eggs in newly parasitized hosts resulted in a reduced reluctance of the parasitoids to oviposit and, a low survival of eggs in hosts that were parasitized ≥24 h resulted in the opposite.

Females of *A. asychis* produce few eggs and invest much time and resources in each egg. If the eggs are not laid, they can be resorbed and the resources are not lost, or not completely lost because conversion may incur a cost. Under such conditions, females showed a strong reluctance to oviposit into parasitized hosts no matter when they were parasitized. The reason is obvious. Even if the laid egg has a good chance of winning competition and of surviving, the risk is still too high for the female. The second egg generally has about a 50% chance of survival if it is laid right after the deposition of the first egg. This chance decreases with the time interval between the two ovipositions (Chapter V). If an egg laid loses out in the competition, a female loses much of the time and resources invested. Thus, it pays for females of this species to avoid risks. This was shown in my earlier experiments (Chapter IV) where *A. asychis* were very reluctant to oviposit even into freshly parasitized hosts, and rejected 95% of them.

The present study not only explains the different oviposition behaviors exhibited by the two aphid parasitoid species, but also offers a useful tool for predicting parasitoid host selection patterns in general. Species that have reproductive strategies similar to those of *Aphelinus* are likely K-selected and have limited numbers of eggs available. Such species in general are risk sensitive; they may lay eggs at a slower rate or show very strong restraints to oviposit in poor hosts (e.g., parasitized hosts). In the field, natural selection will likely mold these types of parasitoids in such a way that they attack mainly those hosts or host stages that provide high survival for their offspring. Evidence for this has been found in communities of ichneumonid parasitoids that attack the Swaine jackpine sawfly (Price 1974 a, b). By contrast, species that reproduce by laying large numbers of eggs, such as *Aphidius*, are probably r-selected. These species generally invest few resources in each egg (Price 1973, 1974b). They tend to be risk insensitive and distribute their eggs at a faster rate (Blackburn 1991b). As a result, their progeny suffer high mortality due to larval competition or host mortality. Therefore, studying parasitoid reproductive biologies may lead to predictions about parasitoid host selection patterns.

CHAPTER IX

GENERAL CONCLUSION

Competitive interactions among insect parasitoids may influence the population dynamics of a parasitoid species and consequently the parasitoid's ability to regulate host populations (Price *et al.* 1986; Taylor 1988; May and Hassell 1988; Mackauer 1990). Studies of competitive interactions may include two aspects: (1) How parasitoids interact directly or indirectly with individuals of the same or a different species, information that may provide insight into mechanisms of parasitoid interactions; and (2) why parasitoids interact in the way they do, information that may explain the underlying evolutionary reasons for certain behavior patterns (Price 1975; van Alphen and Vet 1986; Roitberg 1991). The second aspect is perhaps the more important one because it may not only shed light on a specific host-parasitoid system but also have general implications for the understanding of other systems. In this thesis, I considered first the way by which a female selects hosts for her offspring, i.e., in which host she lays eggs and what criteria she uses to judge if a host is good or bad.

Evolutionary theory assumes that parasitoids select hosts in order to maximize their fitness (e.g., Charnov and Skinner 1985; Charnov and Stephens 1988; Mangel 1989). High quality hosts produce large and fecund parasitoid offspring, which thus provide high fitness returns to the ovipositing females. Therefore, these hosts are expected to be preferred for oviposition over low quality hosts. I investigated the effect of host quality on parasitoid oviposition decisions by examining one aspect--the status of the host, viz., whether or not the host is parasitized and, if so, by the wasp herself, or a conspecific, or a heterospecific. These host types are distinguished because a female may achieve different fitness gains by oviposition in them (Hubbard *et al.* 1987; van Alphen and Visser 1990; Mackauer 1990). Using the pea aphid and two of its parasitoids, *Aphidius ervi* and *Aphelinus asychis*, as my experimental system, I examined conspecific and heterospecific interactions in these species. Insect parasitoids that share the same host species interact not only as immatures (when individuals may compete directly for a host) but also as adults (when females may compete for suitable hosts for oviposition). I examined the interactions during both immature and adult stages.

Host selection (or host acceptance) is affected by several factors, including host quality (e.g., whether the host is parasitized), reproductive strategies, and environment. Selection may involve various mechanisms for assessing host or environmental quality, such as pheromones and visual assessments. Host selection decisions that are adaptive in one species under one set of conditions may not be so in another species, either because the latter has a different reproductive strategy or because the environment or host quality varies.

Hosts selected by *A. asychis* are used not only for oviposition but also for feeding. Patterns of oviposition and host-feeding are influenced by host density (Chapter III). The decision whether to feed on or to oviposit in a host is influenced by the wasp's hunger level and other physiological conditions. Feeding can cause host mortality which may contribute to host population regulation (Kidd and Jervis 1991).

Many parasitic hymenopterans discriminate between unparasitized and conspecifically parasitized hosts and prefer to oviposit in the former because eggs are more likely to survive (van Lenteren 1981). However, an encyrtid egg parasitoid, *Ooencyrtus nezarae*, prefers hosts parasitized by conspecifics over unparasitized hosts (Takasu and Hirose 1991). Although survival of the parasitoid progeny is lower in parasitized than in unparasitized hosts, handling time of the parasitized hosts is extremely short relative to that of unparasitized hosts, because the superparasitizing female can use the punctures made by a previous female. Thus, females prefer the parasitized hosts over unparasitized ones because saving time and energy for drilling is more profitable than that of increasing progeny survival. Although the host selection behavior in *O. nezarae* seems unusual at first glance, it is optimal under these conditions.

When given a choice between unparasitized and conspecific-parasitized (Chapter IV, V) or heterospecific-parasitized (Chapter VI) hosts, females of both *A. ervi* and *A. asychis* discriminate against parasitized aphids and prefer to oviposit in unparasitized ones. Such a host selection pattern is adaptive because oviposition in parasitized hosts results in superparasitism and consequently larval competition, a condition that reduces offspring survival. Therefore, as long as a sufficient number of high quality, unparasitized hosts is available, females are expected to discriminate against the inferior, parasitized hosts. This finding agrees with optimal foraging theory which predicts that parasitoid females should prefer the more profitable hosts (e.g., Skinner 1985; Pak 1986).

When given no choice, with only parasitized hosts available, females of *A. ervi* accept newly parasitized hosts, but they reject those parasitized ≥ 24 h earlier (Chapter IV). Females of *A. asychis* reject all parasitized aphids no matter when they were parasitized (Chapter IV, V). This difference in host selection reflects the respective reproductive strategies of these two species (for a review of reproductive strategies in parasitic Hymenoptera, see Blackburn 1991a, b). *Aphidius ervi* is time-limited but not egg-limited. Females normally carry a large egg load in their ovaries and invest little time or resources in each individual egg (Chapter VIII). Furthermore, any unlaid eggs will be wasted because this species cannot resorb eggs under adverse conditions. If there is a high probability of a second egg surviving in newly parasitized aphids (Chapter IV), it is not surprising that wasps accept such hosts readily. However, when an aphid is parasitized ≥ 24 h earlier by a conspecific, the probability of the second egg surviving competition diminishes. Therefore, it pays for females to be selective under such conditions. In contrast to *A. ervi*, *A. asychis* is egg- but not time-limited. Females usually have only few mature eggs in their ovaries and invest considerable time and resources in each individual egg. Furthermore, these wasps can recover the energy invested in eggs through oosorption (Chapter VIII). These considerations might be expected to influence a female's oviposition decisions and make her selective about hosts, even when her progeny has a good chance (ca. 0.5) of surviving, because the expected payoff does not outweigh the cost of oviposition. Hence it is shown that species with different reproductive strategies behave differently with regard to host selection (Price 1973; Blackburn 1991b).

The ability to discriminate between unparasitized and parasitized hosts is common among parasitic hymenopterans (Mackauer 1990) and other insects that have similar "parasitic" life styles, such as bruchid beetles (Shimada and Ishihara 1990) and tephritid fruitflies (Roitberg and Prokopy 1987). Females of many species can detect the external marking pheromones left on the host by another female (Roitberg and Mangel 1989) or any internal cues that either originate with the host in association with parasitism (Chow and Mackauer 1986; Vinson 1990) or that are injected into the host by the first female (Hubbard *et al.* 1987). Both *A. ervi* and *A. asychis* use the ovipositor to detect internal cues when discriminating between hosts. This suggests that a decision to oviposit is made only after the female has assessed the internal conditions of the host. Although early workers suggested that non-discrimination, a behavior that causes superparasitism, is maladaptive (e.g., van Lenteren 1981), an increasing body of literature supports the concept that superparasitism is not necessarily disadvantageous (Waage 1986; Stand 1986; van Alphen and Visser 1990). Whereas self-superparasitism is seldom functional, conspecific- and heterospecific-superparasitism can be adaptive (van Alphen and Nell 1982; Visser *et al.* 1990). As in some other species (e.g., van

Dijken and Waage 1987; Strand 1988), *A. asychis* does not distinguish between self- and conspecific-parasitized hosts (Chapter III), however, in other species, females may be able to recognize self and conspecifically parasitized hosts (Hubbard *et al.* 1987; Ofuya and Agele 1989; Volkl and Mackauer 1990).

Conspecific and heterospecific interactions in the immature stages involve direct larval competition within the host. Except in the case when a superparasitizing female directly kills the previous brood by ovicide (Smith and Lessells 1985; Strand and Godfray 1989), the offspring must compete for resources. As in many other species (Salt 1961; Mackauer 1990), the first hatched or "oldest" larva usually wins conspecific contests in *A. ervi* and *A. asychis*. In heterospecific competition, *A. ervi* is superior to *A. asychis*, which is usually defeated even when it hatches first. This confirms the observations of Hartley (1922) and Force and Messenger (1965) that aphelinids do not survive when competing with aphidiids. *Aphidius ervi* eliminates conspecific or heterospecific competitors by physical combat in the early first instar and by physiological suppression in later stages; *A. asychis* uses physiological suppression in all larval stages.

The effects of superparasitism on parasitoid fitness components, such as the development rate and adult size, are examined in Chapter VII. A fundamental assumption underlying models of superparasitism as an ESS (van der Hoeven and Hemerik 1990; Visser *et al.* 1990) is that, in solitary species of wasps, superparasitism has no fitness consequences for the surviving larva. I found that, in *A. ervi*, superparasitism does not affect the survivor's development time, but it has a positive effect on adult body size, which is probably due to the increased growth potential of superparasitized aphids (Cloutier and Mackauer 1979, 1980). Therefore, the above assumption in superparasitism models need not be valid. However, in other parasitoid-host associations, the consequences of superparasitism, such as prolonged development time or reduced adult size (Vinson and Sroka 1978; Wylie 1983), must be considered.

My studies attempted to answer questions about both the mechanisms and the possible reasons for parasitoid interactions. Reproductive strategies are likely the underlying reasons that influence the wasps' host selection behavior. Reproductive strategies of a parasitoid species are ultimately moulded by the fitness gains. *Aphidius* and *Aphelinus* have different reproductive strategies probably because they are under different selection pressures in nature. Perhaps the environments or niches in which they live are different. Although the two parasitoid species have roughly similar intrinsic rates of increase (Force and Messenger 1964a; Mackauer 1983), there are many other characters that differ between the two species, for example, longevity, daily fecundity, and the ability to utilize hosts as a food resource (host-feeding). Females of *Aphidius* are short-lived but have a high daily fecundity; adults do not host feed or absorb eggs to prolong life span. Therefore, they tend to be non-selective about newly parasitized hosts when their progeny have some chance of survival and when there are no unparasitized hosts available. The above life history characters suggest that *Aphidius* are adapted to an environment where there are large temporal or spacial variations in host densities. Females often carry a large number of eggs in their ovaries, so that they can exploit hosts quickly when the opportunity arrives.

By contrast, *Aphelinus* are long-lived but have a low daily fecundity; furthermore, females can use hosts or, if hosts are not available, their own eggs as a energy resource to prolong life span. Therefore, they tend to be selective about hosts. Life history parameters of *Aphelinus* suggest that these parasitoids are probably adapted to a relatively stable environment where host supplies are sparse and the parasitoids do not suffer too much instant mortality. Females only carry a dozen mature eggs in their ovaries at any time. Because they can probably live long enough to realize their total fecundity, rejecting a parasitized host at a particular time may not mean a potential loss in total fitness.

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