COMPETITIVE INTERACTIONS BETWEEN THE PEA APHID PARASITOIDS, *APHIDIUS ERVI* AND *PRAON PEQUODORUM* (HYMENOPTERA: APHIDIIDAE): INFLUENCE ON GUILD COMPOSITION IN SOUTHERN BRITISH COLUMBIA

by

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Competitive interactions between the pea aphid parasitoids, *Aphidius ervi* and *Praon pequodorum* (Hymenoptera : Aphidiidae) : Influence on guild composition in southern British Columbia

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ABSTRACT

Competitive interactions between *Aphidius ervi* Haliday and *Praon pequodorum* Viereck (Hymenoptera: Aphidiidae) were studied under controlled laboratory and simulated field conditions. These species are solitary endoparasitoids of the pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), on alfalfa in southern British Columbia.

Parasitoid wasps preferred to attack unparasitized hosts, when this host-type was presented together with newly parasitized aphids. If parasitoids had a choice between self- and conspecific parasitized hosts, *A. ervi* preferred to attack the latter host-type, while *P. pequodorum* preferred the former. The oviposition frequency of *A. ervi* in conspecific parasitized hosts during attacks was lower than expected (i.e. rate of oviposition into unparasitized hosts), but no oviposition restraint was exhibited in self-parasitized aphids. *Praon pequodorum* did not exhibit oviposition restraint.

Wasps of both parasitoid species readily attacked aphids already parasitized by another species. However, *A. ervi* exhibited oviposition restraint when attacking aphids parasitized by *P. pequodorum* $\geq$ 14 h earlier.

In contests between single, same-aged immatures of each species, first-instar larvae of *P. pequodorum* usually killed their *A. ervi* counterparts. In eggs of *P. pequodorum* that developed in heterospecific parasitized hosts, the serosa secreted a layer that functioned as a barrier to physical attack. First-instar larvae of both species were able to kill their older heterospecific competitors.

Aphidius ervi became dominant when equal numbers of female parasitoids were introduced into cages containing plants infested with hosts. The greater abundance of *A. ervi* was, presumably the result of this species' higher numerical

rate of increase. The frequency of oviposition by *A. ervi* was higher than that of *P. pequodorum*, a fact resulting in a greater proportion of hosts parasitized and a higher rate of superparasitism by *A. ervi*. Nevertheless, *P. pequodorum* had a negative effect on the population growth of *A. ervi*.

While *A. ervi* appears to be more effective than *P. pequodorum* as a parasitoid of pea aphid, *P. pequodorum* is expected to co-exist with this species in the field because of its superior larval competitive abilities.

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CHAPTER 1

GENERAL INTRODUCTION

The pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), was accidently introduced into North America in the latter part of the 19th century (Mackauer 1971). It has become a pest of alfalfa (*Medicago sativa* L.) and other economically important Leguminosae (Forbes and Chan 1989; Harper 1986). Pea aphid infestations on alfalfa can be controlled through the application of chemical insecticides (Baenziger *et al.* 1982). However, pesticides are rarely applied on alfalfa in British Columbia (Don Low, pers. comm.). Instead, pea aphid populations are kept in check by natural enemies and abiotic factors.

Prior to the late 1950's, *Aphidius pisivorus* Smith and *Praon pequodorum* Viereck were common solitary endoparasitoids (Hymenoptera: Aphidiidae) of the pea aphid on alfalfa in the Pacific Northwest of the United States (Halfhill *et al.* 1972). Other, less abundant, solitary endoparasitoids included *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae), and the aphidiids, *Ephedrus californicus* Baker, *Monoctonus paulensis* (Ashmead) and *Praon occidentale* Baker. The latter four species infrequently parasitized the pea aphid, and Mackauer and Finlayson (1967) suggested that this aphid may represent only a secondary host for these species.

In the late 1950's, two solitary endoparasitoids (Hymenoptera: Aphidiidae), *Aphidius ervi* Haliday and *Aphidius smithi* Sharma & Subba Rao, were introduced by the United States Department of Agriculture into North America to control the pea aphid (Halfhill *et al.* 1972; Mackauer and Finlayson 1967; Stary 1974). They became established and their range expanded throughout the United States and into Canada. The first survey of aphidiid parasitoids in southern British Columbia was

conducted in 1969 (Campbell 1974). Over the following 21 years, other surveys were conducted in this region, resulting in a record of temporal changes in the relative abundance of aphidiid species (see also Chapter 3). The data indicate that *A. pisivorus*, *A. smithi*, *A. ervi* and *P. pequodorum* were common on alfalfa in southern British Columbia. However, recent surveys indicate that only *A. ervi* and *P. pequodorum* remain as the most predominant parasitoids of the pea aphid.

I chose to examine the pea aphid-aphidiid system because of the documented history and composition of parasitoid species in this system. While there has been considerable change in the relative abundance of aphidiid species over time, *P. pequodorum* has persisted, usually as the least abundant species. Indeed, since 1983, the relative abundance of *P. pequodorum* has never exceeded 6% (Kampbampati 1987; McBrien 1991). The relative abundance of *A. ervi* always exceeded that of *P. pequodorum*, but the former species was always present. This observation raised the following questions: (1) What are some of the attributes that may enable *P. pequodorum* to survive at low relative abundance? (2) Can *A. ervi* and *P. pequodorum* continue to co-exist? To answer these questions, I established as the main objective of this thesis, to examine competition between *A. ervi* and *P. pequodorum*.

To facilitate the examination of heterospecific (= interspecific) competitive interactions between *A. ervi* and *P. pequodorum*, the guild concept can be utilized. Root (1967) defined a guild as "a group of species that exploit[s] the same class of environmental resources in a similar way." The guild concept groups together species that share similar niche requirements, regardless of their taxonomic position. Jaksic and Medel (1990) argued that a guild should include all species whose dietary requirements are not significantly different. The "guild" of natural enemies that utilize the pea aphid includes birds, fungi (*Entomophthora* spp.

[Entomophthorales]), parasitic insects (Aphelinidae, Aphidiidae), predaceous insects (Cecidomyiidae, Coccinellidae, Chrysopidae, Hemerobiidae, Syrphidae) and spiders (Araneidae, Salticidae) (Fluke 1929; Foelix 1982; Frazer and Gilbert 1976; Stary 1970). However, analysis of the interactions among all guild members is beyond the scope of this investigation. For the purposes of my study, I will restrict the term "guild" to include only aphidiid parasitoids.

Populations of organisms are influenced by a number of abiotic and biotic factors (Boughey 1973). However, Force (1970) stated that the most important biotic factor that may affect the structure and function of insect parasitoid communities is competition. Competition has been defined by DeBach and Sundby (1963) as "the attempted or actual utilization by two organisms of common resources." Among individuals of sympatric species of parasitic Hymenoptera, competition for food (eg. nectar, pollen and honeydew) may occur. However, the most crucial competition occurs among female parasitoids for hosts in which to oviposit (Force 1970).

In the field, competition for hosts can result in more than one oviposition per aphid (= superparasitism) (McBrien 1991); superparasitism tends to increase when hosts are scarce (Campbell 1974). Supernumerary larvae are eliminated by physical combat and/or physiological suppression (Chow and Mackauer 1985; Chow and Sullivan 1984). Female fitness may suffer when superparasitism occurs (Roitberg and Mangel 1988; van Alphen and Visser 1990) because, usually, only one parasitoid can successfully develop in a single host. However, superparasitism may be adaptive under certain circumstances (Bakker *et al.* 1985; Hubbard *et al.* 1987; van Alphen and Visser 1990; Visser *et al.* 1992).

Studies on the competitive interaction between parasitoid species may contribute to a greater understanding of ecological communities. However, these

studies are also of practical importance because parasitoids are used in the biological control of insect pests (Force 1970). Legner (1986) stated that, of the various natural enemy taxa that could be utilized, "arthropodophagous arthropods" pose the lowest risk to the environment and future biological control efforts. However, the liberation of exotic species may result in competition between introduced natural enemies (Ehler and Hall 1982) or competitive displacement of native species (Flanders and Oatman 1987). Stary (1970) stated that under natural conditions, displacement of a species occurs over a long period of time, but that the process can be accelerated by actions of humans. The data collected on the relative abundance of aphidiids in British Columbia suggest that *A. smithi* and *A. pisivorus* may have been displaced by *A. ervi*. The question remains, can *P. pequodorum* co-exist with *A. ervi*, or will *P. pequodorum* also be displaced?

This thesis contains six chapters. Chapters 1 and 6 are devoted to the general introduction and general discussion, respectively. Chapter 2 describes the general materials and methods concerning the collection, maintenance and manipulation of live material. In addition, Chapter 2 also includes the rearing history of all insect colonies. Chapter 3 outlines the changes in the relative abundance of aphidiid species that have been observed in southern British Columbia, from 1969 to 1991. Studies on heterospecific competition can benefit from an examination of a guild's ecological history. This exercise can reveal changes in the guild structure, which in turn may be correlated with the competitive ability of individuals in the respective species. Chapter 4 describes the results of experiments that examined host discrimination and larval competition in and between *A. ervi* and *P. pequodorum*. Host discrimination is essential to parasitoids as this ability enables female wasps to discern high quality (unparasitized) hosts from low quality (parasitized) aphids. Female parasitoids are expected to oviposit in those hosts that will give them the

greatest reproductive success (Charnov and Skinner 1985). The abundances of a species may reflect the oviposition decisions made by individual parasitoids. Furthermore, species abundance also may be a function of the probability of survival of immature stages, especially for heterospecific competition. Superparasitism occurs in the field and, in a study of competitive interactions, it is important to know which species is the superior larval competitor. This knowledge may help to explain the present species composition, and to suggest whether the extant species are likely to co-exist. Chapter 5 describes experiments that evaluated competition, at the population level, between *A. ervi* and *P. pequodorum* under controlled and semi-natural conditions. These experiments were conducted to establish which species would out-compete the other when both were simultaneously introduced into cages. The primary benefit of these studies is that an assessment of host exploitation can be obtained. The results of these experiments can be used to support conclusions drawn in the host discrimination and larval competition studies. Using the results from all of the investigations, it may be possible to predict the future composition of species in the parasitoid guild attacking the pea aphid in southern British Columbia.

CHAPTER 2

GENERAL MATERIALS AND METHODS: PLANTS AND INSECTS

Host plant

Broad bean, *Vicia faba* L. cv. "Broad Windsor," served as host for the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). Pots of plants were propagated in a greenhouse by planting approximately 15 seeds in "garden mix" soil in standard 130 mm plastic pots.

Insect colonies

Acyrthosiphon pisum

I established a colony of pea aphids with specimens obtained from a culture that had been continuously reared in the laboratory for over 10 years. All insect colonies were maintained on potted bean plants in clear, Plexiglass and screen cages, measuring 33 cm x 34 cm x 42 cm. Colonies were reared in a controlled environment chamber at a temperature of $20 \pm 1^{\circ}C$, a relative humidity of $60 \pm 10\%$ and continuous light provided by "cool-white" florescent lamps.

New aphid colonies were established at one to three day intervals by separating the adults and nymphs and placing each group onto separate uninfested plants. Aphids reproduced parthenogenetically (asexually) under these rearing conditions, giving rise to viviparous, apteriform, female offspring.

Aphidius ervi

Two *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) colonies were established. The first colony was started with parasitoids that emerged from mummies gathered near Sorrento, British Columbia in 1990 (Figure 2.1 and Table 3.1). Mummies were collected from two alfalfa fields located less than 10 km apart

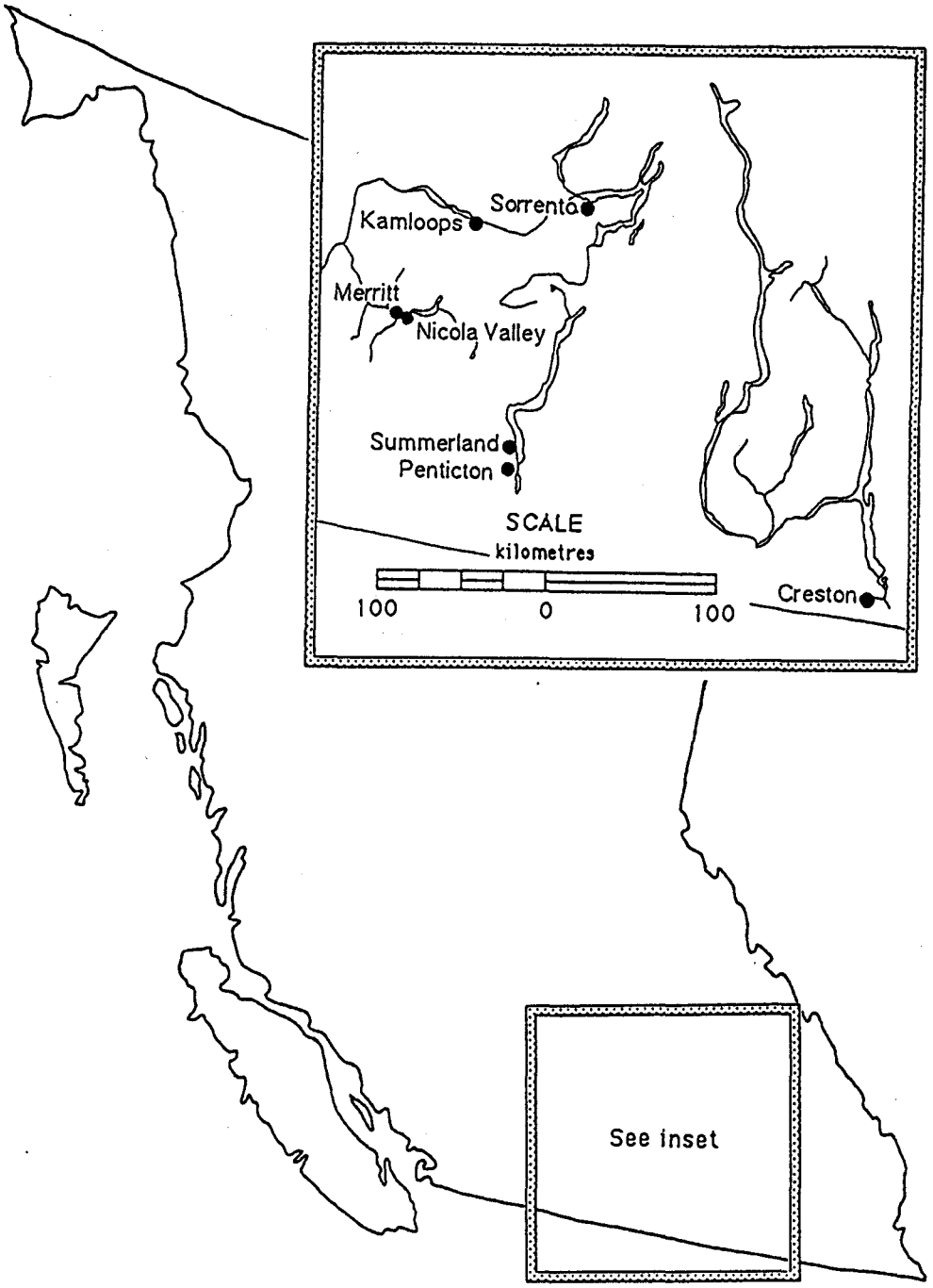


Figure 2.1. Locations in southern British Columbia where parasitoids (Hymenoptera: Aphidiidae) of the pea aphid, *Acyrthosiphon pisum*, were collected. With the exception of Nicola Valley, all sites are identified with the name of a nearby urban centre. Map adapted from Anon. (1986) and Chilton (1981).

and yielded parasitoids that were used to establish 16 isofemale lines. Each isofemale line was started with a different singly-mated female. Parasitoids from the isofemale lines were used in host discrimination experiments (Chapter 4).

The second *A. ervi* colony was established with parasitoids obtained from a laboratory culture that had been started with specimens gathered near Kamloops, British Columbia in 1990. Parasitoids from the second *A. ervi* colony were used in experiments that examined heterospecific competition (Chapter 5).

Praon pequodorum

Two *Praon pequodorum* Viereck (Hymenoptera: Aphidiidae) colonies were established with specimens collected from two different geographic areas of British Columbia. I established two colonies because the host discrimination experiments that I had planned required parasitoids with significant genetic differences. Genetic variation between two colonies established from different collection sites is expected to be greater than the intracolony variation of wasps from a colony founded from a single site. The point-to-point distances between the sites from which the Sorrento and Summerland colonies were established range between 140 km and 160 km (Figure 2.1).

One *P. pequodorum* colony (Summerland strain) was established with parasitoids collected from two sites, located approximately 20 km apart; the mummies obtained from each site were pooled into a single Summerland colony. Mummies were gathered on white sweet clover, *Melilotus alba* Desr., at the Agriculture Canada Research Station in Summerland and on alfalfa southwest of Penticton in 1989 (Figure 2.1 and Table 3.1). The Summerland *P. pequodorum* colony was established with 24 females.

The second *P. pequodorum* colony (Sorrento strain) was founded with parasitoids gathered from two alfalfa fields in 1989. The collection sites were

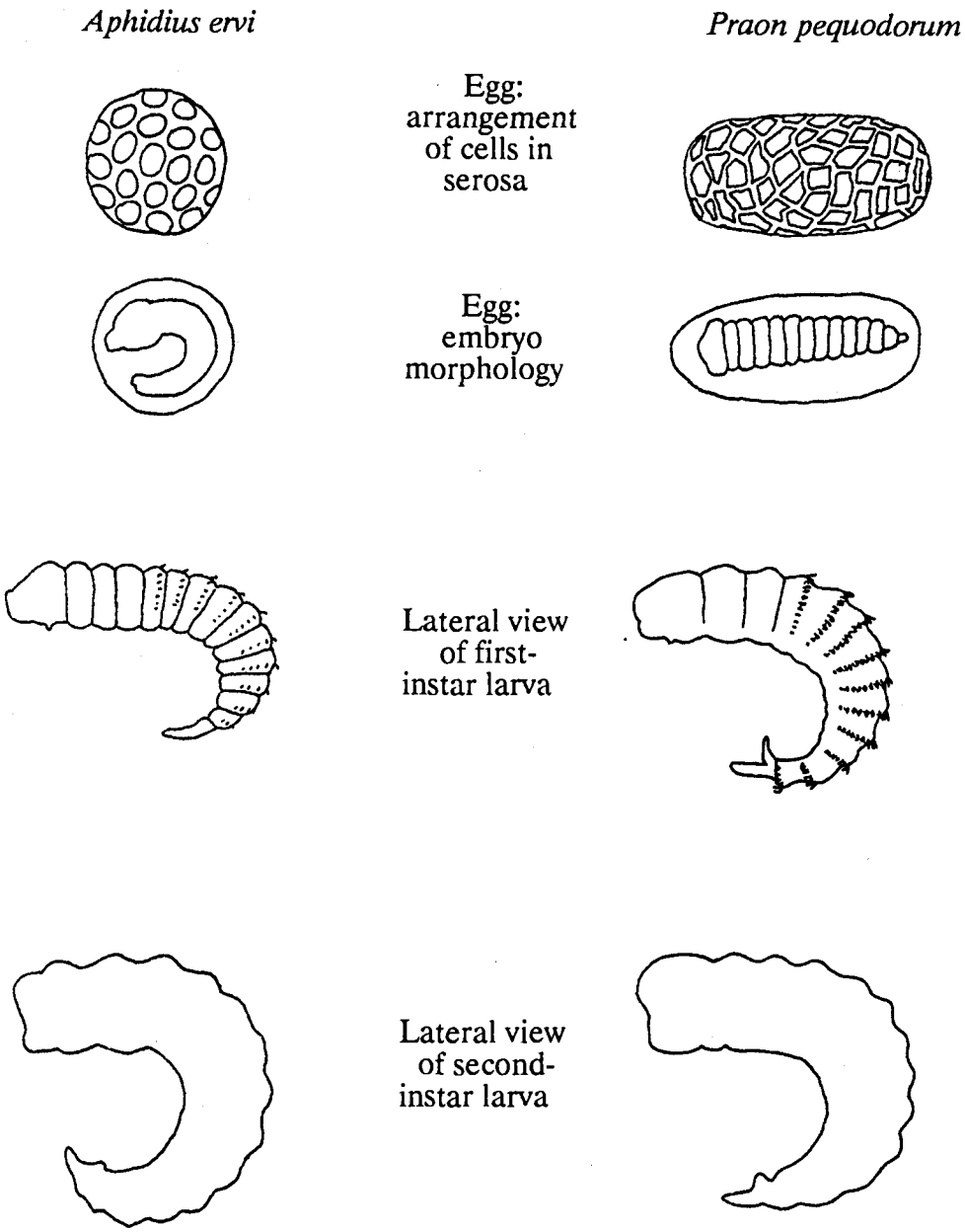


Figure 2.2. Immature stages of *Aphidius ervi* and *Praon pequodorum*.

located southeast of Sorrento and were less than 10 km apart (Figure 2.1); mummies obtained from each field were pooled to establish one Sorrento colony (Table 3.1). The Sorrento *P. pequodorum* colony was founded with 48 females.

Insect dissection

Aphids were dissected to establish if oviposition had occurred and if so, to determine the number, species and stage of development of immature parasitoids. Attacked aphids were reared on bean stalks placed inside small, screened cages (Mackauer and Bisdee 1965). Attacked aphids were not dissected until four to five days after oviposition because parasitoid embryos required this time to eclose from the egg. Dissection before embryo eclosion increased the probability that eggs (evidence of oviposition) could be overlooked, due to their small size.

Dissections were performed in 0.8% $NaCl_{(aq)}$ solution containing surfactant (Micro Liquid Laboratory Cleaner). This solution was not immediately lethal to parasitoid larvae, so it was possible to determine if larvae were alive. Live larvae moved after they were prodded with a dissecting instrument. Another characteristic of live larvae was that they appeared almost transparent, whereas dead immatures appeared translucent to opaque.

Identification of immature parasitoids to species is not difficult because the eggs, first-instar- and second-instar larvae of each species are morphologically distinct (Figure 2.2). Identification of dead larvae is aided by the fact that *P. pequodorum* larvae tended to shrink in length, whereas *A. ervi* larvae retain their shape.

CHAPTER 3

TEMPORAL CHANGES IN THE RELATIVE ABUNDANCE OF PEA APHID PARASITOIDS (HYMENOPTERA: APHIDIIDAE) IN BRITISH COLUMBIA

Introduction

The pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), was accidentally introduced into North America during the middle or the second half of the 19th century. In the United States, the first reported pea aphid infestation occurred in Illinois in 1878 and, within 20 years, the aphid was found in Ottawa (Davis 1915; Johnson 1900). The pea aphid infests many economically important Leguminosae, including alfalfa (*Medicago sativa* L.), beans (*Phaseolus vulgaris* L., *Vicia faba* L.), peas (*Pisum sativum* L.) and clovers (*Melilotus alba* Desr., *Trifolium pratensis* L. and *T. repens* L.) (Forbes and Chan 1989). The pea aphid is a virus vector (Kennedy *et al.* 1962), but most crop damage results from feeding. In alfalfa that is used for forage, pea aphid infestations can lower the plant quality by reducing fresh and dry weight (Harper 1986; Harper and Kaldy 1982).

Previous to 1959, pea aphid populations were attacked by two endoparasitoids (Hymenoptera: Aphidiidae) believed to be indigenous to North America, *Aphidius pisivorus* Smith and *Praon pequodorum* Viereck. However, these parasitoid species did not control pea aphid infestations satisfactorily (Mackauer 1971; Mackauer and Finlayson 1967). As a result, the United States Department of Agriculture imported two exotic species of endoparasitoids (Hymenoptera: Aphidiidae) in an attempt to control the pea aphid biologically. In 1958, *Aphidius smithi* Sharma & Subba Rao was acquired from India and released in the western United States (Mackauer and Finlayson 1967). In 1959, *Aphidius ervi* Haliday was imported from Europe for liberation in the eastern and western United States

(Halfhill *et al.* 1972; Stary 1974). Both species became established and emigrated into alfalfa growing regions throughout Canada and the United States. In British Columbia, *A. smithi* was first discovered near Christina Lake in 1965, while the first reported collection of *A. ervi* occurred near Kamloops in 1970 (Mackauer and Campbell 1972).

Studies assessing heterospecific competition can benefit from a review of the ecological history of competing species. *Aphidius ervi*, *A. pisivorus*, *A. smithi* and *P. pequodorum* belong to the same parasitoid guild. At one time, all four species were present in southern British Columbia (Kambhampati 1987). Examination of temporal changes in the relative abundance of species can reveal variation in diversity. Such a retrospective not only may indicate differences in the competitive ability of guild members, but also contribute to a better understanding of the heterospecific interactions that occur within a guild.

Results

The earliest available records on the relative abundance of aphidiids attacking the pea aphid in British Columbia date from 1969 (Campbell 1974) (Table 3.1 and Figure 3.1). In 1969, collections near Kamloops (Figure 2.1) showed that *A. smithi* comprised 22.0% of the aphidiid guild. The most abundant species was *A. pisivorus* (51.6%), while *P. pequodorum* comprised 26.4% of the samples.

By 1971, the relative abundance of each parasitoid species had changed considerably (Campbell 1974). *Aphidius smithi* was the most prevalent species (75.4%), while the relative abundance of the formerly dominant *A. pisivorus* had decreased to 17.0%. The relative abundance of *P. pequodorum* was 7.6%.

Collections in 1972 indicated the presence of *A. ervi* (0.1%) and revealed that the relative abundance of the other three parasitoid species had not changed

Table 3.1. Numbers of parasitoids (Hymenoptera: Aphidiidae) of the pea aphid, *Acyrthosiphon pisum*, collected in southern British Columbia. Unless otherwise stated, all specimens were collected on alfalfa.

Collection			Nos. of parasitoids collected[a,b]			
Year	Date (M/D)	Site	A.e.	A.p.	A.s.	P.p.
1969[c]		Kamloops	0	47	20	24
1971[c]	06/01-10/06	Kamloops	0	467	2,069	208
	07/30	Summerland	0	2	94	6
1972[c]	07/01-08/10	Kamloops	1	156	566	83
	08/05	Summerland	0	1	32	2
1983[d]		Kamloops	2,990	624	33	53
1984[d]		Kamloops	1,529	226	11	43
1985[e]	05/23-08/28	Kamloops	896	24	8	52
1989	06/11	Summerland[g]	1,000	0	0	55
	06/28	Sorrento and Summerland[g]	2,593	0	0	10
	07/13	Sorrento and Summerland[g]	2,760	0	0	74
1990	08/23	Merritt	9	0	0	1
		Sorrento	252	0	0	4
	08/24	Sorrento	167	0	0	7
		Kamloops	5	0	0	0
		Nicola Valley	58	0	0	3
1991[f]	05/26	Creston	659	0	0	10

a. A.e. = *Aphidius ervi*; A.p. = *A. pisivorus*; A.s. = *A. smithi*; P.p. = *Praon pequodorum*.
b. In all years, values indicate the numbers of parasitoids that emerged from mummies, except values for 1989, 1990 and 1991 that indicate numbers of mummies collected.
c. Campbell (1974).
d. Kambhampati (1987).
e. McBrien (1991).
f. A. Chow and J.P. Michaud (unpublished).
g. Collections in Summerland were on alfalfa (*Medicago sativa*) and white sweet clover (*Melilotus alba*). In Sorrento, mummies were collected only on alfalfa. Where applicable, data from both sites were pooled because temporal, rather than spatial comparisons in the relative abundance of species was of interest.

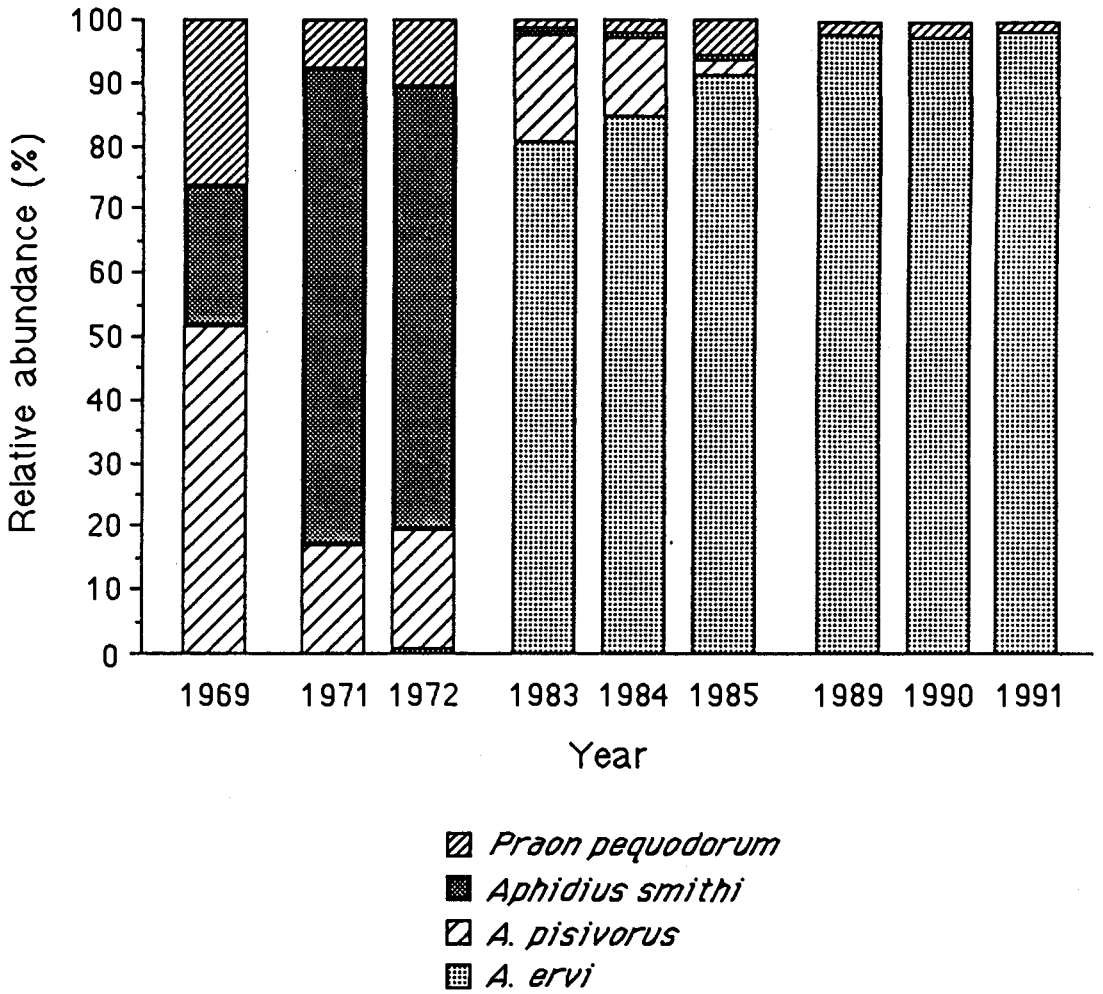


Figure 3.1. Relative abundance of the four main parasitoid species (Hymenoptera: Aphidiidae) of the pea aphid, *Acyrthosiphon pisum*, in southern British Columbia. Specimens collected on alfalfa (*Medicago sativa*) and white sweet clover (*Melilotus alba*). Source: Campbell (1974), Kambhampati (1987), McBrien (1991) and A. Chow and J.P. Michaud (unpublished).

appreciably from the previous year. However, samples gathered in 1983 showed that the relative abundance of *A. ervi* and *A. smithi* had changed significantly. *Aphidius ervi* was the most prevalent species (80.8%), while the formerly dominant *A. smithi* comprised only 0.9% of samples (Kambhampati 1987). From 1972 to 1983, the relative abundance of *A. pisivorus* and *P. pequodorum* decreased, with *P. pequodorum* experiencing the greater decline.

From 1983 to 1985, the relative abundance of *A. smithi* did not exceed 1.0%, while that of *A. pisivorus* decreased from 16.9% to 2.5% (McBrien 1991). Concurrently, the relative abundance of *A. ervi* increased by 11.4% to 91.4% and that of *P. pequodorum* increased by 3.9% to 5.3%.

Collections that I performed in 1989 and 1990 and those of A. Chow and J.P. Michaud (unpublished) in 1991 found only *A. ervi* and *P. pequodorum*.

Discussion

The prevalence of aphidiid species in the southern interior of British Columbia has changed considerably over a 22 year period (Figure 3.1). Before the appearance of the introduced species, *A. pisivorus* and *P. pequodorum* were the main parasitoids of the pea aphid. *Aphidius smithi*, the first introduced species to colonize southern British Columbia, was clearly the most common pea aphid parasitoid in 1971. However, within 10 years, *A. smithi* was displaced by *A. ervi* as the dominant aphidiid. In 1985, it was evident that *A. pisivorus* and *A. smithi* were being replaced by *A. ervi*, leaving *A. ervi* and *P. pequodorum* as the main parasitoids of the pea aphid.

Kambhampati (1987) examined the life history traits of the four aphidiid species and concluded that the decline in the relative abundance of *A. smithi* and *A. pisivorus* could not be explained on the basis of differences in their life history traits.

Compared to *A. ervi*, *A. smithi* was equal or superior in virtually all aspects of reproduction. Indeed, *A. smithi* had the highest intrinsic rate of increase, followed by *A. pisivorus*, *A. ervi* and *P. pequodorum*.

Kambhampati (1987) analyzed for divergence in life history traits between geographically distinct populations of the same parasitoid species. He found that the divergence in traits between populations of *A. ervi* and *A. smithi* was similar and greater than that of different *P. pequodorum* populations. The founding population of *A. smithi* in North America was smaller than that for *A. ervi*, and Kambhampati (1987) suggested that random genetic drift and possibly mutation may have affected the long-term establishment of *A. smithi*. Other factors that may have played a role in the replacement of *A. smithi* by *A. ervi* include climate changes and modifications in crop production and management (Mackauer and Kambhampati 1986).

Mackauer and Kambhampati (1986) recognized that competition between several colonizing species also may have contributed to the usurpation of *A. smithi* by *A. ervi*. For insect parasitoids, competition for food does not generally take place between the adults, but between immature stages contained within hosts (Force 1970). Heterospecific competition among aphidiids will tend to occur most often during periods of low host numbers (Mackauer 1990), particularly in the beginning and at the end of the growing season. The proportion of aphids parasitized in the field may reach 80% (Campbell 1974). Equal or superior larval competitive ability can be an important asset during periods of intense competition for hosts because, usually, only one parasitoid larva can successfully complete its development in a single aphid. McBrien and Mackauer (1990) demonstrated that *A. ervi* was superior to *A. smithi* in terms of larval competitive ability. Chua *et al.* (1990) and McBrien (1991) concluded that this factor may have contributed to the decline of *A. smithi*.

With the decline of *A. smithi*, competition for pea aphids may have declined,

which could have assisted in the establishment of *A. ervi* (Kambhampati 1987). Other factors that may have contributed to the proliferation of *A. ervi* throughout North America include, (1) the pre-adaptation of *A. ervi* to North American climates and, (2) a greater number of specimens were used to found *A. ervi*, which is expected to minimize the deleterious effects of genetic drift.

The explanation behind the disappearance of *A. pisivorus* is best covered in two stages. As there has been some difficulty in classifying *A. pisivorus* (Angalet and Fuester 1977), the systematic position of this species will be addressed first. Mackauer (1969) performed heterospecific and conspecific matings using *A. ervi*, *A. pisivorus* and *A. smithi*. The only combinations that resulted in female offspring (successful hybridization) were matings between conspecific pairs and copula between male *A. pisivorus* and female *A. ervi*. The F_1 *A. pisivorus* x *ervi* hybrids were fertile and, in principle, acceptable as mates for pure-bred male *A. ervi* and *A. pisivorus*. The ability of *A. pisivorus* to hybridize with *A. ervi* and the morphological similarity between these species suggests that *A. pisivorus* may not be a truly near-arctic species, but in fact may be a geographic subspecies of *A. ervi*. Mackauer (1969) suggested that a small founding colony of *A. ervi* could have been accidently introduced into North America by European immigrants some time ago. In the period since the accidental introduction of *A. ervi* and its purposeful re-introduction in 1959, geographic isolation resulted in the evolution of a new species (*A. pisivorus*), complete with morphological differences and limited reproductive isolating mechanisms. Unruh *et al.* (1989) performed enzyme electrophoresis on six *Aphidius* species; they concluded that *A. pisivorus* is a distinct species, but the question of whether there was introgression between *A. ervi* and *A. pisivorus*, subsequent to the introduction of *A. ervi*, remains unanswered.

To explain the decline of *A. pisivorus*, first consider the following conditions

that were in effect, subsequent to the introduction of *A. ervi*: (1) greater relative abundance of *A. ervi* than *A. pisivorus*, (2) reproductive compatibility between *A. pisivorus* males and *A. ervi* females, but not between *A. ervi* males and *A. pisivorus* females and, (3) females of *Aphidius* species mate only once. The differential impact of *A. smithi* and *A. ervi* upon *A. pisivorus* can be assessed if the relative abundances of the "indigenous species" (*A. pisivorus* and *P. pequodorum*) are determined, excluding data from *A. smithi* and *A. ervi*. In 1969, 1971 and 1972, the relative abundance of *A. pisivorus* was 66.2%, 69.2% and 65.3%, respectively, with *P. pequodorum* comprising the remainder. During these years, significant numbers of *A. smithi* were recovered, but the presence of this species did not appear to result in appreciable changes to the relative abundance of *A. pisivorus*. However, in 1983, 1984 and 1985, the period during which time *A. ervi* became the dominant aphidiid, the relative abundance of *A. pisivorus* was 92.2%, 84.0% and 31.6%, respectively. The degree of change in the relative abundance of *A. pisivorus* appears to vary depending upon whether *A. smithi* or *A. ervi* was the dominant species. Keeping this in mind and the fact that *A. smithi* cannot hybridize with *A. pisivorus*, these results suggest that competitive displacement by *A. ervi* may not be the sole reason for the decline of *A. pisivorus*. Rather, *A. pisivorus* appears to have been absorbed by the parental species (*A. ervi*), primarily as a consequence of the ability to hybridize with *A. ervi*. Indeed, Angalet and Fuester (1977) predicted that *A. pisivorus* may be eliminated as a distinct species.

Heterospecific competition may have also played a role in the decline of *A. pisivorus*. There are no reports on the competitive ability of *A. pisivorus* larvae, so it is difficult to compare the larval competitive ability of this species with that of the other *Aphidius* species. However, inferences may be drawn about the larval competitive ability of *A. pisivorus* larvae with that of *P. pequodorum* larvae. The

competitive ability of *P. pequodorum* larvae is superior to that of *A. smithi* (Chow and Mackauer 1984) and *A. ervi* (Chapter 4). Given the morphological similarity between the three *Aphidius* species (Mackauer 1969; Mackauer 1971; Mackauer and Finlayson 1967), it is reasonable to suggest that the competitive ability of *P. pequodorum* larvae would be superior to that of *A. pisivorus*. A synergistic interaction between the competitive superiority of *P. pequodorum* larvae and the ability of *A. ervi* to hybridize may have contributed to the decline of *A. pisivorus*.

Samples collected since 1989 suggest that the fluctuation in diversity within the aphidiid guild has stabilized. Presently, *A. ervi* and *P. pequodorum* are the most prevalent pea aphid parasitoids in alfalfa. Since 1983, the relative abundance of *P. pequodorum* has remained virtually unchanged, amounting to less than 6%, while that of the *Aphidius* species has varied considerably. The large disparity in the relative abundance of the two remaining parasitoid species would be expected to lead to the competitive displacement of the less abundant species (DeBach 1966). However, *P. pequodorum* possesses attributes that enable this species to compete successfully with *A. ervi* when hosts are scarce (Chapter 4). These qualities should permit *P. pequodorum* to co-exist with *A. ervi* in southern British Columbia.

CHAPTER 4

HOST DISCRIMINATION AND LARVAL COMPETITION: *APHIDIUS ERVI* AND *PRAON PEQUODORUM*

Introduction

The ability of parasitoids to distinguish between parasitized and unparasitized hosts has been described as host discrimination (Salt 1961). This definition may be expanded to include the ability of parasitoids to differentiate between unparasitized, self-, conspecific- and heterospecific parasitized hosts.

Insect parasitoids utilize external cues (marking pheromones) to differentiate between distinctive host-types (Salt 1937). Marking pheromones may be placed on or in a host before, during or after an oviposition (van Lenteren 1981). External cues appear to be active in the early stages of parasitism (Chow and Mackauer 1986) and may inhibit ovipositional attacks on marked hosts (Salt 1937). Internal cues are utilized during the latter stages of parasitism and indicate the presence of a parasitoid egg or larva (Chow and Mackauer 1986; van Lenteren 1981). Internal cues may include substances that are injected by females (van Lenteren 1981) and physiological changes in the host brought about by parasitization (Beckage 1985; Chow and Mackauer 1986; Stoltz 1986; Stoltz and Vinson 1979; Vinson 1990). Detection of internal cues occurs while the ovipositor is inserted into the host and may result in the avoidance of oviposition (= oviposition restraint) (Salt 1937).

Host discrimination is expected to prevent the wastage of eggs (via superparasitism) and hosts (frequent attacks may increase host mortality) (Bakker *et al.* 1985; Charnov and Skinner 1985; van Alphen 1988; van Lenteren 1976; van Lenteren 1981; Vinson 1976). However, host discrimination is not absolute and parasitoids may superparasitize under certain conditions. Superparasitism in solitary

endoparasitoids leads to competition between eggs and/or larvae. Normally, a single host can support the successful development of only one parasitoid. Supernumerary larvae are usually eliminated during the first larval stage (Mackauer 1990; Polaszek 1986) by physical combat and/or physiological suppression (Chow and Mackauer 1985; Chow and Sullivan 1984). In physical combat, a first instar larva uses its mandibles to attack and kill a competitor. Physiological suppression is often invoked to explain the death of larvae when evidence of physical combat is lacking (Hagvar 1988). Physiological suppression may occur as a result of starvation (Salt 1961), asphyxiation (Fisher 1961b; Fisher 1971) or the action of a toxin such as a cytolytic enzyme (Mackauer 1990).

The increment in fitness from an egg laid into a parasitized host is expected to be less than that of an egg laid into an unparasitized host (Roitberg and Mangel 1988; van Alphen and Visser 1990; Visser *et al.* 1992). Accordingly, superparasitism was once thought to result from oviposition mistakes as parasitoids were not expected to waste offspring (van Lenteren 1981). However, superparasitism may be adaptive in certain situations if the second egg has a greater than zero chance of survival (Bakker *et al.* 1985; Hubbard *et al.* 1987; van Alphen and Visser 1990). Adaptive superparasitism may especially apply to time-limited species, as female parasitoids likely do not realize their potential fecundity in the field (Gilbert and Gutierrez 1973). The alecithal eggs of time-limited species are considered to be "inexpensive" (Bai 1991); females that encounter host patches containing a low proportion of unparasitized hosts are expected to remain and superparasitize, rather than migrate (Hubbard *et al.* 1987; van Alphen 1988; van Strien-van Liempt and van Alphen 1981). In other words, it is better to risk an egg for the chance of producing an offspring than to refrain from oviposition.

Aphidius ervi Haliday and *Praon pequodorum* Viereck (Hymenoptera:

Aphidiidae) are time-limited, solitary endoparasitoids of the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). The pea aphid is an exotic species (Mackauer 1971) that is a pest of many economically important Leguminosae in British Columbia (Forbes and Chan 1989). In 1959, *A. ervi* was introduced into the United States from Europe to control the pea aphid (Halfhill *et al.* 1972; Stary 1974). Subsequent to its introduction, *A. ervi* extended its range throughout the United States and Canada. *Praon pequodorum* is indigenous to North America and favors pea aphid as a host (Johnson 1987; Mackauer and Finlayson 1967). *Aphidius ervi* and *P. pequodorum* are sympatric in alfalfa (*Medicago sativa* L.) in southern British Columbia (Campbell 1974). Field collections indicate that since 1983, the relative abundance of *P. pequodorum* has remained below 6%, while that of *A. ervi* has mainly comprised the remainder (Chapter 3; A. Chow and J.P. Michaud [unpublished]; McBrien 1991).

Studies of competition between *A. ervi* and *P. pequodorum* benefit from an examination of host discrimination and larval competition. Due to the solitary parasitic nature of these species, all ovipositions will not produce the same increment in female fitness. Rather, fitness can vary, depending on whether the host has been parasitized. Comparing the oviposition decisions made by females of both species can assist in explaining patterns of host utilization. This may help in understanding the factors that contribute to the observed disparity in the relative abundance of *A. ervi* and *P. pequodorum* in the field.

Materials and Methods

Conspecific Host Discrimination: *Aphidius ervi* and *Praon pequodorum*

Seven experiments were performed to assess conspecific host discrimination in *A. ervi* and *P. pequodorum*. Methodology varied slightly between experiments due

to procedural constraints imposed by conditions inherent to each experiment (Table 4.1). Unless otherwise indicated, all materials and methods were identical in each experiment and are as follows.

Aphids used in experiments were classified according to whether they were (1) attacked by wasps whose behavior was measured (i.e. the test females) (self-parasitized host-type), (2) attacked by parasitoids whose behavior was not measured (conspecific parasitized host-type) or (3) not attacked (unparasitized). Parasitized hosts were prepared by placing third-instar pea aphids individually into gelatin capsules together with one of several unmated, 2 to 5 day-old female parasitoids. Aphids in a common host-type were similarly marked by amputation of the distal portion of one antenna (Mackauer 1972).

The prepared parasitized aphids were divided into two cohorts. The first cohort was used in the experiment, while the second cohort (control) was set aside to estimate the percent parasitism of the first cohort. The proportion of aphids parasitized in the control was used to calculate the expected frequency of (super)parasitism.

Parasitized aphids were used within one hour following their preparation. Experiments were conducted in an arena consisting of a clear, plastic petri dish measuring 1.8 cm high by 8.5 cm in diameter. Equal numbers of aphids from each host-type were placed in the arena and an unmated, 2 to 4 day-old female parasitoid (i.e. the test female) was introduced into the petri dish. The test female was allowed to remain in the arena until she had attacked 20 to 33 aphids, depending on the experiment (Table 4.1). Attacked aphids were removed and replaced with one of the same host type. All attacked aphids were placed onto broad bean stalks, reared for 4 days and dissected.

Table 4.1. Experimental design for tests of conspecific host discrimination in *Aphidius ervi* and *Praon pequodorum*.

Exp.	Parasitoid species[a]	Contrast[b]	Nos. of aphids of each host-type in arena	Total nos. of aphids attacked
1	A.e.	U vs.C	10	33
2	A.e.	U vs. S vs. C	6	20
3	A.e.	S vs. C	10	20
4	A.e.	S vs. C	10	20
5	P.p.	U vs. C	8	20
6	P.p.	U vs. S	8	20
7	P.p.	S vs. C	10	20

a. A.e. = *Aphidius ervi*, P.p. = *Praon pequodorum*.
b. Host-type abbreviations: U = unparasitized aphid, S = self-parasitized aphid, C = conspecific paraitized aphid.

Experiment 1

This experiment examined host discrimination by *A. ervi* females when parasitoids were presented with unparasitized and conspecific parasitized aphids. Before the experiment, parasitoids were allowed to attack fewer than 10 aphids to gain experience. Ten aphids of each host-type were placed in the arena and each test female was allowed to attack a total of 33 aphids. Twelve test females were used.

Experiment 2

Aphidius ervi females were presented with unparasitized, self- or conspecific parasitized aphids. Test females were experienced with fewer than 10 aphids before they were used to prepare self-parasitized hosts. Six aphids of each host-type were placed in the arena, and each test female was allowed to attack a total of 20 aphids. Fourteen test females were used.

Experiment 3

Aphidius ervi females were presented with conspecific- and self-parasitized aphids. Test females were placed individually into covered, waxed-paper cups with unparasitized and conspecific parasitized aphids (fewer than 15 aphids in total) for one hour to gain experience. The experienced test females were removed from the cups and used to prepare self-parasitized aphids. Ten aphids of each host-type were placed in the arena and each test female was allowed to attack a total of 20 aphids. Fourteen test females were used.

Experiment 4

This experiment was similar to Experiment 3, except that *A. ervi* females were given a longer period of experience. Each test female was placed individually into covered, waxed-paper cups with a total of 30 aphids on a bean stalk overnight. Following confinement with aphids, the experienced test females were used to

prepare self-parasitized aphids. Ten aphids of each host-type were placed in the arena and each test female was allowed to attack a total of 20 aphids. Twelve test females were used.

Experiment 5

Praon pequodorum females were presented with unparasitized and conspecific parasitized aphids. Preliminary work suggested that *P. pequodorum* females may use external cues in host discrimination. To quantify this observation, the behavior of test females was videotaped in the arena. This procedure permitted me to collect data on the number of times females encountered each host-type and the behaviour associated with specific host-types. I defined a host encounter to be contact (physical or otherwise) between the test female and an aphid. Evidence of a host encounter included the following behaviors: (1) physical contact between a parasitoid and an aphid, (2) directed movement of the parasitoid's antennae toward a host and, (3) deflection in the parasitoid's movement upon walking by an aphid. Host encounters could result in either acceptance (an attack, with or without an accompanying oviposition) or rejection (no attack) of the host.

Test females were allowed to attack fewer than 10 aphids to gain experience. Eight aphids of each host-type were placed in the arena and arranged into a four by four Latin square pattern measuring 4 cm x 4 cm. All aphids were anesthetized with CO_2 to restrict their movement. Test females were allowed to attack a total of 20 aphids. Fifteen test females were used.

Experiment 6

Females of *P. pequodorum* were presented with unparasitized and self-parasitized aphids. Test females were allowed to attack fewer than 10 aphids prior to the experiment to gain experience. Eight aphids of each host type were placed into the arena and each test female was allowed to attack a total of 20 aphids. Ten

test females were used.

Experiment 7

Praon pequodorum females were presented with self- and conspecific parasitized aphids in this experiment. The results of two studies were pooled for analysis because a Chi-square test of independence indicated that the numbers of aphids attacked from each host-type did not differ significantly between the two studies ($X^2 = 2.59$, $P > 0.1$, df = 1). In each replicate, test females and females used to prepare conspecific parasitized aphids were obtained from different strains (see Chapter 3). Ten aphids of each host-type were placed into the arena and each test female was allowed to remain until a total of 20 aphids was attacked or 1 hour had elapsed, whichever came first. Thirty-four test females were used.

Statistical analysis

In Experiments 1 to 7, host discrimination was assessed by comparing the number of aphids of each host-type attacked. The results from individual females were pooled to assess the species' response to the host-types offered. Statistical differences between the numbers of aphids attacked from each host-types was tested with paired *t*-tests (StatView512+, version 1.0; Abacus Concepts, Inc. 1986).

Host discrimination and oviposition restraint was examined by analyzing the frequency of oviposition in particular host-types. In each experiment, analyses were performed on pooled results. Attacked aphids may contain 0, 1 or 2 eggs; the numbers of aphids that contain the given numbers of eggs comprise the observed frequency of (super)parasitism. The numbers of aphids expected to contained 0, 1 or 2 eggs can be calculated using Equations (1), (2) and (3).

$$N_0 = N(1 - p_A)(1 - p_B), \quad (1)$$

$$N_1 = N[(p_A(1 - p_B)) + (p_B(1 - p_A))], \quad (2)$$

$$N_2 = N(p_A \times p_B), \quad (3)$$

where N_0, N_1 and N_2 = expected numbers of aphids containing 0, 1 and 2 eggs, respectively; N = total number of aphids that were attacked in the arena and; p_A and p_B = proportion of attacked aphids that were parasitized by specific groups of parasitoids. The same formulae were used in all experiments, but the parasitoids that contributed to the proportions (p_A and p_B) varied between and among experiments, depending on the host type analyzed and the information available (see Table 4.2).

The observed and expected frequencies of oviposition in particular host-types was comparatively analyzed with *G*-tests for goodness of fit; Williams' correction was used to adjust the values of *G* for continuity (Sokal and Rohlf 1981). Based on a null hypothesis of no discrimination between unparasitized and parasitized aphids (no oviposition restraint), the frequency of ovipositions into unparasitized hosts should equal the frequency in parasitized hosts. A significant difference between the observed and expected frequencies of (super)parasitism suggests discrimination. Oviposition restraint was concluded if the number of ovipositions observed (i.e. number of larvae found) was less than the expected frequency.

Heterospecific Larval Competition: *Aphidius ervi* and *Praon pequodorum*

Heterospecific larval competition between *A. ervi* and *P. pequodorum* was examined to assess the ability of each parasitoid species to survive in the presence of the other. I chose to examine competition between larvae of different ages because this method provided an opportunity to demonstrate whether the relative age of competing larvae could influence the outcome of competition.

To obtain an unbiased measurement of the larval competitive ability of the two species, competing larvae should be the same age. Competition between same-

Table 4.2. Source of data used in calculations of the expected frequencies of (super)parasitism in Experiments 1 to 7. See Tables 4.3 and 4.4.

Exp.	Host type[a]	Source of data for given proportions
1	C	p_A - conspecific parasitized control p_B - proportion of unparasitized aphids parasitized during the experiment
2	S	p_A - self-parasitized control p_B - proportion of unparasitized aphids parasitized during the experiment
	C	p_A - conspecific parasitized control p_B - proportion of unparasitized aphids parasitized during the experiment
3	S	p_A - self-parasitized control p_B - self-parasitized control
	C	p_A - conspecific parasitized control p_B - self-parasitized control
4	S	p_A - self-parasitized control p_B - self-parasitized control
	C	p_A - conspecific parasitized control p_B - self-parasitized control
5	C	p_A - conspecific parasitized control p_B - proportion of unparasitized aphids parasitized during the experiment
6	S	p_A - self-parasitized control p_B - proportion of unparasitized aphids parasitized during the experiment
7	S	p_A - self-parasitized control p_B - self-parasitized control
	C	p_A - conspecific parasitized control p_B - self-parasitized control

a. S = self-parasitized aphid, C = conspecific parasitized aphid.

aged larvae can be arranged by using an appropriate length of time between successive attacks (= oviposition interval). This procedure will lead to the simultaneous hatching of *A. ervi* and *P. pequodorum* eggs. Competition between different-aged larvae was accomplished by varying the order of attack and oviposition interval. Published measurements of median developmental times for *A. ervi* at 21°C (McBrien and Mackauer 1990) and *P. pequodorum* at 21.1°C (Chow and Mackauer 1984) were used to establish appropriate oviposition intervals (Figure 4.1). From oviposition, *A. ervi* embryos require 80.1 h (95% C.I., 79.4 h- 80.8 h) to eclose. First instar larvae of *A. ervi* molt 115.2 h (95% C.I., 114.1 h- 116.2 h) post-oviposition. Embryos of *Praon pequodorum* eclose 76.2 h (95% C.I., 74.7 h- 78.0 h) after oviposition. First instar larvae molt into the second instar 94.1 h (95% C.I., 93.7 h- 94.5 h) post-oviposition.

Parasitized aphids were prepared by placing third instar pea aphids into a petri dish and introducing a 4 to 6 day-old, mated female parasitoid into the container. Aphids attacked by one parasitoid species were removed and reared until presentation to females of the other species. Aphids attacked first by *A. ervi* were presented to *P. pequodorum* 0 h ($\leq$10 min.), 4 h ($\pm$15 min.), 14 h ($\pm$10 min.) and 24 h ($\pm$10 min.) later-- the AP_0, AP_4, AP_{14} and AP_{24} oviposition intervals, respectively. Aphids attacked first by *P. pequodorum* were presented to *A. ervi* 14 h ($\pm$15 min.) and 24 h ($\pm$10 min.) later-- the PA_{14} and PA_{24} oviposition intervals, respectively. Aphids attacked by both parasitoid species were reared and a portion were dissected after 3 days. The remaining aphids were reared to allow larvae that survived competition to fully develop and mummify the host.

Statistical analysis

Heterospecific larval competition was examined using an indirectly method

because this study was also designed to examine the reaction of *P. pequodorum* eggs to a heterospecific competitor. The main assumption used in the analysis of data from the indirect method is that in a randomly selected sample of parasitized aphids (2 heterospecific attacks per aphid), the ratio of the numbers of larvae of each parasitoid species found at dissection should be proportionally equivalent to the numbers of mummies formed in the group of undissected aphids. An additional assumption is that the larval competitive ability of each parasitoid species is equivalent.

The numbers of mummies expected to form at each oviposition interval can be calculated with Equations (4) and (5).

$$N'_{mA} = N_m(p_A + 0.5p_{AB}), \qquad (4)$$

$$N'_{mB} = N_m(p_B + 0.5p_{AB}), \qquad (5)$$

where N'_{mA} = the expected number of *A. ervi* mummies, N'_{mB} = the expected number of *P. pequodorum* mummies, N_m = total number of mummies that formed from the undissected aphids, p_A = proportion of dissected aphids that contained only *A. ervi*, p_B = proportion of dissected aphids that contained only *P. pequodorum* and p_{AB} = proportion of dissected aphids that contained both parasitoid species.

I tested the null hypothesis that the competitive ability of larvae did not differ between the two parasitoid species. *G*-tests for goodness of fit were used to test for statistically significant differences between the observed and expected numbers of mummies; values of *G* were adjusted for continuity with Williams' correction (Sokal and Rohlf 1981). Significant differences indicate that the larval competitive ability of the two parasitoid species differs.

Response of *Praon pequodorum* Eggs to a Heterospecific Competitor

The response of *P. pequodorum* eggs to an *A. ervi* competitor was a focus of the previously described experiment. In addition to the aforementioned oviposition intervals, *P. pequodorum* females were used to prepare singly and conspecific superparasitized aphids; the oviposition interval between conspecific attacks was 18 h (± 15 min.). The purpose for including the conspecific parasitized treatment was to investigate whether the species of a competitor would influence the response of *P. pequodorum* eggs.

Attacked aphids were dissected and all *P. pequodorum* eggs found were removed and placed in dissecting saline containing a low concentration of Janus Green B (<0.5% w/w). Stained eggs were examined with a compound microscope at magnifications of up to 1,000x. An ocular micrometer was used to measure the sizes of structures.

Results

Conspecific Host Discrimination: *Aphidius ervi* and *Praon pequodorum*

Experiment 1

Aphidius ervi attacked a greater number of unparasitized hosts than conspecific parasitized aphids (t = 3.23, P = 0.004, df = 11) (Table 4.3). This result suggests that *A. ervi* females discriminate between these host-types, preferring to attack unparasitized hosts. Observation of the *A. ervi* females' behaviour in the arena reveals that the frequency with which hosts escaped attack was low.

The number of conspecific parasitized aphids observed to be superparasitized was lower than expected (G_{adj} = 11.50, P < 0.001, df = 2). There is no evidence to suggest that ovicide in *A. ervi* is responsible for the reduced

Table 4.3. Results of host discrimination experiments with *Aphidius ervi*.

Exp.	Mean no. of aphids attacked per female (s.e. in brackets)[w,x]			Nos. of aphids containing given no. of larvae[w,y]									Proportion parasitism in control[w]	
				U		S			C					
	U	S	C	0	1	0	1	2	0	1	2		S	C
1	17.7a (1.03)	- -	15.2b (0.82)	30	175[z]	-	-	-	1	62	116***		-	0.863
2	6.1a (0.49)	7.5a (0.45)	6.4a (0.43)	16	70	8	40	56	2	43	41**		0.787	0.762
3	- -	8.9a (0.44)	11.1b (0.38)	-	-	1	26	94	5	48	99**		0.900	0.858
4	- -	9.3a (0.54)	10.7a (0.54)	-	-	3	25	80	2	50	77***		0.875	0.917

w. Host type: U = unparasitized aphid, S = self-parasitized aphid, C = conspecific parasitized aphid.

x. Data analyzed with paired t-test. Means within rows followed by different letters are significantly different at $P < 0.05$. See text for levels of significance.

y. The observed frequencies of oviposition into aphids of each host type are listed in the table. The expected frequencies of oviposition (not shown) are determined using Equations (1),(2) and (3) in the text. Significant differences between the observed and expected values are tested utilizing contingency tables for *G*-tests for goodness of fit (with Williams' correction). Levels of significance: **, $P < 0.01$; ***, $P < 0.001$.

z. Analysis not performed on these data.

frequency of superparasitism. Therefore, I assumed that all eggs laid developed normally, and that the presence of a larva during aphid dissection was evidence of an oviposition. These results suggest that females exhibited oviposition restraint and thus were able to discriminate between unparasitized and conspecific parasitized hosts.

Experiment 2

The mean numbers of attacks by *A. ervi* females on unparasitized, self- and conspecific parasitized hosts did not differ statistically (One way analysis of variance; F = 2.54, P = 0.092) (Table 4.3). This result indicates that females did not preferentially attack particular host-types.

Aphid dissections revealed that the frequency of ovipositions into unparasitized hosts was not significantly different from that expected (G_{adj} = 0.40, P > 0.5, df = 1). Similar to the results of Experiment 1, the number of conspecific parasitized hosts superparasitized by the test female was lower than expected (G_{adj} = 10.05, P < 0.01, df = 2). However, the frequency of self-superparasitism was equal to that expected (G_{adj} = 5.85, P > 0.05, df = 2). This suggests that females restrained oviposition into hosts attacked by conspecifics, but not into self-parasitized hosts.

Experiment 3

Aphidius ervi females attacked a greater number of conspecific parasitized aphids than self- parasitized hosts (t = 2.75, P = 0.008, df = 13) (Table 4.3). The preference for conspecific parasitized hosts suggest that females can discriminate between the host types.

Dissections revealed that the number of self-superparasitized aphids was equal to the number expected (G_{adj} = 0.93, P > 0.9, df = 2). However, the number of conspecific parasitized hosts that contained two eggs was less than expected (G_{adj}

= 11.24, $P < 0.005$, df = 2). These results are consistent with those found in Experiments 1 and 2 and suggest that oviposition behavior is influenced by host-type.

Experiment 4

The numbers of self- and conspecific parasitized aphids attacked by *A. ervi* females was not statistically different ($t = 1.26$, $P = 0.118$, df = 11) (Table 4.3). Parasitoids exhibited oviposition restraint when they attacked conspecific parasitized hosts ($G_{adj} = 28.67$, $P < 0.001$, df = 2), but not when they attacked self-parasitized hosts ($G_{adj} = 0.99$, $P > 0.1$, df = 2). The oviposition behavior of females in this experiment was consistent with results in Experiments 1, 2 and 3.

Experiment 5

Praon pequodorum females attacked significantly more unparasitized hosts than conspecific parasitized aphids ($t = 13.83$, $P < 0.001$, df = 14) (Table 4.4). The significant difference in the response of females toward these host types cannot be explained by a difference in host encounter rate, as females encountered each host-type an equal number of times ($t = 1.35$, $P = 0.099$, df = 14).

Of the hosts that test females encountered, parasitoids rejected conspecific parasitized hosts more often than unparasitized aphids (*G*-test test for independence with Williams' correction; $G_{adj} = 228.31$, $P < 0.001$, df = 1). Parasitoids had an equal opportunity to attack or reject encountered aphids, so the difference in response toward the two hosts-types suggests a high degree of discrimination utilizing external cues. In many instances, females rejected conspecific parasitized aphids without touching the hosts, suggesting that the external cue may be volatile.

Dissection of attacked conspecific parasitized hosts revealed that the number of aphids superparasitized was equal to the number expected ($G_{adj} = 0.25$, $P > 0.5$,

Table 4.4. Results of host discrimination experiments with *Praon pequodorum*.

Exp.	Mean no. of aphids attacked per female (s.e. in brackets)[w,x]			Nos. of aphids containing given no. of larvae[w,y]									Proportion parasitism in control[w]	
				U		S			C					
	U	S	C	0	1	0	1	2	0	1	2		S	C
5	17.1a (0.55)	- -	2.7b (0.49)	7	167[z]	-	-	-	0	4	26		-	0.912
6	17.6a (0.54)	2.4b (0.54)	- -	29	143	1	5	18	-	-	-		0.909	-
7	- -	11.3a (0.61)	7.7b (0.48)	-	-	0	39	329	0	26	225		0.944	0.952

w. Host type: U = unparasitized aphid, S = self-parasitized aphid, C = conspecific parasitized aphid.

x. Data analyzed with paired *t*-test. Means within rows followed by different letters are significantly different at $P < 0.05$. See text for levels of significance.

y. The observed frequencies of oviposition into aphids of each host type are listed in the table. The expected frequencies of oviposition (not shown) are determined using Equations (1),(2) and (3) in the text. Significant differences between the observed and expected values are tested utilizing contingency tables for *G*-tests for goodness of fit (with Williams' correction). Levels of significance: undesignated values are not significantly different from expected frequencies ($P > 0.05$).

z. Analysis not performed on these data.

df = 2). This result suggests that females did not exhibit oviposition restraint when attacking conspecific parasitized hosts.

Experiment 6

Praon pequodorum females attacked significantly more unparasitized hosts than self-parasitized aphids (t = 14.03, $P < 0.001$, df = 9) (Table 4.4), suggesting that females discriminate between these host-types.

Dissection of self-superparasitized hosts revealed that the number of aphids that contained two eggs was equal to the number expected (G_{adj} = 0.76, $P > 0.5$, df = 2). This result suggests that females do not exhibit oviposition restraint in self-superparasitized hosts.

Experiment 7

This experiment took considerably longer to accomplish than either Experiments 5 or 6 because females of *P. pequodorum* were offered a choice between host-types that they did not prefer. Test females attacked significantly more self- than conspecific parasitized aphids (t = 4.65, $P < 0.001$, df = 33) (Table 4.4). This result suggests that females could discriminated between self- and non-self-parasitized aphids.

Dissection of superparasitized aphids revealed that females did not exhibit oviposition restraint, either when attacking self-parasitized hosts (G_{adj} = 2.31, $P >$ 0.1, df = 2) or conspecific parasitized aphids (G_{adj} = 1.41, $P > 0.1$, df = 2).

Heterospecific Larval Competition: *Aphidius ervi* and *Praon pequodorum*

At oviposition intervals AP_{24} and AP_{14}, *A. ervi* larvae were expected to hatch before their *P. pequodorum* counterparts; younger *P. pequodorum* larvae competed with older *A. ervi* larvae (Figure 4.1). At the AP_4 oviposition interval, *A. ervi* and *P. pequodorum* were expected to eclose synchronously-- the competitive elimination of

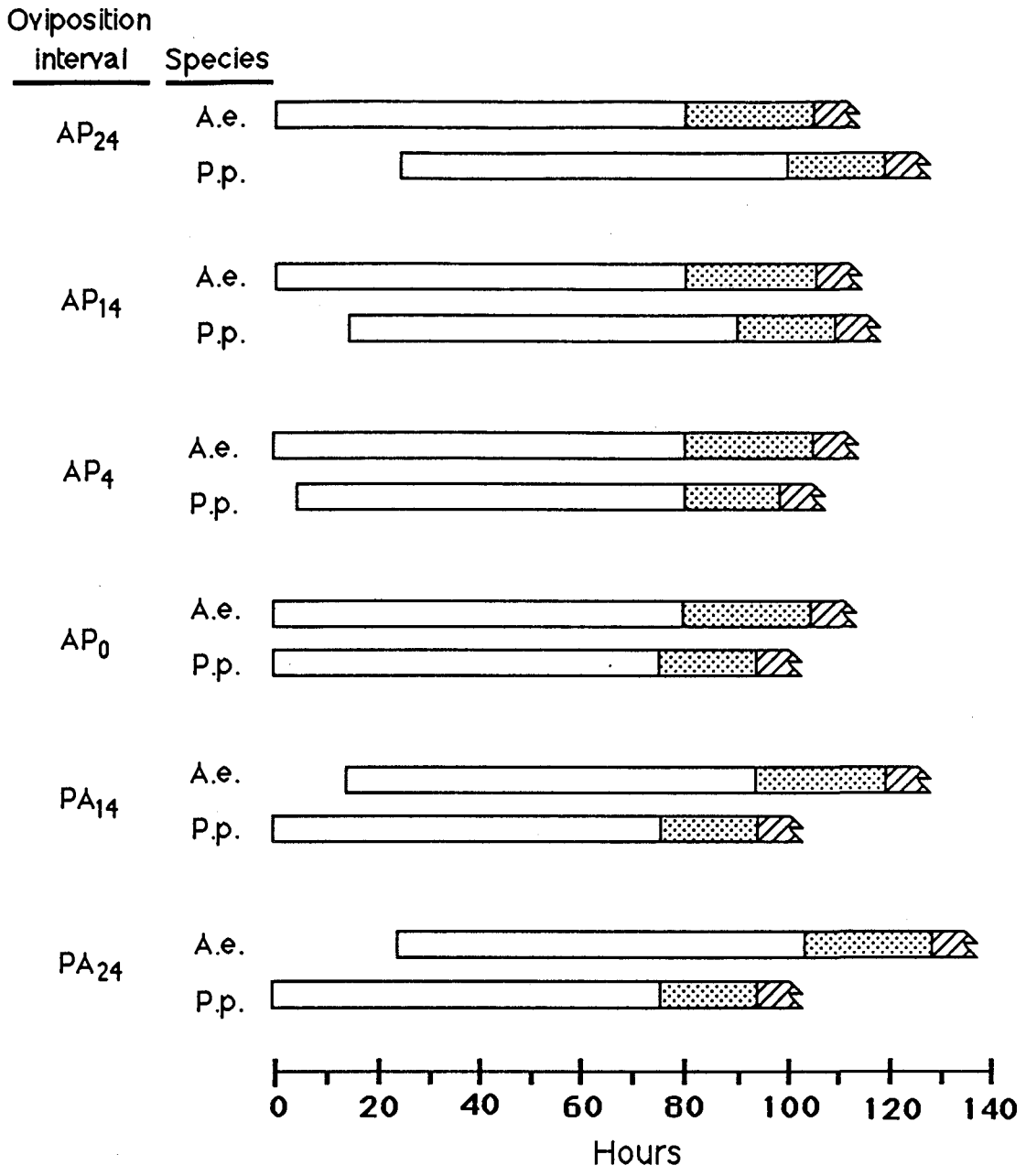


Figure 4.1. Expected stage of development for *Aphidius ervi* and *Praon pequodorum* in heterospecific superparasitized aphids. Oviposition interval: order of attack indicated by order of letters (A = *A. ervi*, P = *P. pequodorum*); numbers in subscript indicate time (h) between successive attacks on the same aphid. Species abbreviation: A.e. = *A. ervi*, P.p. = *P. pequodorum*. Clear, stippled and hatched portions of bars represent median developmental time (h) of the egg, first- and part of the second-instar stages, respectively. At each oviposition interval, oviposition by first species to attack aphids occurs at 0 h; time between successive ovipositions represented by a displacement of one bar along the time scale. Developmental times of larvae in singly parasitized aphids (@ 21°C for *A. ervi* [McBrien and Mackauer 1990]; @ 21.1°C for *P. pequodorum* [Chow and Mackauer 1984]).

supernumerary larvae would occur when both species were first instar larvae. At these intervals, the number of *A. ervi* mummies observed to form was significantly lower than the number expected (AP_{24} interval: G_{adj} = 14.03, $P < 0.001$, df = 1; AP_{14} interval: G_{adj} = 24.05, $P < 0.001$, df = 1; AP_4 interval: G_{adj} = 12.43, $P < 0.001$, df = 1) (Table 4.5). These results suggest that *P. pequodorum* larvae won more competitions. The result for the AP_4 interval, in particular, indicates that *P. pequodorum* is superior to *A. ervi* in larval competition. At this interval, larvae were expected to hatch at the same time, resulting in an unbiased test of larval competitive ability. Data from aphid dissections confirmed that more *P. pequodorum* larvae survived larval competition.

In oviposition intervals AP_0 and PA_{14}, *P. pequodorum* larvae were expected to hatch before their *A. ervi* counterparts (Figure 4.1). Dissections of aphids in the AP_{14} interval confirmed this, but dissections from the AP_0 interval revealed that *A. ervi* larvae hatched before *P. pequodorum* larvae. However, earlier eclosion did not benefit *A. ervi* larvae because the superiority of first instar *P. pequodorum* compensated for the apparent temporal eclosion advantage. At these intervals, the number of *A. ervi* mummies observed to form was equal to the number expected (AP_0 interval: G_{adj} = 0.25, $P > 0.5$, df = 1; PA_{14} interval: G_{adj} = 0.90, $P > 0.1$, df = 1) (Table 4.5). These results suggest that neither parasitoid species was superior to the other. Aphid dissections revealed that approximately the same number of larvae died in both species.

At the PA_{24} interval, *P. pequodorum* larvae were expected to hatch before *A. ervi*. The results show that the number of *A. ervi* mummies observed to form was significantly greater than the number expected (G_{adj} = 5.31, $P < 0.05$, df = 1), suggesting that *A. ervi* won more larval competitions.

Table 4.5. Results of heterospecific larval competition between *Aphidius ervi* and *Praon pequodorum*.

Order of attack[a]	Time between attacks (hours)	Oviposition interval	No. of aphids dissected	Proportion of dissected aphids containing given species[a]			Nos. of mummies observed to formed from undissected aphids[a,b]	
				A.e. (p_A)	P.p. (p_B)	A.e.+P.p. (p_{AB})	A.e. (N_A)	P.p. (N_B)
A.e. then P.p.	24	AP_{24}	44	0	0.182	0.818	6	36***
	14	AP_{14}	44	0	0.136	0.864	2	32***
	4	AP_4	41	0.097	0.123	0.780	9	32***
	0	AP_0	29	0	0.241	0.759	14	27
P.p. then A.e.	14	PA_{14}	46	0.044	0.630	0.326	3	20
	24	PA_{24}	28	0	0.893	0.107	7	42*

a. A.e. = *A. ervi*; P.p. = *P. pequodorum*.
b. The numbers of mummies observed to form from undissected aphids are listed. The numbers of mummies expected to form from the dissected aphids if they were allowed to develop (not shown) are determined using Equations (4) and (5) in the text. Significant differences between the observed and expected frequencies are tested utilizing 2x2 contingency tables and *G*-tests for goodness of fit (with Williams' correction). Levels of significance: *, $P < 0.05$; ***, $P < 0.001$.

Response of *Praon pequodorum* Eggs to a Heterospecific Competitor

Eggs of *A. ervi* and *P. pequodorum* differ in gross morphology (Figure 2.2). Microscopic examination of stained *A. ervi* eggs that contain a well developed embryo reveal that they possess two main layers, the serosa and chorion. Eggs of *P. pequodorum* in a similar state of development possess three major layers (Figure 4.2). The *P. pequodorum* embryo is surrounded by a serosa and chorion, but between these layers exists a clear, elastic, apparently non-cellular layer. Eggs of *A. ervi* may possess the extra-serosa envelope (E.S.E.), but in a much reduced state.

The E.S.E. in *P. pequodorum* eggs is easily separated from the serosa and the chorion. In eggs that were placed in a concentrated $NaCl_{(aq)}$ solution, the serosa recessed from the E.S.E., leaving concave impressions from individual serosa cells on the inner surface of the E.S.E. With some manipulation, the chorion can be removed from the egg, leaving the E.S.E. intact. These results suggest that the E.S.E. may not be tightly bound to either the serosa or the chorion.

The depth of the E.S.E. in *P. pequodorum* eggs, three days after oviposition, varied between host-types (Table 4.6). In aphids that were singly and conspecifically superparasitized, the thickness of the E.S.E. in eggs from these host-types was not significantly different ($P > 0.05$). However, the deepness of the E.S.E. in *P. pequodorum* eggs was significantly greater if eggs were found in the presence of an *A. ervi* competitor ($P < 0.05$). These results indicate that species of competitor may be the primary factor that initiates the response in *P. pequodorum* eggs; the E.S.E. developed only when *P. pequodorum* was present with a heterospecific competitor.

Some *P. pequodorum* eggs in heterospecific superparasitized hosts possessed puncture marks in the chorion. These marks may have been inflicted by the mandibles of first instar *A. ervi* larvae, which were frequently observed attacking *P. pequodorum* eggs. The *P. pequodorum* embryos inside the eggs did not appear to

Figure 4.2. Egg of *Praon pequodorum*, about 40 h after oviposition in pea aphid. A. Morphology of egg in singly or conspecific superparasitized host. B. Egg from host also containing one egg of *Aphidius ervi*. Abbreviations: C = chorion, E = embryo, ESE = extra-serosa envelope, S = serosa.

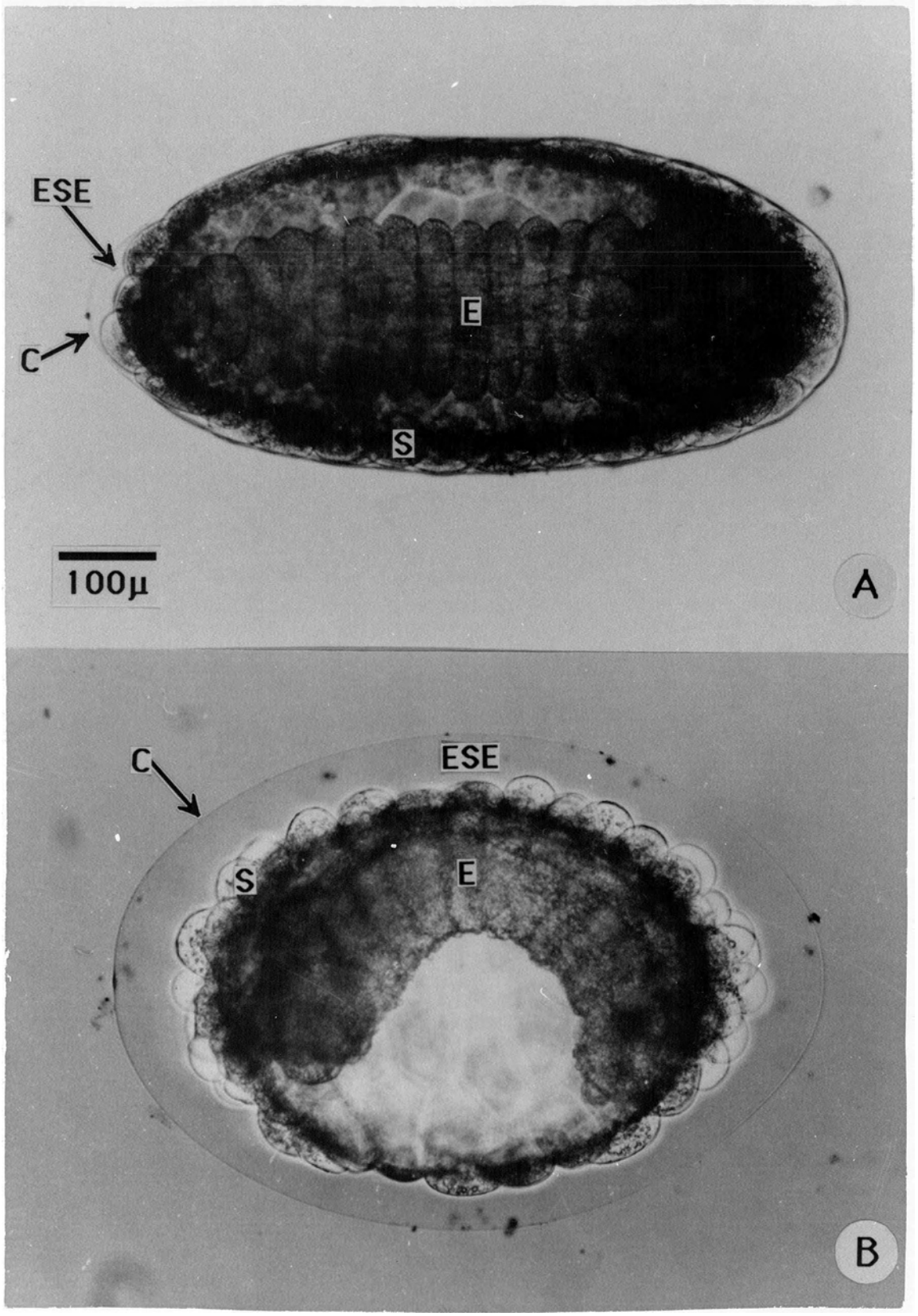

ESE
C
E
S
100µ
A
C
ESE
S
E
B

Table 4.6. Thickness of the extra-serosa envelope in *Praon pequodorum* eggs three days after oviposition.

No(s). of parasitoid eggs per dissected aphid[v,w]		Time between successive attacks (h)	N[x]	Mean thickness (±s.e.) of the E.S.E. (μ)[z]
A.e.	P.p.			
0	1	N/A	14	1.25±0.912a
0	2	18[y]	11	1.27±0.604a
1	1	0[y]	10	33.60±5.797bc
1	1	4[y]	10	26.70±1.955b
1	1	14[y]	11	62.36±1.216d
1	1	24[y]	12	48.83±6.224cd

v. Only aphids that contained the given number(s) of eggs were included in the analysis.
w. Abbreviations: A.e. = *Aphidius ervi*, P.p. = *Praon pequodorum*, E.S.E. = extra-serosa envelope.
x. N = number of eggs examined.
y. Time between successive attacks $\leq$ 15 min.
z. Means within column followed by different letters are significantly different at $P = 0.05$. The T'-method of unplanned, multiple comparisons (Sokal and Rohlf 1981).

suffer any visible deleterious effects. Indeed, the results of heterospecific larval competitions and these observations suggest that the E.S.E. may protect the *P. pequodorum* embryos from attack by *A. ervi* larvae. As a result, the E.S.E. gives *P. pequodorum* a competitive advantage over *A. ervi* in larval competition.

Discussion

Host discrimination is the ability of female wasps to differentiate between similar individuals. In the social Hymenoptera, recognition between nestmates and non-nestmates appears to be a rule (Carlin and Holldobler 1983). Nestmate recognition appears to be mediated purely chemically (Holldobler and Michener 1980), and has been demonstrated in *Apis mellifera* L. (Hymenoptera: Apidae) (Moritz 1988; Page, Jr. and Erickson, Jr. 1984), *Lasioglossum zephyrum* (Buckle and Greenberg 1981), *Polistes fascatus* (Hymenoptera: Vespidae) (Gamboa 1988) and *Pseudomyrmex ferruginea* (Mitzner 1982). It has been suggested that in *A. mellifera* (Breed 1983; Visscher 1986), *Dolichovespula maculata* (Hymenoptera: Vespidae) (Ryan *et al.* 1985), *L. zephyrum* (Greenberg 1979) and *P. fuscatus* (Gamboa 1988; Klahn and Gamboa 1983), individual-specific cues may assist insects in distinguishing between similar individuals.

Among the parasitic Hymenoptera, host discrimination appears to be a common phenomenon (Vinson 1976) and has been studied by a number of investigators. Most solitary parasitoid species exhibit host discrimination (van Alphen and Visser 1990; van Lenteren 1981), and examples include *Leptopilina heterotoma* Thomson (Hymenoptera: Cynipidae) (Bakker *et al.* 1990) *Nemeritis* (= *Venturia*) *canescens* (Grav.) (Hymenoptera: Ichneumonidae) (Fisher 1961a; Hubbard *et al.* 1987; Rogers 1972), *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) (Takasu and Hirose 1991), *Pseudeucoila bochei* Weld (Hymenoptera:

Cynipidae) (van Lenteren 1976; van Lenteren and Bakker 1975) and *Trichogramma evenescens* Westwood (Hymenoptera: Trichogrammatidae) (Salt 1937). Host discrimination has been reported in a number of species of aphid parasitoids (Hymenoptera: Aphidiidae) (Mackauer 1990), including *A. ervi* (Bai 1991; Bai and Mackauer 1991; McBrien and Mackauer 1990, 1991), *Aphidius nigripes* Ashmead (Cloutier *et al.* 1984), *A. smithi* Sharma & Subba Rao (McBrien and Mackauer 1990, 1991), *Ephedrus californicus* Baker (Chow and Mackauer 1986; Volkl and Mackauer 1990), *Ephedrus cerasicola* Stary (Hofsvang 1988; Hofsvang and Hagvar 1983), *Praon palitans* Muesebeck (Schlinger and Hall 1960) and *Trioxys* (= *Binodoxys*) *indicus* Subba Rao & Sharma (Singh and Sinha 1981, 1982); in addition, Bai and Mackauer (1990, 1991) reported host discrimination in *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae).

Bakker *et al.* (1985) concluded that host discrimination evolved because it was of strong selective advantage. Host discrimination is expected to prevent the wastage of eggs and hosts (Bakker *et al.* 1985; Charnov and Skinner 1985; Roitberg and Mangel 1988; van Alphen 1988; van Lenteren 1976; van Lenteren 1981; Vinson 1976). In solitary endoparasitoids, supernumerary larvae are eliminated by physical combat and/or physiological suppression (Chow and Mackauer 1985; Chow and Sullivan 1984). As a result, female fitness can benefit if parasitoids can recognize the difference between unparasitized and parasitized hosts and preferentially oviposit into the former (Roitberg and Mangel 1988; van Alphen and Visser 1990; Visser *et al.* 1992).

When *A. ervi* females were given a choice between unparasitized and conspecific parasitized hosts, females preferentially attacked the former host-type. These results suggest that females utilize an external cue in host discrimination

(McBrien and Mackauer 1990, 1991). The preference expressed by females for unparasitized hosts is consistent with the accepted view that parasitoids tend to avoid superparasitism (Bakker *et al.* 1990). However, my results are in contrast to those of Bai (1991) who utilized the same host-types and found no difference in the number of attacks, suggesting that females did not use external cues in host discrimination. The experimental procedures used by Bai (1991) are similar to those used in my study, so it is unlikely that the difference arose due to this factor. The disparity in the results between the studies may be explained by differences in the degree of relatedness between individual wasps. Bai (1991) used wasps from a laboratory colony that was cultured for many generations, whereas the wasps used in my study were from isofemale lines established with field collected specimens. In my experiment, the test females and parasitoids used to prepare conspecific parasitized aphids came from different lines. This procedural difference between the studies may be significant because Unruh *et al.* (1983) demonstrated that genetic variation of laboratory colonies of *A. ervi* can decrease over time. Assuming that marking pheromones (or their blends) are influenced by genotype (Crozier and Dix 1979; Wilson 1987), it is possible that the differences in the marking pheromones between individuals may have been greater in my study than in that of Bai (1991). The degree of relatedness between two parasitoids is expected to influence their ability to discriminate between self- and non-self-parasitized hosts (Hubbard *et al.* 1987).

Aphidius ervi females did not express a statistically significant preference when presented with unparasitized, self- and conspecific parasitized hosts. When the supply of unparasitized hosts is sufficient (Experiment 1), parasitoids should preferentially attack unparasitized hosts (Hubbard *et al.* 1987). However, the ratio of unparasitized to parasitized hosts was 1:2 in this experiment-- unparasitized hosts were in short supply. Under these conditions, it would be adaptive for time-limited

parasitoids to superparasitize because the second egg has a greater than zero chance of survival (Cloutier 1984; Hubbard *et al.* 1987; van Alphen and Visser 1990; van Dijken and Waage 1987). Statistical differences may have become evident with further replication.

Aphidius ervi females presented with self- and conspecific parasitized hosts preferred to attack the latter host-type. Firstly, these results suggests that host discrimination in *A. ervi* may be mediated by external cues. McBrien and Mackauer (1990, 1991) suggested that *A. ervi* females utilized a marking pheromone in heterospecific host discrimination; *A. ervi* females given a choice between conspecific parasitized hosts and hosts attacked by *A. smithi*, preferred to attack the latter host-type. In my experiment, female parasitoids were not supplied with unparasitized hosts, so under these conditions superparasitism of either host type is adaptive because the second egg has a greater than zero chance of survival (Cloutier 1984; Hubbard *et al.* 1987; van Alphen and Visser 1990; van Dijken and Waage 1987). Secondly, the difference in the frequency of attacks on self- and conspecific parasitized hosts suggests that the external marker may be individual-specific. Mackauer (1990) stated that there is no experimental proof to suggest that marking pheromones in aphidiids are individual and unique. However, discrimination between self- and conspecific parasitized hosts was demonstrated in *E. californicus* (Volkl and Mackauer 1990); females preferentially oviposited into conspecific parasitized hosts.

The oviposition behaviour of *A. ervi* was consistent throughout all experiments. *Aphidius ervi* females exhibited oviposition restraint when they attacked conspecific parasitized hosts. Conversely, females did not refrain from ovipositing into self-parasitized hosts. McBrien and Mackauer (1990) stated that

evidence of oviposition restraint by females that attack newly parasitized hosts is indicative of a response to an external marker, rather than an internal cue. The short interval between successive attacks would not have be long enough to result in significant changes in host physiology (i.e. development of internal cues). The difference in oviposition behaviour towards self- and conspecific parasitized aphids further suggests that the pheromone markers used by *A. ervi* are individual-specific.

The tendency of females to self-superparasitize hosts is difficult to explain as this behaviour results in competition among siblings and wastage of eggs. However, this behavior may be adaptive under three conditions that include (1) parasitoid species is time-limited (eggs are inexpensive), (2) suitable hosts are in short supply and (3) the probability of superparasitism by conspecifics is significant. All three conditions may not have to be in effect concurrently, but conditions (1) and (2) are important. Firstly, *A. ervi* can be described as a time-limited species (Mackauer *et al.* 1992). Secondly, with host replacement, the average ratio between unparasitized and parasitized hosts was 1:1, so unparasitized hosts were in short supply. Test females may have percieved the shortage of unparasitized hosts to be indicative of a high degree of competition with conspecifics for hosts. Bakker *et al.* (1985) stated that self-superparasitism is not favorable for parasitoids that optimize the allocation of time and eggs. However, under my experimental conditions, the oviposition behavior exhibited by *A. ervi* may be adaptive. While only one egg survives in self-superparasitized hosts, the presence of two or more eggs may increase the probability of producing an offspring if the host is subsequently attacked by a conspecific (condition [3]) or a wasp of a different species (Mackauer *et al.* 1992).

Bai and Mackauer (1992) provided physiological evidence that suggested that conspecific superparasitism by *A. ervi* may benefit the surviving parasitoid offspring.

They found that the developmental time (oviposition to adult eclosion) of *A. ervi* from conspecific superparasitized and singly parasitized aphids did not differ, but the dry mass of adults was significantly greater from the former host-type. Likewise, the dry mass of surviving larvae from self-superparasitized hosts may be higher than that from singly parasitized hosts because the physiology and larval competitive mechanisms possessed by members of the same species are expected to be similar. However, Bai and Mackauer (1992) state that in the case of self-superparasitism, a gain in adult dry mass is unlikely to compensate for a 50% or greater reduction in egg survival. These results, in combination with my findings, suggest that conspecific superparasitism in *A. ervi* may by adaptive.

Praon pequodorum females always preferred to attack unparasitized hosts when given a choice between this host-type and conspecific parasitized or self-parasitized hosts. These results suggest that *P. pequodorum* utilizes an external marker in host discrimination. The preference displayed for unparasitized hosts is expected if the supply of unparasitized hosts is not limiting (Bakker *et al.* 1985; Hubbard *et al.* 1987). In contrast to the behavior of *A. ervi*, the higher proportion of unparasitized hosts attacked by *P. pequodorum* females suggests that females in the latter species may have a higher expectation of encountering a large number of unparasitized hosts. This may be important to the explanation of the behavior associated with parasitized hosts.

Of the hosts encountered by parasitoids, *A. ervi* females attacked a greater proportion of aphids than did *P. pequodorum* females. For example, 52.8% of unparasitized-host encounters resulted in attacks, while the value for conspecific parasitized hosts was 9.1% (Experiment 5). Conversely, the proportion of host encounters that result in an attack by *A. ervi* females was greater than 90% for all

host-types. The explanation for this disparity may involve inherent differences in oviposition behavior. *Praon pequodorum* females are less active than their *A. ervi* counterparts. Oviposition by *A. ervi* occurs almost instantaneously after females encounter a host; generally, host handling time (time spent examining and ovipositing in the host) is less than one second. On the other hand, *P. pequodorum* females may spend up to ca. 15 seconds with the host prior to oviposition, which takes less than a second. The greater time spent by *P. pequodorum* females examining hosts may enable them to make a more thorough assessment of host quality than is possible by *A. ervi* females. This innate difference in host handling behavior may be responsible for the disparity in host acceptance/rejection rates between the species.

Praon pequodorum females attacked more self-parasitized hosts than conspecific parasitized aphids. This result suggests that discrimination between these host-types may be facilitated by oviposition markers that are individual-specific. However, similar to the self-superparasitizing behavior of *A. ervi*, the same behavior in *P. pequodorum* is difficult to explain. The evolutionary explanation may depend on the degree of expectation that wasps have about the frequency with which they will encounter hosts. Earlier, I suggested that *P. pequodorum* may have a high expectation about the availability of unparasitized hosts. In this experiment, such an expectation may have been created when the wasp attacked only unparasitized aphids during the preparation of the self-parasitized host-type. On encountering only parasitized host types in the arena, females had two choices: leave without ovipositing or stay and superparasitize. Frequently, parasitoids attempted to fly away, but were prevented from doing so by the petri dish lid, indicating that they would have preferred to refrain from oviposition. Hubbard *et al.*

(1987) concluded that wasp fitness would benefit more if conspecific parasitized hosts were attacked. However, wasps with a high expectation about the availability of "good quality" hosts may perceive a large proportion of parasitized hosts to be indicative of a significant risk of superparasitism by other females. In this case, self-superparasitism will benefit female fitness more (Mackauer *et al.* 1992). Having two (or more) eggs in a host can increase the probability of producing an offspring if conspecifics parasitize the host later (Mackauer *et al.* 1992).

Observations of the behavior of *P. pequodorum* females with parasitized and unparasitized hosts suggests that females do not require physical contact with hosts to discriminate. This indicates that the external cue is volatile. Schlinger and Hall (1960) reported similar behavior with *P. palitans*. Dissection of females of *A. ervi* and *P. pequodorum* revealed that parasitoids in both species possess a Dufour's gland that contains an oily substance. In the parasitoids *Campoletis perdistinctus* (Viereck) (Hymenoptera: Ichneumonidae) (Guillot and Vinson 1972) and *N. canescens* (Harrison *et al.* 1985), it has been suggested that the Dufour's gland contains the marking pheromones; hosts topically treated with Dufour's gland secretions were avoided by searching females. Mudd *et al.* (1982) demonstrated that the Dufour's gland of *N. canescens* contains an oily mixture of saturated and monosaturated hydrocarbons that may act as a carrier for marking pheromones. The response of *A. ervi* and *P. pequodorum* females to various host-types indicates that females detect a marking pheromone.

I did not conduct a separate experiment to test heterospecific host discrimination between *A. ervi* and *P. pequodorum*. However, observations and data relating to the heterospecific larval competition study can be used to infer whether heterospecific host discrimination occurs in these two species. *Aphidius ervi* females

readily attacked aphids previously attacked by *P. pequodorum*, suggesting that females do not recognize, or ignored, the external cues left by *P. pequodorum*. Dissection of heterospecific attacked aphids (PA_{14} and PA_{24} oviposition intervals) revealed that in 44 aphids containing *P. pequodorum*, only 13 contained *A. ervi*. The rate of oviposition by *A. ervi* females was less than that expected (Table 4.3), suggesting that females exhibited oviposition restraint. McBrien and Mackauer (1990, 1991) reported that *A. ervi* discriminated against aphids parasitized by *A. smithi*; as discrimination was observed when the interval between successive attacks was as large as 72 hours, internal cues were likely responsible for host-type recognition. Discrimination of aphids from long oviposition intervals is facilitated by the perception of physiological changes (i.e. internal cues). Chow and Mackauer (1984) demonstrated that *A. smithi* females detected internal cues in hosts previously parasitized by *P. pequodorum*, resulting in oviposition restraint; oviposition restraint was observed only if the time between successive attacks was 14 hours or more. The oviposition restraint demonstrated by *A. ervi* in my experiment indicates that female parasitoids can discriminate if internal cues are utilized.

In all oviposition intervals, *P. pequodorum* females readily attacked aphids previously attacked by *A. ervi*, suggesting that females may not recognize, or ignored, external cues deposited by *A. ervi*. Dissection of attacked aphids revealed that the frequency of oviposition by *P. pequodorum* females into hosts previously attacked by *A. ervi* was consistent with that expected (Table 4.4). This result suggests that *P. pequodorum* females do not discriminate against aphids previously attacked by *A. ervi*. Similarly, Chow and Mackauer (1984) reported that *P. pequodorum* did not discriminate against aphids previously attacked by *A. smithi*.

The lack of heterospecific host discrimination based upon recognition of external cues is not entirely unexpected, due to the apparent difference in the

phylogeny between *Aphidius* and *Praon*. Edson and Vinson (1979) stated that differences in the venom apparatus of wasps from these genera may indicate that *Aphidius* and *Praon* evolved along two very distinct lines. The chemical composition (or blend) of the marking pheromone(s) may share a greater similarity among conspecifics than among females of different species. Crozier and Dix (1979) and Wilson (1987) stated that chemical cues utilized in kin recognition are genetically determined in insects. For this reason, heterospecific host discrimination based on the recognition of external cues is rare (Bakker *et al.* 1985); van Alphen and Visser (1990) stated that heterospecific host discrimination may only occur between closely related species. The occurrence of heterospecific host discrimination may be explained by the recognition of internal cues (i.e. physiological changes). Cues of this type are probably not associated with individual species, but rather, they may be common to parasitized hosts in general (Vinson 1976).

The larval competitive ability of *P. pequodorum* was superior to that of *A. ervi*; in competitions between same-aged larvae, *P. pequodorum* won more larval competitions than *A. ervi*. Similarly, Chow and Mackauer (1984) reported that *P. pequodorum* was superior to *A. smithi*, a species related to *A. ervi*.

In heterospecific contests between larvae of different ages, the order of attack and time between successive ovipositions influenced the outcome. Generally, *P. pequodorum* larvae were able to out-compete their *A. ervi* counterparts. *Aphidius ervi* larvae could out-compete *P. pequodorum* only if the former species oviposited into aphids previously attacked by the latter species (PA_{24} oviposition interval). In this situation, competition ensued between young (i.e. mandibulate first-instar) *A. ervi* larvae and older (i.e. amandibulate second-instar) *P. pequodorum* larvae. The advantage arises in this particular contest due to the fact that first-instar *A. ervi* larvae are mandibulate, while their second-instar counterparts are amandibulate.

The comparative advantage afforded younger larvae in heterospecific larval competitions is in contrast to the relative superiority of older larvae in conspecific larval competitions (Mackauer 1990). Taking into account the apparent competitive advantages of each species, *P. pequodorum* is the species that possesses competitively superior larvae.

A factor that may increase the chances for survival of immature *P. pequodorum* in competition is a characteristic of the *P. pequodorum* egg. Unlike the eggs of *A. ervi*, *P. pequodorum* eggs have an E.S.E. that becomes prominent during competition with heterospecific eggs or larvae.

One explanation for the presence of the E.S.E. is that *P. pequodorum* eggs are being subjected to an immune reaction from the host. Insects have three main physiological defence mechanisms against invading organisms: coagulation, humoral reaction and cellular response (Vinson 1990). Coagulation of insect hemolymph involves cell agglutination and coagulation of hemolymph proteins. This immune reaction is observed in wound healing and likely does not contribute directly to an immune response in parasitized hosts. Humoral reactions involve the production and/or release of protein/carbohydrate-type compounds, primarily to combat bacterial infection. A cellular immune response (encapsulation) is a common reaction of insect hosts to an invasion by foreign organisms that include bacteria, fungi, protozoa and parasitoid eggs or larvae. Encapsulation involves the deposition of several layers of host hemocytes around a foreign body; often, the inner layer of hemocytes melanizes. In aphids, a cellular immune response to endoparasitization has been reported in *Acyrthosiphon kondoi* Shinji (Carver and Woolcock 1985), *Aulacorthum circumflexum* Buckt. (Griffiths 1961) and *Neomyzus circumflexus* (Buck.) (El-Shazly 1972). However, encapsulation is not a consistent phenomenon across, or within, aphid species. For example, *A. asychis* is encapsulated in *A. kondoi*,

whereas *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae), *A. ervi*, *Aphidius pisivorus* Smith (Hymenoptera: Aphidiidae) and *Praon volucre* (Haliday) (Hymenoptera: Aphidiidae) develop successfully (Carver and Woolcock 1985). *Acyrthosiphon pisum* has not been reported to encapsulate *A. asychus* (Bai 1991; Bai and Mackauer 1990; Carver and Woolcock 1985), *A. ervi* (Bai 1991; Chow and Mackauer 1986, 1992; McBrien and Mackauer 1990, 1991), *A. pisivorus* (Chow and Mackauer 1992), *A. smithi* (Campbell and Mackauer 1975; Chow and Mackauer 1984, 1985, 1992; McBrien and Mackauer 1990, 1991), *E. californicus* (Chow and Mackauer 1986; Volkl and Mackauer 1990) or *P. pequodorum* (Chow and Mackauer 1984, 1985, 1992; Sequeira and Mackauer 1987, 1988). Thus, the E.S.E. in *P. pequodorum* eggs is unlikely to be a result of encapsulation.

An alternative explanation for the E.S.E. is that the layer is generated within the egg. Discussion of this explanation is aided by reviewing embryogenesis in insects. As I have not encountered descriptions of the embryology in *A. ervi* and *P. pequodorum*, examples of development in other holometabolous species will be utilized. Studies of embryogenesis in *Drosophila melanogaster* Meig. (Diptera: Drosophilidae) (Mahowald 1963) and the apocritan hymenoptera, *A. mellifera* (Anderson 1972, DuPraw 1967), *Mesoleius tenthredinis* Morl. (Hymenoptera: Ichneumonidae) (Bronskill 1964) and *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae) (Bronskill 1959), provide good examples of holometabolous insect development.

The typical insect egg is composed of a central mass of cytoplasm contained within a non-cellular vitelline membrane which is surrounded by a non-cellular chorion (Counce 1973; Hinton 1981). At oviposition, egg cytoplasm contains yolk vacuoles (small or absent in parasitic hymenoptera), nuclear material, organelles and other constituents. This description is consistent with the egg morphology of the

insect parasitoids *Apanteles glomeratus* (L.) (Hymenoptera: Braconidae) (King *et al.* 1969), *M. tenthredinis* (Bronskill 1964), *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) (King *et al.* 1968), *P. turionellae* (Bronskill 1959) and *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) (King *et al.* 1968). The egg morphology of *A. ervi* and *P. pequodorum* is similar to that of other insect parasitoids.

Development of insect embryos begins soon after oviposition, irrespective of whether eggs have been fertilized. Mitotic divisions of the nuclear material (without cell division) ensue, resulting in a large number of cleavage energids. The majority of the energids migrate from the central cytoplasm/yolk mass to the periplasm (less dense cytoplasm located adjacent to the vitelline membrane). There, each energid is surrounded by a membrane (now called a plasma membrane) which is derived from the vitelline membrane. Concomitant with the enclosure of each energid, a single membrane is laid down around the central yolk mass. At this stage of development, a blastula is formed; the embryo consists of a central yolk mass contained within a single membrane that is surrounded by a blastoderm (one cell thick) and a chorion. Soon thereafter, cells in the blastoderm differentiate into the embryonic primordium (consisting of embryonic ectoderm and mesoderm) and extra-embryonic ectoderm. In the apocritan Hymenoptera, the development of the serosa differs from that observed in other holometabolous species, as usually there is no trace of an amnion; in the eggs of most holometabolous species, an amnion forms beneath the serosa. The serosa in apocritan Hymenoptera is formed in three main steps (see Fleig and Sander 1988): (1) attenuation of the extra-embryonic ectoderm (presumptive serosa), (2) separation of the dorsal margins of the extra-embryonic ectoderm from the embryonic ectoderm in the embryonic primordium and (3) migration of the extra-embryonic ectoderm over the embryonic primordium. Further development results in the separation of the embryo from the serosa,

leaving a free embryo surrounded by a complete serosa (one cell thick) and a chorion. The central yolk mass is internalized by the developing embryo after being encased in embryonic tissue.

The explanation that the E.S.E. arises within the egg, rather than being an indication of encapsulation, is supported by two lines of evidence. Firstly, according to the description of the embryogenesis in other apocritan Hymenoptera, cells of the serosa in *P. pequodorum* eggs should lay adjacent to the chorion. In fact, the E.S.E. is positioned between the serosa and chorion. The presence of a layer in an area that should not contain a major structure suggests that the layer arises from, or its construction is aided by, the serosa. Secretion of a distinct layer by the serosa has been observed in the Collembola (Jura 1972),. the grasshopper, *Melanoplus differetialis* (Slifer [1937] cited in Miller [1940]), and the stonefly, *Pteronarcys proteus* Neuman (Miller 1940). Secondly, examination of stained eggs does not suggest that the E.S.E. is composed of cells, as would be observed in encapsulation. Rather, the E.S.E. appears to be a single mass. The scarcity of published reports on serosa secretions in the apocritan Hymenoptera (or endoparasitoids, in particular) suggests that this phenomenon may be rare in insects. In any event, the E.S.E. in *P. pequodorum* eggs appears to play a role in embryo survival.

The E.S.E. may increase the probability of survival of *P. pequodorum* embryos during heterospecific competition of immature stages. First-instar larvae of *A. ervi* attack *P. pequodorum* eggs, but are unable penetrate the E.S.E. and serosa. Hinton (1981) reported that in a number of insect species, layers in or around the egg (eg. cuticle secreted by serosa, thickened chorion) act as a mechanical barrier to physical attack. The protection afforded *P. pequodorum* embryos by the E.S.E. provides this species with a competitive advantage. This attribute, in combination with superior larval competitive ability, may assist immature stages of *P.*

pequodorum to survive in heterospecific superparasitized hosts.

It is reasonable to suggest that, if the E.S.E. can withstand physical attacks on its exterior surface, the structural integrity of the layer may impede hatching of *P. pequodorum* embryos. This statement would be true if it was not for a significant reduction in the elasticity of the layer observed prior to embryo eclosion. Degradation of the E.S.E. suggests that the layer may undergo enzymatic catabolysis during hatching. Dissolution of egg membranes in a number of Hymenoptera species, across six related Families, has been reported by DuPraw (1967); in *A. mellifera*, the embryo was reported to produce a "hatching enzyme" that was responsible for dissolution of the chorion. The source of the lytic enzymes in eggs of *P. pequodorum*, may be the serosa. Dahlman (1990) demonstrated that trophocytes (disassociated serosa cells) have a secretory function. Therefore, it is possible that cell that comprise the serosa in *P. pequodorum* eggs may express a secretory role prior to their separation. As a result, the serosa may facilitate eclosion of *P. pequodorum* embryos from eggs that possess an E.S.E.

Parasitoids are expected to exhibit behaviors that allow them to achieve the greatest reproductive success (Charnov and Skinner 1985). Host discrimination decisions directly influence the reproductive success of parasitoids and such decisions may be strongly shaped by natural selection. The oviposition behaviors displayed by *A. ervi* and *P. pequodorum* appear to be adaptive. In the field, superparasitism of pea aphids by *A. ervi* and *P. pequodorum* is not common (McBrien 1991), except when hosts are scarce (Campbell 1974). In instances when the two species compete, the attributes possessed by *P. pequodorum* are favorable to its survival and may assist this species to survive at low population levels. Even though *A. ervi* is a more aggressive colonizer, it is unlikely that this species will competitively displace *P. pequodorum* in southern British Columbia.

CHAPTER 5

COMPETITION BETWEEN *APHIDIUS ERVI* AND *PRAON PEQUODORUM*

Introduction

The pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), is an economically important pest of Leguminosae, including alfalfa (*Medicago sativa* L.) (Forbes and Chan 1989; Harper 1986). The solitary endoparasitoids (Hymenoptera: Aphidiidae), *Aphidius ervi* Haliday and *Aphidius smithi* Sharma & Subba Rao, were introduced into North America during the late 1950's to control the pea aphid (Halfhill *et al.* 1972; Mackauer and Finlayson 1967; Stary 1974). These species were imported to increase the level of control effected by the extant solitary endoparasitoids (Hymenoptera: Aphidiidae), *Aphidius pisivorus* Smith and *Praon pequodorum* Viereck (Mackauer 1971; Mackauer and Finlayson 1967).

In British Columbia, *A. ervi*, *A. pisivorus*, *A. smithi* and *P. pequodorum* were all common parasitoids of pea aphid in alfalfa (Campbell 1974; Kambhampati 1987; McBrien 1991). However, recent collections from southern British Columbia indicate that *A. ervi* is the dominant parasitoid of the pea aphid, comprising 90% or more of samples, with *P. pequodorum* constituting the remainder (Chapter 3).

Force (1970) stated that the most important biotic factor that may affect the structure and function of insect parasitoid communities is competition. Competition has been defined as "the attempted or actual utilization by two organisms of common resources" (DeBach and Sundby 1963). Competition between individuals of different species that share one or more resource requirements may result in the competitive displacement of one of the species (DeBach 1966). The apparent absence of *A. smithi* and *A. pisivorus* suggests that these species may have been competitively displaced by *A. ervi*.

Griffiths and Holling (1969) stated that competition influences parasitism on two occasions. Initially, parasitoids must compete for hosts in which to oviposit. Female fitness benefits if parasitoids preferentially oviposit into unparasitized hosts (Roitberg and Mangel 1988; van Alphen and Visser 1990; Visser *et al.* 1992). However, if unparasitized hosts are in short supply, the fitness of time-limited parasitoids can be enhanced if females attack parasitized hosts (Bakker *et al.* 1985; Hubbard *et al.* 1987; van Alphen and Visser 1990; Visser *et al.* 1992). The second type of competitive interaction identified by Griffiths and Holling (1969) was larval competition. Usually, only one parasitoid is able to complete its development in a single host. While the two stages of interaction occur independent of one another, the solitary parasitic nature of the aphidiids results in an inexorable link between them. Larval competition was the subject of investigation in the previous chapter. In this Chapter, the competitive interaction between *A. ervi* and *P. pequodorum* at the population level will be evaluated. The main objectives of this study are to (1) elucidate the response of each parasitoid species to different competitive regimes and (2) identify some life history traits that may influence the heterospecific interaction. In addition, the results may demonstrate the relative level with which each species can exploit their host resource. Using this information and the results from the host discrimination and larval competition studies, it may be possible to predict the future composition of species in the pea aphid parasitoid guild in southern British Columbia.

Materials and Methods

Controlled Environment

Competition between *A. ervi* and *P. pequodorum* was evaluated under controlled environment conditions in screen cages, measuring 100 cm x 50 cm x 50

cm (Appendix 1). Experiments were performed at a temperature of 22±2°C, 25±10% relative humidity and continuous light using "cool white" florescent lamps. Eight pots of broad bean plants were infested with a total of 800 second-instar pea aphids (100 aphids per pot) and placed into a screen cage (Appendix 1). Each pot was fitted with devices that enabled aphids that had dropped off a plant to return to it (Appendix 2). After aphids were placed on the host plants, one to two hours were allowed to pass before 2- to 4-day old parasitoids were released into the cage (Table 5.1). This time period was allowed to enable aphids to disperse to feeding sites. Parasitoids were not provided with food after their release because they could obtain nourishment from aphid honeydew and extrafloral nectaries on the bean plants. Water was sprayed onto the plants and cage screen daily to supply parasitoids with moisture.

Mummification of aphids signified the completion of the first parasitoid generation. At this point, a decision was made whether to establish subsequent generations. Trials were terminated when the percentage of *A. ervi* declined to less than 10%. Preliminary work indicated that when the relative abundance of *A. ervi* decreased to approximately 10%, the continued existence of this species was tenuous.

Subsequent generations of parasitoids were established in the following manner. Two to three days after mummies formed, the eight pots of plants in the screen cage were removed. Every mummy on each pot was removed and placed into one of eight empty containers, one containers for each pot. Two containers were randomly selected and their mummies (cohort A) served as parents for the following generation. Mummies from the remaining six containers (cohort B) were saved to estimate the percentage of mortality and the sex ratio of cohort A. Percent mortality is the percentage of mummies in cohort B from which parasitoids did not eclose. Sex

Table 5.1. Experiment to evaluate competition, between *Aphidius ervi* and *Praon pequodorum*, in screen cages under controlled environment conditions.

Trial	No. of female parasitoids at beginning of trial[a,b]		No. of parasitoid generations until end of trial[c]
	A.e.	P.p.	
1	19	7	6
2	(34)	15	1
3	16	(11)	3
4	7	7	3
5	5	5	6
6	12	(40)	1
7	8	(47)	1

a. A.e. = *A. ervi*, P.p. = *P. pequodorum*
b. Values in brackets are estimated numbers of females; parasitoids were released into the cage as pupae.
c. Trial terminated when the relative abundance of *A. ervi* was less than 10% in one generation.

ratio is the percentage of female parasitoids that eclosed from mummies in cohort B.

The relative abundance of *A. ervi* and *P. pequodorum* was measured in each parasitoid generation by summing the numbers of mummies in cohort A and B, plus those found adhering to the cage.

The oviposition activity of parasitoids was measured in each parasitoid generation through oviposition activity samples that were performed in the following manner. Two days after wasps were released or had eclosed from mummies, two pots of plants were randomly selected and removed from the screen cage to make room for two sampling pots. Aphids from the two pots removed from the cage were reared and, depending upon the number of aphids, either all or 50% of them were dissected. Two sampling pots, each infested with 100 third-instar aphids, were placed into the cage to assess oviposition activity. Aphids on the sampling pots were marked by amputation of the distal portion of one antenna before being placed into the cage (Mackauer 1972). The sampling pots were left in the cage for 24 hours and then removed. Of the aphids found on the sampling pots, the marked aphids were separated from the rest, reared for 4 days and dissected. Two pots of plants, each containing 100 third instar nymphs, were placed into the cage immediately after the sampling pots were removed from the cage.

Statistical analysis

Of the trials I performed (Table 5.1), I analyzed only those trials where *A. ervi* and *P. pequodorum* coexisted for three or more generations following the release of female parasitoids. Seven population variables were measured in each parasitoid generation (see Appendix 3 for data): generation number (Gen), number of mummies in cohort A (*A. ervi*, No_{aem}; *P. pequodorum*, No_{ppm}), sex ratio in cohort B (*A. ervi*, SR_{ae}; *P. pequodorum*, SR_{pp}) and percent mortality in cohort B (*A. ervi*,

Mo_{ae}; *P. pequodorum*, Mo_{pp}). Data were analyzed to examine the relationship between the relative abundance of *A. ervi* and the population variables. I chose to use the relative abundance of *A. ervi* rather than that of *P. pequodorum* because *A. ervi* was more abundant in the field (Chapter 3) and I expected that this species would become the dominant species in heterospecific competitions. All data that were recorded as proportions (percentages) were angular transformed for analysis. This transformation modifies the data in a manner that gives greater weight to the assumption that treatment effects are additive, rather than multiplicative.

Utilizing some data from Appendix 3 and the results of the oviposition activity samples, the data table in Appendix 4 was constructed to assess if the number of female parasitoids released into the cage in each generation influenced the relative abundance of *A. ervi*. Five population variables were measured in each generation (Appendix 4): generation number (Gen), number of female parasitoids used to start each generation (*A. ervi*, No_{aef}; *P. pequodorum*, No_{ppf}) and mean number of parasitoid larvae per dissected aphid in the oviposition activity samples (*A. ervi*, $Ovip_{ae}$; *P. pequodorum*, $Ovip_{pp}$). The numbers of female parasitoids initially released into a cage is known. For subsequent generations, the numbers in each generation had to be estimated using Equations (1) and (2).

$$No_{aef} = No_{aem}\,[(Mo_{ae} / 100) \times (SR_{ae} / 100)], \qquad (1)$$

$$No_{ppf} = No_{ppm}\,[(Mo_{pp} / 100) \times (SR_{pp} / 100)], \qquad (2)$$

where for *A. ervi* and *P. pequodorum*, respectively, No_{aef} and No_{ppf} = numbers of female parasitoids in generation F_x, No_{aem} and No_{ppm} = numbers of mummies in cohort A of generation F_x, Mo_{ae} and Mo_{pp} = percent mortality in cohort B of generation F_{x-1} and SR_{ae} and SR_{pp} = sex ratio in cohort B of generation F_{x-1}. Only untransformed data were used in Equations (1) and (2).

Data from Appendix 3 and 4 were analyzed using the forward stepwise

multiple linear regression and correlation analysis options provided in StatView512+, version 1.0 (Abacus Concepts, Inc. 1986). The levels of significance for the correlation coefficients were determined using procedures described in Sokal and Rohlf (1981). Oviposition activity data were analyzed (1) utilizing procedures explained by Sokal and Rohlf (1981) to test for a difference between two sample means and (2) utilizing techniques outlined by Johnson (1980) for making inferences concerning two proportions using the *z* test statistic. *G*-tests for goodness of fit and independence, incorporating Williams' correction, were applied to frequency data (Sokal and Rohlf 1981).

Semi-natural Environment

Competition between *A. ervi* and *P. pequodorum* was examined under semi-natural conditions in a screen-house located outdoors. The screen-house consisted of four screen walls, each measuring 4.5 m long x 2.5 m high, with a screen and clear plastic roof. The screen-house covered an area of ground measuring approximately 20 m^2.

Inside the screen-house, one 130 mm plastic pot was placed into each of 48 holes dug into the earth (Appendix 5). Twelve broad bean plants in "garden mix" soil were transplanted into each pot. A hygrothermograph was placed inside a Stevenson screen within the screen-house to record the temperature and relative humidity. Appendix 6 outlines the daily range of temperature and relative humidity throughout the experiment.

On May 27, 1991, I infested each pot of plants with 60 third instar pea aphids. On the following day (day 0), 35 *A. ervi* females and 35 *P. pequodorum* females were released into the screen-house. Parasitoids were 2 to 4 days old and presumably mated. On day 5, a second aphid infestation was performed, but with each pot receiving half the number of second instar aphids as in the first infestation.

The relative abundance of *A. ervi* and *P. pequodorum* was measured by conducting abundance samples. Nine and 10 pots of plants, respectively, were randomly selected and harvested in the abundance samples that were performed on day 28 and 47; the numbers and species of mummies found on all plant material were determined. For each sample, data from individual pots of plants were pooled to estimate the relative abundance of *A. ervi*. Uninfested broad bean plants were transplanted into the screen-house to replace those removed in the abundance sample.

The oviposition activity of parasitoids was assessed by placing 6 sample pots of plants on the ground inside the screen-house (Appendices 5 and 7). Each sample pot contained 6 broad bean plants that were infested with a total of 50 third instar aphids. A transparent plastic funnel, painted with a band of Fluon, was affixed to each sample pot to reduce the loss of aphids. After 48 hours, the sample pots were removed from the screen-house, the aphids reared for 4 days and 50% of them were dissected. The remaining aphids were reared until mummies formed whereupon they were counted. Oviposition activity samples were conducted on six occasions: days 32-34, 41-43, 50-52, 61-63, 72-74 and 85-87. For each sample, dissection data from all sample pots were pooled for analysis. The experiment was terminated on day 95.

Statistical analysis

Statistical differences in the relative abundance of *A. ervi* between successive parasitoid generations was assessed utilizing the procedure outlined by Johnson (1980) for making inferences concerning two proportions using the z test statistic. G-tests for independence, using Williams' correction, were used to analyze data from the oviposition activity samples (Sokal and Rohlf 1981).

Results

Controlled Environment

Aphidius ervi became the dominant species in trials 1 and 5, whereas *P. pequodorum* competitively displaced *A. ervi* in all other trials (Figure 5.1).

The relative abundance of *A. ervi* is positively correlated with generation number ($P < 0.01$) and the number of *A. ervi* mummies ($P < 0.05$) (Table 5.2). These results indicate that *A. ervi* experienced an increase in number over time, while *P. pequodorum* did not, suggesting that the relatively higher population growth rate of *A. ervi* may be responsible for the increase in the relative abundance of this species.

The relative abundance of *A. ervi* is negatively correlated with the number of *P. pequodorum* mummies in each generation ($P < 0.01$). This result suggests that *P. pequodorum* is effectively competing for hosts, thereby reducing the number of aphids available to *A. ervi* for development and *vice versa.*

The sex ratios and percent mortalities of *A. ervi* and *P. pequodorum* are not correlated with the relative abundance of *A. ervi* suggesting that, under my experimental conditions, these variables may not influence the competitive interaction between the parasitoid species. Percent mortality of *A. ervi* and *P. pequodorum* was positively correlated, suggesting that both species may be sensitive to similar mortality factors.

Analysis of data in Appendix 3 indicates that generation number and the number of *P. pequodorum* mummies in each generation contribute most significantly to variation in the relative abundance of *A. ervi* (Table 5.3). The F-to-enter values of the remaining population variables did not warrant their inclusion into the regression equation. Equation (3) is the final regression equation, describing 93.3% ($r = 0.966$) of the variation in y utilizing the included population variables.

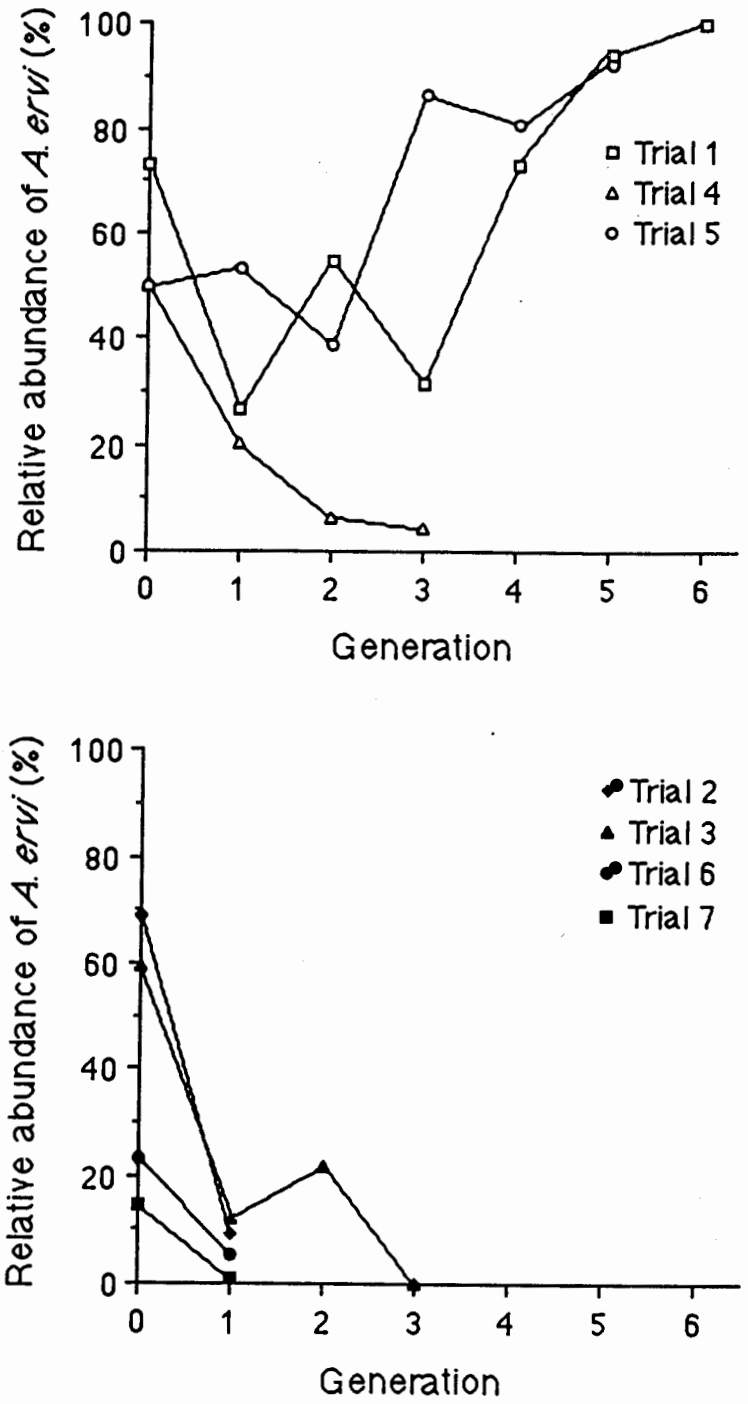


Figure 5.1. Temporal change in the relative abundance of *Aphidius ervi* in experiment to evaluate competition between *A. ervi* and *Praon pequodorum* in screen cages under controlled environment conditions. The relative abundance in generation number 0 represents the percentage of *A. ervi* among the introduced parasitoids (see Table 5.1 for numbers of parasitoids released).

Table 5.2. Competition between *Aphidius ervi* and *Praon pequodorum* in screen cages under controlled environment conditions: correlations between selected population parameters[x].

a) Correlations between the relative abundance of *A. ervi* and selected population variables[y,z]

	Gen	No_{aem}	No_{ppm}	SR_{ae}	SR_{pp}	Mo_{ae}	Mo_{pp}
	0.832**	0.644*	-0.714**	0.033	-0.064	0.024	-0.153
df	11	11	11	11	11	10	10

b) Correlation matrix of selected population variables[y,z]

	Gen	No_{aem}	No_{ppm}	SR_{ae}	SR_{pp}	Mo_{ae}	Mo_{pp}
Gen	1						
No_{aem}	0.730**	1					
No_{ppm}	-0.460	-0.460	1				
SR_{ae}	-0.111	-0.451	-0.158	1			
SR_{pp}	-0.176	-0.361	-0.300	0.160	1		
Mo_{ae}	-0.016	-0.188	0.092	-0.060	-0.194	1	
Mo_{pp}	-0.219	-0.330	0.071	0.314	0.052	0.683*	1

x. See Appendix 3 for data.
y. Gen = generation number, No_{aem} = number of *A. ervi* mummies, No_{ppm} = number of *P. pequodorum* mummies, SR_{ae} = sex ratio of *A. ervi*, SR_{pp} = sex ratio of *P. pequodorum*, Mo_{ae} = percent mortality of *A. ervi*, Mo_{pp} = percent mortality of *P. pequodorum*, df = degrees of freedom.
z. Levels of significance: *, $P < 0.05$; **, $P < 0.01$.

Table 5.3. Competition between *Aphidius ervi* and *Praon pequodorum* in screen cages under controlled environment conditions: results of forward stepwise multiple linear regression analysis[a].

Analysis of Variance Table

Source	df	Sum of squares	Mean square	F	
Regression	2	7116.97	3558.49	55.60	$p < 0.001$
Residual	8	512.05	64.01		
Total	10	7629.02			

Variables Included in Regression Equation[b]

Parameter	Value	Standard error	F-to-remove value
Intercept	19.801		
Gen	11.993	1.853	41.877
No_{ppm}	-0.213	0.048	19.499

Variables Not Included in Regression Equation[b]

Parameter	Partial correlation coefficient	F-to-enter value
No_{aem}	-0.085	0.051
SR_{ae}	-0.164	0.193
SR_{pp}	-0.454	1.820
Mo_{ae}	0.296	0.671
Mo_{pp}	0.101	0.072

a. See Appendix 3 for data; F-to-enter value = 4.000 and F-to-remove value = 3.996.

b. Gen = generation number, No_{aem} = number of *A. ervi* mummies, No_{ppm} = number of *P. pequodorum* mummies, SR_{ae} = sex ratio of *A. ervi*, SR_{pp} = sex ratio of *P. pequodorum*, Mo_{ae} = percent mortality of *A. ervi*, Mo_{pp} = percent mortality of *P. pequodorum*.

$$y = 19.801 + 11.993Gen - 0.213No_{ppm}, \qquad (3)$$

where y = relative abundance of *A. ervi*, Gen = generation number and No_{ppm} = number of *P. pequodorum* mummies in each generation. The high degree of correlation is supported by a uniform distribution of the standardized residuals about the linear regression line (Figure 5.2). The regression analysis suggests that the relative abundance of *A. ervi* is predicted to increase with generation number, but that *P. pequodorum* may act to reduce the population growth of *A. ervi*.

Regression analysis of the data in Appendix 4 reveals that the relative abundance of *A. ervi* is positively correlated with generation number ($P < 0.01$) which, in turn, is positively correlated with the number of *A. ervi* females ($P < 0.05$) (Table 5.4). These results are similar to those obtained from the previous regression analysis, indicating that the relative abundance and number of *A. ervi* are expected to increase with time. This result suggests that in the competitive interaction between *A. ervi* and *P. pequodorum*, *A. ervi* may play the major role in influencing the outcome of the interaction between the two species.

The effect of *P. pequodorum* on the relative abundance of *A. ervi* becomes evident using the data from Appendix 4. The relative abundance of *A. ervi* is not correlated with the number of *A. ervi* females ($P > 0.05$), but is negatively correlated with the number of *P. pequodorum* females ($P < 0.01$) (Table 5.4). Furthermore, it is interesting to note that the oviposition activity in both species is positively correlated with the numbers of females in the respective species ($Ovip_{ae}$, $P < 0.05$; $Ovip_{pp}$, $P < 0.01$). However, the only correlation demonstrated between oviposition activity of either parasitoid species and relative abundance of *A. ervi* is the negative relationship involving *P. pequodorum* ($P < 0.01$). These results suggest that in heterospecific competition, *P. pequodorum* may be more injurious to the population growth of *A. ervi* than the converse situation.

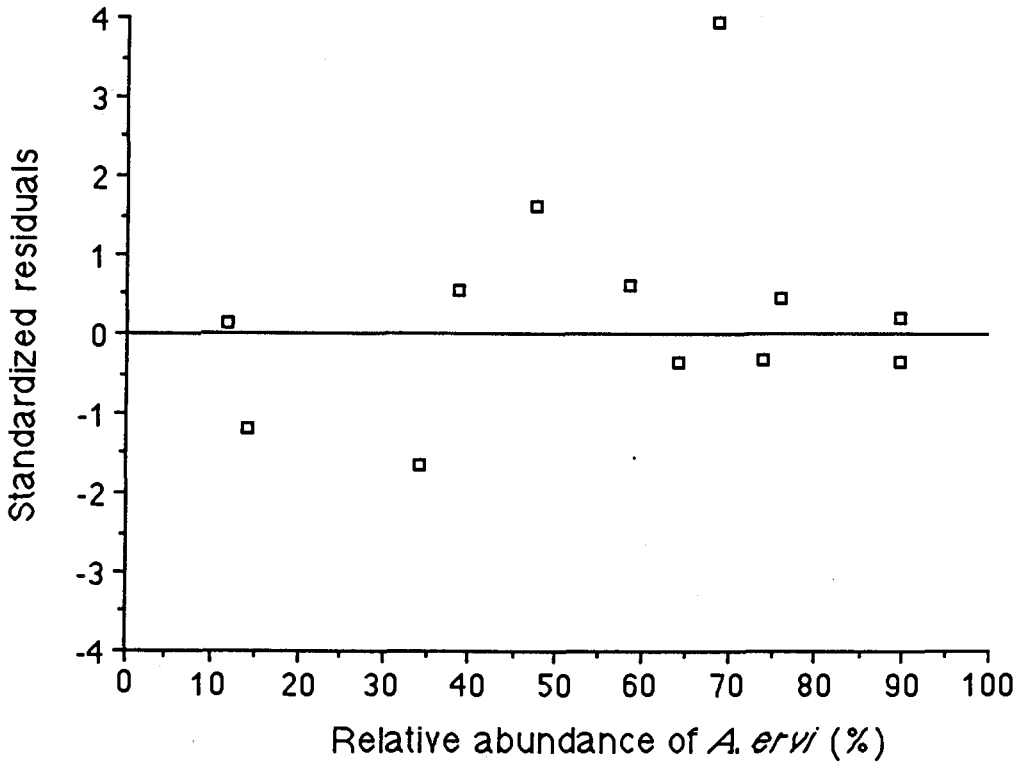


Figure 5.2. Diagram of standardized residuals associated with the final regression equation from experiment to evaluate competition between *Aphidius ervi* and *Praon pequodorum* in screen cages under controlled environment conditions. The values of relative abundance are angular transformed.

Table 5.4. Competition between *Aphidius ervi* and *Praon pequodorum* in screen cages under controlled environment conditions: correlations between selected population variables[x].

a) Correlations between the relative abundance of *A. ervi* and selected population variables[y,z]

	Gen	No_{aef}	No_{ppf}	$Ovip_{ae}$	$Ovip_{pp}$
	0.805**	0.524	-0.696**	0.373	-0.867**
df:	14	13	13	14	14

b) Correlation matrix of population variables[y,z]

	Gen	No_{aef}	No_{ppf}	$Ovip_{ae}$	$Ovip_{pp}$
Gen	1				
No_{aef}	0.583*	1			
No_{ppf}	-0.256	-0.354	1		
$Ovip_{ae}$	0.350	0.634*	-0.607*	1	
$Ovip_{pp}$	-0.531*	-0.258	0.749**	-0.287	1

x. See Appendix 4 for data.
y. Gen = generation number, No_{aef} = number of *A. ervi* females, No_{ppf} = number of female *P. pequodorum*, $Ovip_{ae}$ = oviposition activity of *A. ervi* (mean no. larvae/ dissected aphid), $Ovip_{pp}$ = oviposition activity of *P. pequodorum* (mean no. larvae/ dissected aphid), N = number of observations.
z. Levels of significance: *, $P < 0.05$; **, $P < 0.01$.

Some interesting differences between *A. ervi* and *P. pequodorum* become evident when oviposition data are analyzed further. When 10 female parasitoids of each species were released into two separate cages, a greater proportion of the aphids from the *A. ervi*-only cage were parasitized as compared to aphids removed from the *P. pequodorum*-only cage ($P < 0.001$) (Table 5.5). Similarly, when 5 females from both species were released simultaneously into the same cage, a greater proportion of the aphids contained *A. ervi* larvae ($P < 0.001$). Data from the 7 females density were not significantly different ($P > 0.1$). As the results from *P. pequodorum* were consistent at all densities, the non-significant result at the 7 females density may have been due to the death of one or more *A. ervi* females. Fewer females would have resulted in fewer ovipositions and therefore, fewer *A. ervi* larvae.

Examining the distributions of larvae among parasitized aphids in the 10 females density allows an assessment of the oviposition rates of each species in the absence of heterospecific competition (Figure 5.3) (see Appendix 8 for data). The distributions for the 5 and 7 females densities were similar, but the data were not amenable to analysis because these aphids were heterospecifcally superparasitized. At the 10 females density, the species differ with respect to the degree of (super)parasitism at the 0 to 5 larvae per aphid densities ($P < 0.001$). At the 6 and 7 larvae per aphid densities, the differences are not significant ($P > 0.05$). These results suggest that *A. ervi* females tend to superparasitize hosts more often than their *P. pequodorum* counterparts.

At the 5, 7 and 10 parasitoid densities, there is no statistical difference between the species in terms of mean number of larvae found per dissected aphid ($P > 0.05$) (Table 5.6). However, at all parasitoid densities, *A. ervi* females oviposited significantly more eggs than did *P. pequodorum* females ($P < 0.001$) (Table 5.6).

Table 5.5. Distribution of *Aphidius ervi* and *Praon pequodorum* larvae in a sample of aphids exposed to female parasitoids in screen cages for 48 hours under controlled environment conditions.

Exp	Nos. of parasitoids per cage[a]	Total no. of aphids dissected	No. of aphids containing 0 or ≥1 larvae of A.e. : P.p.[b]				G_{adj}[c,d]
			0:0	≥1:0	0:≥1	≥1:≥1	
1	5+5	199	8	94	18	79	21.644***
2	7+7	104	1	19	6	78	0.932
3	10+10	159	3	156	-	-	57.370***
		144	46	-	98	-	

a. In experiment 1 and 2, 5 and 7 females, respectively, of each species were placed into the same cage. In experiment 3, 10 females of each species were placed into separate cages.
b. A.e. = *A. ervi*, P.p. = *P. pequodorum*.
c. In each experiment, *G*-test for goodness of fit was used to test whether there was a difference between the numbers of aphids found to contain larvae of each species (Sokal and Rohlf 1981). The numbers of aphids that contained larvae of each species comprise the observed frequencies; the respective expected frequencies are calculated by multiplying the total number of aphids in each experiment by 0.5. (H_0 = no difference in the numbers of aphids attacked by females of both species).
d. Level of significance: ***, $P < 0.001$.

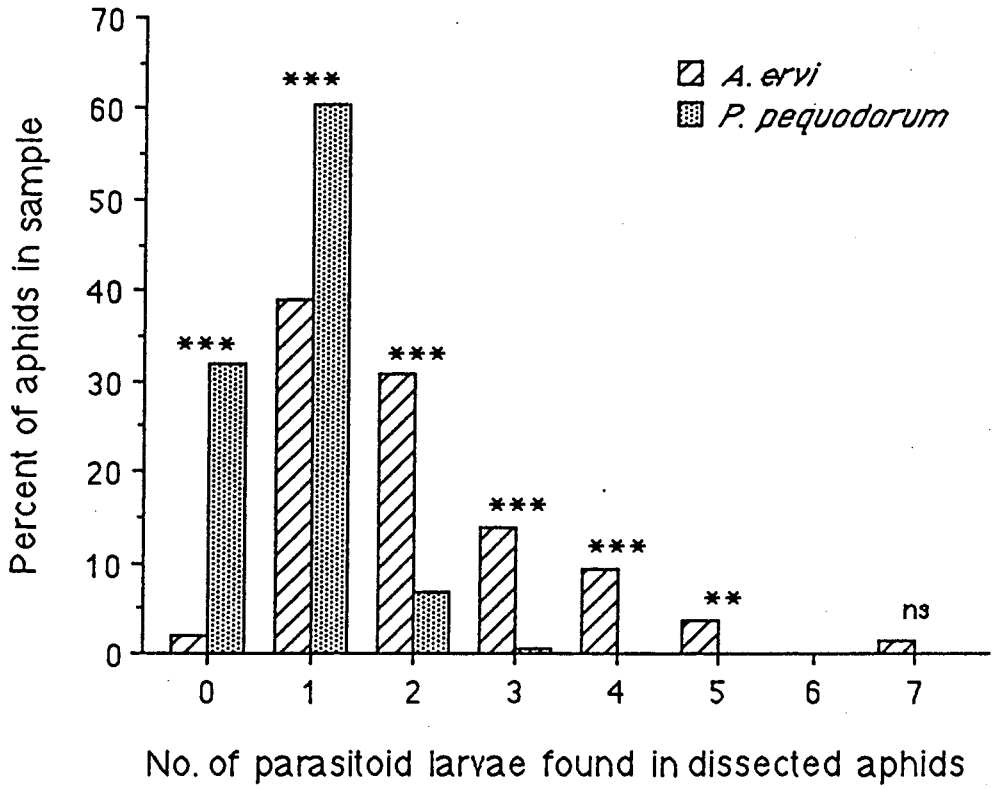


Figure 5.3. Distribution of parasitoid larvae in aphids that were exposed to 10 *Aphidius ervi* and 10 *Praon pequodorum* females for 48 hours in screen cages under controlled environment conditions. Each species of parasitoid was released into a separate cage. Numbers of aphids dissected: N = 159 from *A. ervi*-only cage and N = 144 from *P. pequodorum*-only cage. Inferences concerning statistical differences between proportions of aphids found to contain various numbers of larvae established by using procedures outlined by Johnson (1981). Levels of significance: **, $P < 0.01$; ***, $P < 0.001$; ns = not significant ($P \geq 0.05$).

Table 5.6. Oviposition activity of *Aphidius ervi* and *Praon pequodorum* under controlled environment conditions during a 48 hour period after introduction.

Exp	No. of parasitoids per cage[a]	Total no. of aphids dissected	Parasitoid species[b]	Mean no. of larvae per dissected aphid ± s.e.	$t^{c,e}$	Total nos. of larvae found	$G_{adj}^{d,e}$
1	5+5	199	A.e.	1.121 ± 0.0470	0.685	223	44.249***
			P.p.	0.523 ± 0.0402		104	
2	7+7	104	A.e.	1.981 ± 0.1366	0.341	206	26.785***
			P.p.	1.096 ± 0.0791		114	
3	10+10	159	A.e.	2.075 ± 0.1016	0.026	330	114.984***
		144	P.p.	0.764 ± 0.0502		110	

a. In Experiment 1 and 2, 5 and 7 females, respectively, of each species are placed into the same cage. In Experiment 3, 10 females of each species are placed into separate cages.
b. A.e. = *A. ervi*, P.p. = *P. pequodorum*.
c. In each experiment, the procedure that tests for a statistical difference between two sample means (Sokal and Rohlf 1981) was used to establish whether there was a difference between the mean number of parasitoid larvae of each species found per dissected aphid.
d. In each experiment, a *G*-test for goodness of fit was used to test if there was a difference between the total number of larvae of each species found in the sample of dissected aphids (Sokal and Rohlf 1981). The numbers of larvae of each species comprised the observed frequencies; the respective expected frequencies were calculated by multiplying the total number of larvae found in each experiment by 0.5. (H_0 = no difference in the frequency of ovipositions [i.e. numbers of larvae found] by females of both species).
e. Level of significance: ***, $P < 0.001$.

The results from Tables 5.5 and 5.6 suggest that under similar conditions, *A. ervi* females may find more aphids and oviposit more often in discovered hosts than *P. pequodorum* females.

The relative abundance of a parasitoid species may vary with the proportion of aphids successfully parasitized by that species. There is a significant positive curvilinear correlation between the percent change in relative abundance of *A. ervi* between successive generations and the proportion of *A. ervi* larvae in aphids (P = 0.01) (Figure 5.4).The equation fitted to the data, Equation (4), explains 54.0% (r = 0.735) of the variation in y.

$$y = 6.390 - 71.486x + 79.795x^2, \qquad (4)$$

where y = percent change in the relative abundance of *A. ervi* and x = proportion of *A. ervi* larvae in parasitized aphids. Substituting y = 0 into Equation (4) reveals that the proportion of *A. ervi* larvae in a sample of aphids must equal 0.80 in order for the relative abundance of *A. ervi* to remain the same between successive generations. Theoretically, if the proportion of a given parasitoid species within aphids must exceed 0.5 to maintain a heterospecific equilibrium, then this indicates that the larvae of this species are competitively inferior. The rationale is that if two species are competitively equivalent, the larvae of one species would not require a numerical advantage in order to win 50% of larval competitions (relative abundance of species in equilibrium). The results suggest that *A. ervi* larvae are competitively inferior to those of *P. pequodorum* and that the chances of *A. ervi* winning larval competitions are increased if *A. ervi* larvae have a numerical advantage.

Semi-natural Environment

The relative abundance *A. ervi* on day 28 was 68.31%. Assuming that the relative abundance of *A. ervi* on day 0 was 50%, then the 18.31% increase is statistically significant (z = 2.965; P < 0.01) (Table 5.7). The second abundance

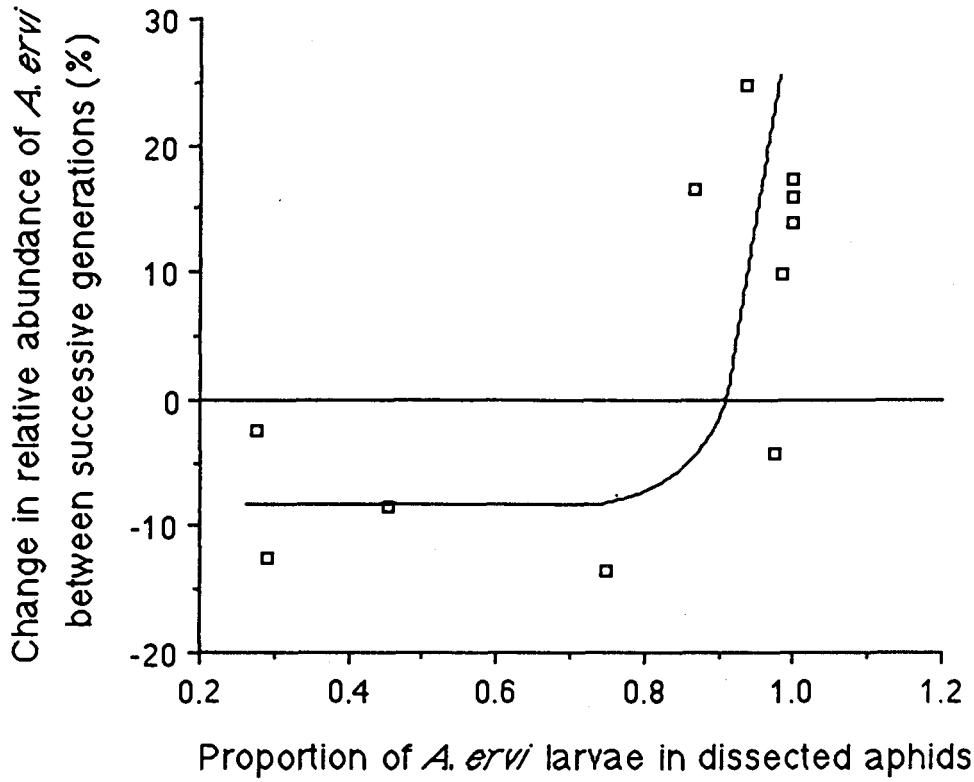


Figure 5.4. Relationship between the proportion of *Aphidius ervi* larvae and the change in the relative abundance of *A. ervi* between successive generations. Curve was fitted by eye. Source of data: values of oviposition activity in experiment to evaluate competition between *A. ervi* and *Praon pequodorum* in screen cages under controlled environment conditions.

Table 5.7. Relative abundance of parasitoid species in experiment conducted under semi-natural conditions to examine competition between *Aphidius ervi* and *Praon pequodorum*.

Time after beginning of trial (days)	No. of plants harvested	Nos. of mummies on plants[a]		Relative abundance (%)[a]	
		A.e.	P.p.	A.e.	P.p.
28	108	263	122	68.31	31.69
47	103	3,426	516	86.91	13.09

a. A.e. = *A. ervi*, P.p. = *P. pequodorum*.

sample, conducted on day 47, showed that the relative abundance of the second generation of *A. ervi* also increased significantly to 86.91% (z = 9.824; $P < 0.001$). These results are similar to those obtained in the controlled environment studies and indicate that after the liberation of equal numbers of *A. ervi* and *P. pequodorum*, *A. ervi* will become the dominant species.

The number of insects (aphids or parasitoids) recorded in the abundance samples can be used to establish the degree to which populations increased between successive generations. The number of individuals in the screen-house is estimated using Equation (5).

$$N_{est} = (N_s / P_s)(P_p \times T), \qquad (5)$$

where N_{est} = the estimated number of insects, N_s = number of individuals (aphids, *A. ervi* mummies or *P. pequodorum* mummies) in the abundance sample, P_s = number of plants harvested in the abundance sample, P_p = the number of plants per pot (12) and T = total number of pots of plants in the screen-house (48). Substituting 576 for the total number of plants in the screen-house, Equation (5) simplifies to Equation (6).

$$N_{est} = (576 \times N_s)/ P_s \qquad (6)$$

After the second release of aphids on day 5, the population of aphids is estimated to have been about 4,300. On day 28, the aphid population was estimated to be about 13,400 and although the number of aphids was not determined on day 47, a large number of hosts was observed. These calculations suggest that aphids were abundant and that host supply should not have limited the population growth of either parasitoid species.

The parasitoid population in the first generation was estimated to be about 1,400 *A. ervi* and 650 *P. pequodorum*. In the second parasitoid generation, the population of wasps was estimated to have increased to about 19,160 *A. ervi* and

2,890 *P. pequodorum*. Relative to the first generation, the population of *A. ervi* increased by 13.7 times, while that of *P. pequodorum* increased by only 4.4 times. Similar to the results from the controlled environment studies, these results indicate that *A. ervi* may increase in numbers faster than *P. pequodorum*.

The results from the activity sample show that *A. ervi* was more prevalent in the first, third and fifth samples, whereas *P. pequodorum* was more common in the second and fourth samples (Table 5.8 and Figure 5.5). The alternating predominance of each parasitoid species in successive samples may be attributed to different numbers of parasitoids present during the sampling period. Asynchronous eclosion due to disparities in developmental time can explain differences in the number of parasitoids; *A. ervi* was observed to become active before *P. pequodorum* in the first parasitoid generation. These results suggest that, in the field, the shorter developmental time of *A. ervi* may enable this species to complete more generations than *P. pequodorum* during the growing season. In turn, this may contribute to population growth for *A. ervi* and result in its greater relative abundance.

No parasitoid larvae were found in aphids dissected from the sixth oviposition activity sample, indicating that the parasitoid population had all but disappeared when the sample was taken. Only a few *A. ervi* and *P. pequodorum* were observed in the screen-house after day 80. Factors such as environmental conditions, host-plant quality or aphid density are not believed to be responsible for the massive decline in parasitoid numbers. Rather, large numbers of hyperparasitoids and an epizootic of *Entomophthora* sp(p). (Entomophthorales) in the pea aphid population likely contributed to the decimation of the parasitoid population.

Pooling the dissection data from the oviposition activity samples (Table 5.8) reveals differences in the oviposition behaviour of *A. ervi* and *P. pequodorum* females. Of the aphids containing larvae of only one parasitoid species,

Table 5.8. Oviposition activity of parasitoids in experiment conducted to examine competition between *Aphidius ervi* and *Praon pequodorum* under semi-natural conditions.

Time after beginning of experiment (days)	No. of aphids dissected[a]	Nos. of aphids containing 0, 1 or more larvae of A.e. and P.p.[d]								Mummy sample (control)		G_{adj}[e,f]
		0:0	1:0	2:0	3:0	0:1	0:2	1:1	2:1	A.e.	P.p.	
32-34	128	83	38	6	0	1	0	0	0	52	0	1.030
41-43	140	99	2	0	0	36	0	3	0	4	40	0.541
50-52	142	31	68	16	2	9	1	12	3	62	9	2.835
61-63	110[b]	89	0	0	0	19	2	0	0	0	17	0
72-74	115[c]	105	9	1	0	0	0	0	0	8	0	0
85-87	212	212	0	0	0	0	0	0	0	-	-	-

a. Total of 300 aphids placed on sample plants.
b. Not included: 3 aphids, each containing 1 larva of *Monoctonus paulensis* Ashmead (Hymenoptera: Aphidiidae); 1 aphid containing 1 larva of *P. pequodorum* and 1 larva of *M. paulensis*.
c. Not included: 1 aphid containing 1 larva of *M. paulensis*.
d. Ratios in column heading express categories of aphids containing given numbers of parasitoid larvae (A.e.: P.p.); A.e.= *A. ervi* and P.p.= *P. pequodorum*.
e. *G*-test of independence in each sample between frequencies of mummies in control and the frequencies expected from dissected aphids, assuming 1) *P. pequodorum* larvae win all heterospecific larval competitions and 2) all singly parasitized aphids mummify.
f. Values are not significantly different ($P \geq 0.05$).

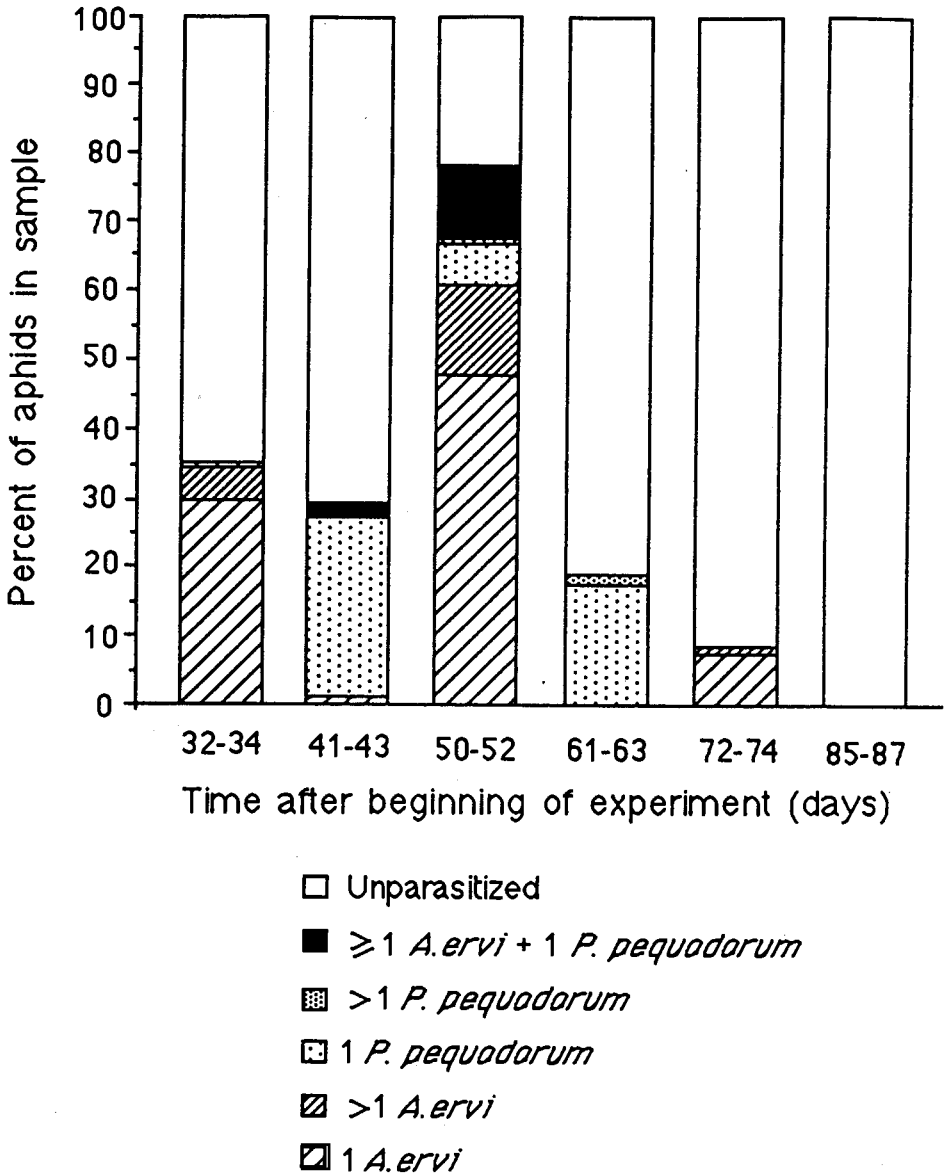


Figure 5.5. Percent unparasitized and parasitized aphids in oviposition activity samples from experiment to evaluate competition between *Aphidius ervi* and *Praon pequodorum* under semi-natural conditions.

the frequency of conspecific superparasitism is higher for *A. ervi* than for *P. pequodorum* (G_{adj} = 7.806; $P < 0.01$). The difference in the rate of superparasitism between the species suggests that host discrimination by *P. pequodorum* may influence the distribution of eggs under field-like conditions.

Heterospecific superparasitism was not common in the oviposition activity samples; of the aphids dissected, 2.84% contained one or more larvae of both parasitoid species. Even though the rate of heterospecific superparasitism is low, the consequences of larval competition between the parasitoid species may be significant. The outcome of larval competitions can be examined indirectly using data from the second and third oviposition activity samples; these are the only samples where heterospecific superparasitism by *A. ervi* and *P. pequodorum* was observed. The numbers of mummies formed by each species from undissected aphids (control) can be compared to the numbers of mummies expected to develop from dissected aphids, had they been allowed to develop. For the analysis, two assumption are necessary. Firstly, all conspecifically (super)parasitized aphids develop into to mummies and secondly, *P. pequodorum* wins all heterospecific larval competitions. The *G*-test values for the second and third samples are not significant ($P > 0.05$), indicating that *P. pequodorum* won all (or most) larval competitions (Table 5.8). This result suggests that under field-like conditions, if the age difference between *A. ervi* and *P. pequodorum* is 24 hours or less, larvae of the latter species may have a competitive advantage.

Discussion

I examined competition between *A. ervi* and *P. pequodorum* using two competitive regimes that differed in the intensity of the heterospecific interactions. In the first regime, *A. ervi* and *P. pequodorum* were placed together in 0.25 m^3

screen cages to compete under controlled conditions. This environment produced a high level of interaction and assisted in the elucidation of factors that may be important in the interaction between the two parasitoid species in the field. In the second regime, *A. ervi* and *P. pequodorum* were released into a 50 m^3 screen-house containing an abundant host supply. This experiment was performed under semi-natural conditions and allowed the two species to express their intrinsic abilities under field-like conditions.

The pea aphid is a common host of *A. ervi* and *P. pequodorum* (Campbell 1974). As these parasitoid species share a common resource, placing them together in the same environment should result in competition for hosts (DeBach and Sundby 1963). The data reveal that competition occurred between the two parasitoid species and that the competitive interactions between *A. ervi* and *P. pequodorum* were asymmetrical. Trials conducted under controlled conditions indicate that *P. pequodorum* had a greater negative influence on the relative abundance of *A. ervi* than the converse situation. Differences in the life history traits between the two parasitoid species appear to explain the observed competitive asymmetry in the trials. These differences may provide some insight into explaining the disparity in the relative abundance of *A. ervi* and *P. pequodorum* observed in the field.

The main advantage that *A. ervi* appears to have is that this species is capable of greater increases in numbers than *P. pequodorum*. As a result, the greater capacity for population increase enables *A. ervi* to attain a higher relative abundance than *P. pequodorum*. Heterospecific differences in the capacity for population increase can be discussed in terms of parasitoid natality and parasitoid mortality.

For the purposes of this discussion, aphidiid natality will be considered to be the number of ovipositions performed by females of a particular parasitoid species.

Under controlled conditions, there were two consistent observations found when aphids were dissected after exposure to equal numbers of parasitoid females. Firstly, more aphids were parasitized by *A. ervi* than by *P. pequodorum* and secondly, *A. ervi* females oviposited more eggs into parasitized aphids than did *P. pequodorum* females. The greater oviposition frequency of *A. ervi* females can be attributed to factors that have a high degree of interaction. One factor that may be significant is egg load; upon dissection of three day old parasitoids, *A. ervi* females contain a greater mean number of eggs than their *P. pequodorum* counterparts. *Aphidius ervi* females contain a total (mean±s.e.) of 262.2±13.2 eggs (N = 12 females), whereas *P. pequodorum* females contain a total (mean±s.e.) of 138.6±3.3 eggs (N = 22 females) (experiment not described in thesis). In and of itself, egg load may not necessarily result in more ovipositions. However, if egg load is considered together with parasitoid activity, the result can be meaningful. During experiments that examined host discrimination utilizing parasitized and unparasitized hosts, the mean (±s.e.) host attack rate was higher for *A. ervi* females (2.73±0.34 attacks/ minute; N = 12 females) than for *P. pequodorum* females (0.803±0.082 attacks/ minute; N = 15 females) (Chapter 4). Using a different measure of parasitoid activity, oviposition activity under controlled conditions for equal numbers of females was consistently higher for *A. ervi* than for *P. pequodorum* (Appendix 4, Gen 1 of trials 4 and 5). My results indicate that *A. ervi* has a higher oviposition frequency than *P. pequodorum*. This conclusion concurs with that of Kambhampati (1987) who found that the mean number of ovipositions per day (±s.e.) is significantly higher for *A. ervi* (48.06±1.87) than for *P. pequodorum* (44.04±2.81). The oviposition frequency/egg load/ parasitoid activity interaction may result in more ovipositions from *A. ervi* females in the field and therefore, enable *A. ervi* to achieve a higher relative abundance than *P. pequodorum*.

Another factor that may result in a higher oviposition frequency is the occurrence of superparasitism. Under controlled conditions, *A. ervi* females superparasitized hosts more frequently than *P. pequodorum* females. Similarly, Kambhampati (1987) found that *A. ervi* superparasitized a greater proportion of aphids than *P. pequodorum*. The higher rate of superparasitism exhibited by *A. ervi* females cannot be explained by the lack of host discrimination by *A. ervi* because this species has been shown to exhibit a reduced frequency of oviposition into parasitized hosts (Chapter 4). Instead, the heterospecific difference in attack behavior toward parasitized hosts may be responsible. *Aphidius ervi* females tend not to refrain from attacking self-, conspecific- and heterospecific parasitized hosts, while *P. pequodorum* females do (Chapter 4). Even though *A. ervi* females can discriminate, a higher attack rate on parasitized hosts will produce more ovipositions than if females refrained from attacking the hosts. Incidents of superparasitism by either species is not surprising in trials conducted under controlled conditions because of the high intensity of heterospecific- and conspecific competition. However, it is interesting to note that superparasitism was still observed under semi-natural (field-like) conditions, where presumably, hosts were not limiting. This result is consistent with DeBach's (1966) statement that the abundance of a resource does not preclude competition. In comparison to *P. pequodorum*, the higher rate of superparasitism observed by *A. ervi* in the screen-house may be explained if: (1) upon encountering a host, a higher proportion of *A. ervi* females oviposit more than one egg in the aphid before abandoning it, (2) *A. ervi* females encounter more hosts per unit time than their *P. pequodorum* counterparts and (3) *A. ervi* females oviposit into a greater proportion of hosts that they encounter. Experimental evidence supporting these explanations is outlined in Chapter 4. All results seem to suggest that: (1) given an encounter with a particular

aphid, exhibition of host discrimination by *P. pequodorum* females will result in fewer ovipositions compared to *A. ervi* females, (2) the searching capacity (number of hosts located per unit time) might be higher for *A. ervi* females and (3) the proportion of aphids rejected for oviposition may be lower for *A. ervi* females than for *P. pequodorum* females. The relative differences in oviposition behavior between *A. ervi* and *P. pequodorum* may give *A. ervi* a higher capacity for population growth and could contribute to the dominance of this species in alfalfa.

Another factor that is important in the regulation of parasitoid population size is mortality, or more specifically, the death of parasitoid larvae. If hosts survive long enough to allow the parasitoid larvae to complete their development, mortality of larvae in singly parasitized hosts probably is not common and thus, would have only a negligible effect on the parasitoid population. However, mortality may be an important factor in heterospecific superparasitized hosts. *Aphidius ervi* and *P. pequodorum* are solitary endoparasitoids; usually, only one parasitoid larva completes development in a single host. In heterospecific larval competitions, *P. pequodorum* larvae have been shown to be competitively superior to *A. ervi* larvae in two ways (also see Chapter 4). Firstly, *P. pequodorum* larvae have a physical competitive advantage over their *A. ervi* counterparts. Secondly, in part due to the previous advantage, *P. pequodorum* larvae have a temporal competitive advantage. *Praon pequodorum* larvae win as many or more heterospecific larval contests if the age difference between larvae is 24 hours or less (also see Chapter 4). In the field, less than 3% of aphids are heterospecifically superparasitized (McBrien 1991), but the differential larval competitive ability of *A. ervi* and *P. pequodorum* could affect the competition for host resources, and benefit the latter species. Indeed, the superior larval competitive ability of *P. pequodorum* may offset the apparent numerical advantage of *A. ervi*. In turn, this may permit *P. pequodorum* to remain

competitive with *A. ervi* in the field, even at low relative abundances.

Competition for common host resources will occur between members of different species in a guild. The intensity of competition is positively correlated with the degree with which the two or more species utilize a common resource (Flanders and Oatman 1987; Schoener 1974). Competition is a selection pressure that can lead to the evolution of the competitors; the mechanisms that evolve can influence the co-existence between species, thereby having a potential to alter the diversity of the parasitoid guild (Mitchell and Wallace 1991). Evolution of superior competitive mechanisms in single species within a guild may result in an ever decreasing acquisition of host resources by the remaining, competitively inferior, species. Over time, this may eventually result in the competitive displacement of one or more species in the guild (DeBach 1966).

In response to competition among co-evolving individuals, different mechanisms have been developed to secure limited resources (Force 1974). With insect parasitoids, the most critical competition for food (i.e. survival) does not occur among adults, but between immature stages (Force 1970). Partitioning of host resources is an effective adaptation that competing species have evolved to reduce heterospecific competition (Schoener 1974). The ways in which parasitoids have partitioned host resources include diversification of host ranges (Price 1970), exploitation of the same host species on different parts of a plant (Yu *et al.* 1990), utilization of identical host species in different habitats (DeBach and Sundby 1963; Price 1970) and asynchrony of diapause (Carton *et al.* 1991).

When exotic parasitoid species are introduced into an ecosystem, there is always a chance that novel species will compete with indigenous species (Flanders and Oatman 1987) or with those previously released in biological control programs (Ehler and Hall 1982). In these situations, successful co-existence between

parasitoid species may depend more on inherent differences in behavior and reproduction, rather than an adaptation of the species to a new competitive interaction (Price 1970). Such appears to be the case with *A. ervi* and *P. pequodorum*. These are species that are members of the same guild, but they have evolved on different continents. Results of the competition experiments indicate that *A. ervi* has a higher reproductive capacity than *P. pequodorum*. Although *A. ervi* has a higher intrinsic rate of increase than *P. pequodorum* (*A. ervi*, 0.371 and *P. pequodorum*, 0.321 [Kambhampati 1987]), this advantage is balanced by the superior larval competitive ability of *P. pequodorum*. Thus, *A. ervi* is able to more fully exploit the pea aphid, but *P. pequodorum* possesses attributes that should enable it to resist competitive displacement by *A. ervi* from alfalfa in southern British Columbia.

CHAPTER 6

GENERAL DISCUSSION

The main objective of this thesis was to evaluate competition between two species of solitary endoparasitoids, *Aphidius ervi* Haliday and *Praon pequodorum* Viereck (Hymenoptera: Aphidiidae). These species are members of a guild of parasitoids that attack the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), on alfalfa in southern British Columbia. The pea aphid-parasitoid complex is useful as a system to examine heterospecific interactions, for two reasons. Firstly, over the past three decades, there has been considerable change in the diversity and relative abundance of guild members (Chapter 3). Prior to the introduction of *A. ervi* and *Aphidius smithi* Sharma & Subba Rao in the late 1950's, *Aphidius pisivorus* Smith and *P. pequodorum* were the predominant parasitoids of the pea aphid on alfalfa (Halfhill *et al.* 1972). In the following years, the relative abundances of *A. pisivorus* and *A. smithi* declined markedly, and recent collections failed to discover either species. Currently, *A. ervi* and *P. pequodorum* are the most common parasitoids of the pea aphid in southern British Columbia.

The second aspect that makes the pea aphid-aphidiid system interesting (and perhaps unique among conventional agro-ecosystems) is that chemical pesticides are not used on alfalfa (Don Low, pers. comm.). Certain agricultural practices, such as harvesting, may exacerbate the effects that abiotic and biotic factors have on parasitoid communities (van den Bosch *et al.* 1966, 1967). Harvesting an alfalfa crop leads to a decline in the number of aphids in the field (Campbell 1974; McBrien 1991), which reduces host availability and leads to increased competition for aphids. Force (1970) stated that competition is an important factor that may affect the structure and function of parasitoid communities. Competition has been defined by

DeBach and Sundby (1963) as "the attempted or actual utilization by two organisms of common resources." Among the aphidiids, competition for hosts may lead to superparasitism (Campbell 1974; McBrien 1991). Supernumerary larvae are eliminated by physical combat and/or physiological suppression (Chow and Mackauer 1985; Chow and Sullivan 1984).

In order to fulfil the main objective of the thesis, I performed experiments to answer two basic questions. The first question was: "What are some of the attributes that may enable *P. pequodorum* to survive at low relative abundance?" I addressed this question by evaluating host discrimination and larval competition in and between *A. ervi* and *P. pequodorum*. It was necessary to examine these factors in both species because *A. ervi* and *P. pequodorum* are sympatric. Oviposition decisions of female parasitoids directly influence their fitness. In heterospecific interactions, the behaviors of individuals may affect the numerical abundance of the species.

Host discrimination is the ability of parasitoids to distinguish between parasitized and unparasitized hosts (Salt 1961). Among the parasitic Hymenoptera, host discrimination is found in many species of solitary parasitoids (van Alphen and Visser 1990; van Lenteren 1981; Vinson 1976). Host discrimination is expected to prevent the wastage of eggs and hosts (Bakker *et al.* 1985; Charnov and Skinner 1985; van Alphen 1988; van Lenteren 1976; van Lenteren 1981; Vinson 1976).

Aphidius ervi females discriminated between unparasitized and conspecific parasitized hosts, attacking more of the former host-type (Chapter 4). In addition, female parasitoids attacked more conspecific parasitized aphids than self-parasitized hosts. During attacks, wasps consistently exhibited a lower frequency of oviposition in conspecific parasitized hosts, than in either unparasitized or self-parasitized aphids. As the interval between successive ovipositions was about one hour, the results suggest that parasitoids used external marking pheromones in host

discrimination (McBrien and Mackauer 1990, 1991).

Praon pequodorum females preferred to attack unparasitized hosts when this host-type was presented together with either self- or conspecific parasitized aphids (Chapter 4). In addition, wasps attacked more self-parasitized than conspecific-parasitized aphids. The results suggest that parasitoids utilized oviposition markers in host discrimination. Females of *P. pequodorum* did not demonstrate oviposition restraint in aphids of any host-type.

Some interesting similarities and differences are evident in the oviposition behavior of *A. ervi* and *P. pequodorum*. Females of both species preferred to attack unparasitized hosts when this host-type was presented together with parasitized aphids. Preference for unparasitized hosts is expected because, of all host-types available to wasps, oviposition in this class of aphids offers the greatest chance for offspring survival (Bakker *et al.* 1985; Hubbard *et al.* 1987). When only self- and conspecific parasitized aphids were presented to wasps, *A. ervi* females attacked more conspecific parasitized hosts. In contrast, *P. pequodorum* females attacked more self-parasitized aphids. Superparasitism in time-limited species, like *A. ervi* and *P. pequodorum,* may be adaptive because female parasitoids likely do not realize their potential fecundity in the field (Gilbert and Gutierrez 1973). Bai (1991) considered the alecithal eggs of time-limited species to be "inexpensive," so females are expected to superparasitize, rather than refrain from ovipositing in parasitized hosts (Hubbard *et al.* 1987; van Alphen 1988; van Strien-van Liempt and van Alphen 1981). Mackauer *et al.* (1992) stated that as the number of eggs laid in a host increases, the mean rate of larval survival declines. Consequently, a female's decision to oviposit should reflect a balance between maximizing fitness gain per host (favoring increased superparasitism), while minimizing wastage of eggs (favoring decreased superparasitism). For *A. ervi* females, superparasitism of hosts

previously attacked by conspecifics may be adaptive if the second egg has a greater than zero chance of survival (Bakker *et al.* 1985; Cloutier 1984; Hubbard *et al.* 1987; Mackauer *et al.* 1992; van Alphen and Visser 1990; van Dijken and Waage 1987). Under the conditions of the host discrimination experiments experiment, the time between successive ovipositions was about one hour. Under similar conditions, Visser *et al.* (1992) found that the "pay-off" from a second egg of *Leptopilina heterotoma* (Thompson) (Hymenoptera: Eucoilidae) was 0.43 offspring per egg; the probability of producing an offspring decreased with increasing intervals between successive attacks. Similarly, a pay-off of about 0.50 offspring per egg would be expected from conspecific superparasitism by aphidiid parasitoids.

The tendency towards self-superparasitism by *P. pequodorum* females is difficult to explain as this behavior results in the death of one offspring (Mackauer 1990; Polaszek 1986). However, this oviposition strategy may be adaptive, if attacked hosts are subsequently subject to oviposition by other females (Mackauer *et al.* 1992; van Alphen and Visser 1990); a greater number of eggs laid by the first females may increase the probability that one of her offspring will survive. In contests between larvae of *A. smithi* and *Ephedrus californicus* Baker (Hymenoptera: Aphidiidae), when the ratio of larvae was 1:1, respectively, *A. smithi* larvae won 16.2% of the contests, whereas if the ratio was 2:1, respectively, *A. smithi* won 44.4% of the contests (Mackauer *et al.* 1992).

Aphidius ervi and *P. pequodorum* differed with respect to heterospecific host discrimination (Chapter 4). Neither females of *A. ervi* nor *P. pequodorum* refrained from attacking heterospecific parasitized hosts. However, whereas females of *A. ervi* exhibited oviposition restraint in heterospecific parasitized hosts, *P. pequodorum* females did not refrain from ovipositing in hosts previously attacked by *A. ervi*. These results are consistent with those of McBrien and Mackauer (1990, 1991), who

utilized *A. ervi* and *A. smithi*, and Chow and Mackauer (1984), who worked with *P. pequodorum* and *A. smithi*.

Larvae of *P. pequodorum* are superior to their *A. ervi* counterparts in larval competition (Chapter 4). In heterospecific parasitized hosts, the difference in larval competitive ability determines any fitness gains for the female. For example, Mackauer *et al.* (1992) found that in contests between one larva each of *A. smithi* and *E. californicus*, the latter species survived in 82.1% of cases, regardless of oviposition sequence. As a result of oviposition in heterospecific parasitized host, the increment in fitness for an *A. smithi* female is lower than the gain by an *E. californicus* female. Similarly, the fitness gain by *P. pequodorum* females will be higher after heterospecific superparasitism than the increment in fitness received by females of *A. ervi*.

The results from the host discrimination experiments can be used to explain the out-come of competition between *A. ervi* and *P. pequodorum* in screen cages. The evidence from experiments described in Chapter 4 suggest that, in the field, *A. ervi* females may utilize a greater proportion of the aphid resource than females of *P. pequodorum*. In contrast to females of *P. pequodorum*, *A. ervi* females attacked a high proportion of hosts and attacks were made in quick succession. As pea aphids tend to drop from a plant in response physical contact from parasitoids (McBrien 1991), the characteristic oviposition behaviors of each species may permit *A. ervi* females to exploit a host patch more thoroughly. Indeed, this was the finding from experiments conducted under controlled and semi-natural conditions (Chapter 5); a greater number of hosts were (super)parasitized by *A. ervi* than by *P. pequodorum*. These experiments were not designed to follow the behavior of specific females, so the larvae in superparasitized hosts may be the offspring of one or several females. The greater propensity of *A. ervi* females to superparasitize hosts may also be a

reflection of their higher egg load. In theory, greater egg load, combined with the oviposition behavior of *A. ervi* females, may enable wasps of this species to lay more eggs. Under controlled environment conditions, more aphids contained larvae of *A. ervi* than of *P. pequodorum* (Chapter 5).

An adaptation that may allow *P. pequodorum* females to compensate for their slow behavior is that females use one or both front legs to hold onto the host during an attack. Indeed, even if an aphid releases itself from the substrate in an attempt to fall, wasps can still hang onto and attack the aphid.

Under semi-natural conditions, 2.8% of aphids were heterospecific parasitized (Chapter 5), whereas McBrien (1991) reported that the proportion in the field was 6.4%. The exact proportion is not as important as the fact that, under field conditions, there will be occasions when an aphid contains larvae of both species, resulting in larval competition. Given that the larvae of *P. pequodorum* are superior in competitive ability to those of *A. ervi*, it would be fair to assume that larval competitive superiority would give *P. pequodorum* an advantage in the field. Indeed, under semi-natural conditions, the results indicate that *P. pequodorum* larvae won all (or most) of the larval competitions (Chapter 5).

The second question that I asked, in order to fulfil the main objective of the thesis was: "Can *A. ervi* and *P. pequodorum* continue to co-exist?" The results from my studies suggests a positive answer.

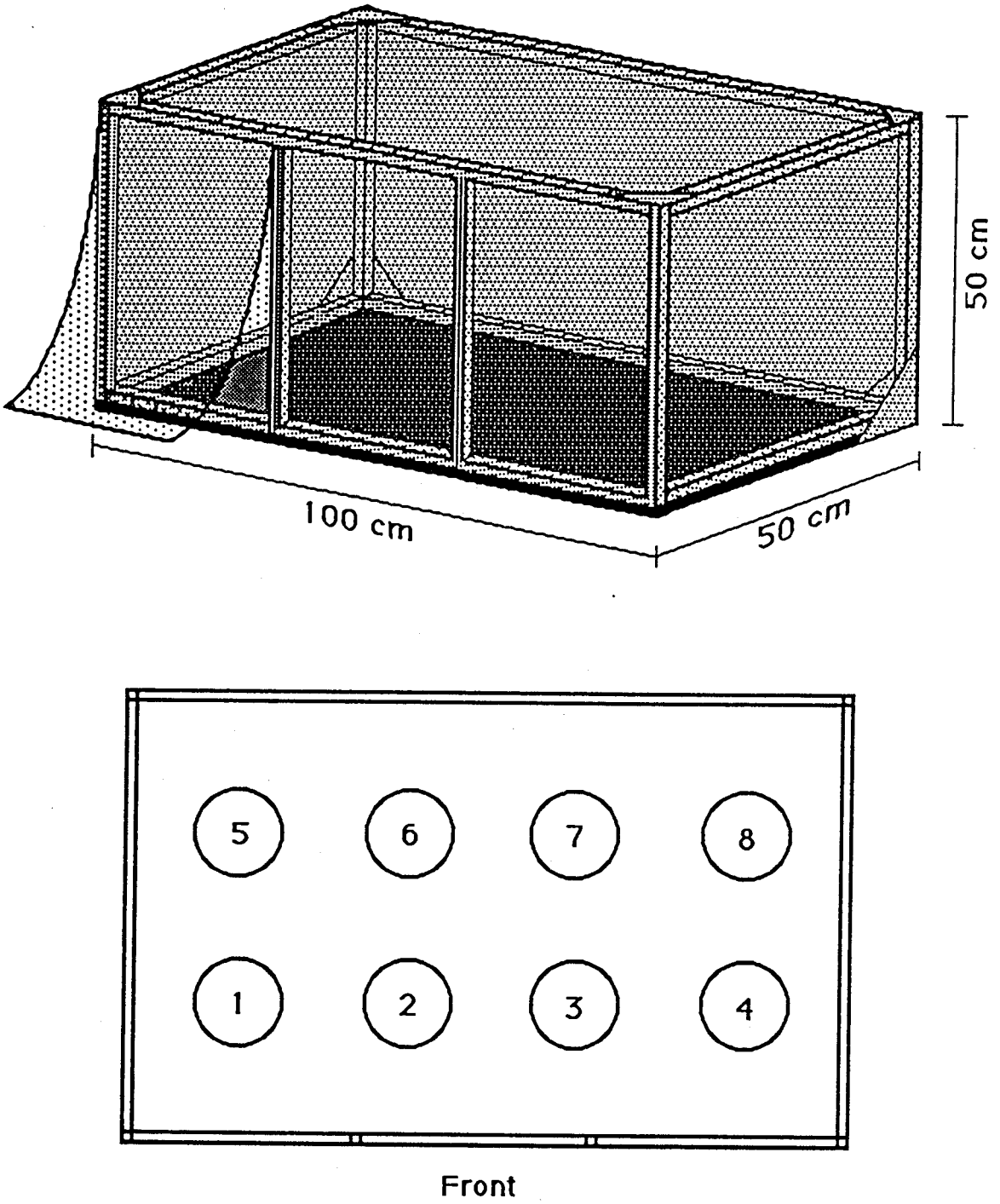
In addition to the intrinsic qualities of each species, other factors may contribute to the coexistence of *A. ervi* and *P. pequodorum*. Firstly, there is a surplus of unparasitized aphids in the field. In aphids collected on alfalfa by Campbell (1974) and McBrien (1991), the proportion that were unparasitized varied between 20 and 100%. A surplus of aphids permits females of both parasitoid species to have access to "high quality hosts," with a minimum of competition. However, should

hosts be parasitized by both species, the intrinsic attributes in *P. pequodorum* may give this species a competitive advantage. Nevertheless, host availability may be more important for females of *P. pequodorum* as the relative abundance of this species is less than 10%. Competitive displacement of a less abundant species is expected to occur over time (DeBach 1966).

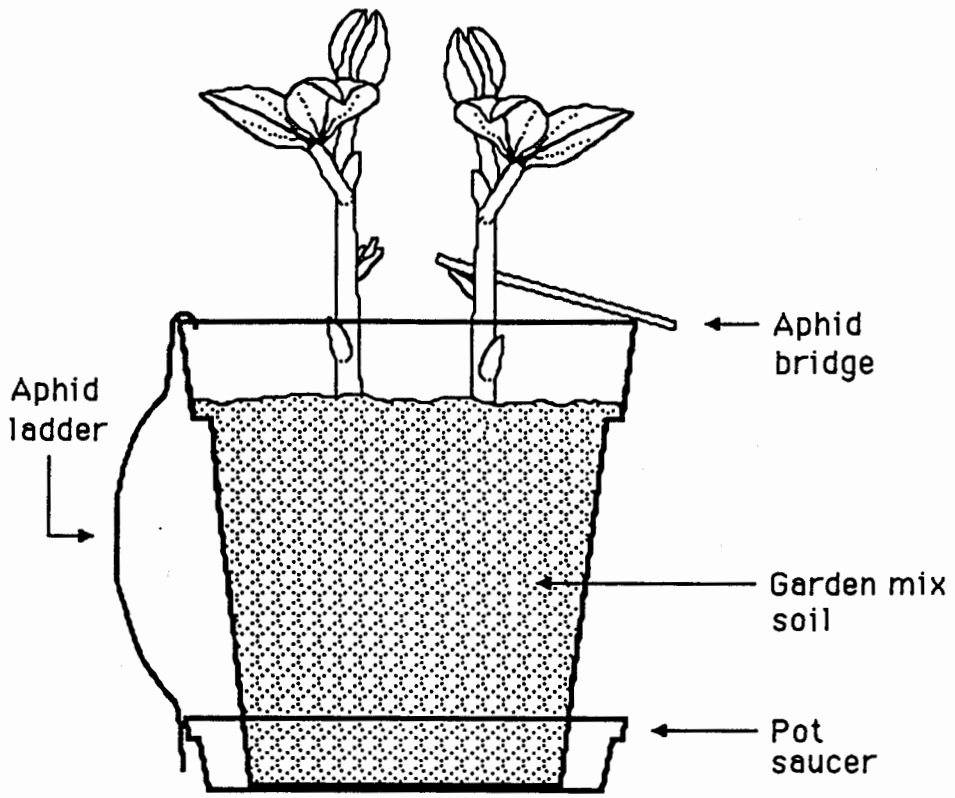
Secondly, the availability of a host reservoir may be beneficial to *P. pequodorum*. *Aphidius ervi* and *P. pequodorum* are polyphagous species (Pungerl 1986; Wilkes 1965), but Stary (1970) suggested that *A. ervi* has a smaller host range than *P. pequodorum*. Forbes and Chan (1989) have documented the presence of a number of the aphid species in southern British Columbia that are common to the host ranges of both parasitoid species. In southern British Columbia, host reservoirs may exist on plants growing on the road-side and on the boarder of agricultural land. Typically, these areas receive less water (via irrigation) than cultivated alfalfa. Halfhill *et al.* (1972) stated that *P. pequodorum* tended to be more abundant than *A. pisivorus* in unirrigated alfalfa; the converse situation was true in irrigated fields. The climate of southern British Columbia is dry; precipitation during the growing season provides only about 50% of the total water required by vegetation during this period (Farley 1979). *Aphidius ervi* is closely related to *A. pisivorus* (Mackauer 1969), and these species may share similar microclimate adaptations. Exploitation of a host reservoir in dry-land areas may permit immigration of *P. pequodorum*, enabling *P. pequodorum* to remain at low levels of abundance within fields of cultivated alfalfa.

Aphidius ervi and *A. smithi* were introduced into North America to augment the control of the pea aphid. As has been noted in other systems (Ehler and Hall 1982; Flanders and Oatman 1987), importation of exotic species may have resulted in disruption of the existing guild of parasitoids. After a period of great variation in

diversity and abundance, *A. ervi* emerged as the predominant, or "most effective" (Unruh *et al.* 1989), aphidiid parasitoid of the pea aphid. *Aphidius ervi* is more common in the field because this species may exploit the host resource to a greater extent than *P. pequodorum* (Chapter 4 and 5). Coexistence between species in a parasitoid guild may depend on inherent differences in behavior and reproduction (Price 1970). The qualities that may contribute to the success of *A. ervi* contrast with those of *P. pequodorum*. Nevertheless, *P. pequodorum* appears to possess many attributes that enable this species to compete successfully with *A. ervi* in the field. As a result, both *A. ervi* and *P. pequodorum* should remain part of the guild of parasitoids that attacks the pea aphid in southern British Columbia.



Appendix 1. Design of screen cage used in experiment to evaluate competition between *Aphidius ervi* and *Praon pequodorum* under controlled environment conditions. Lower diagram indicates approximate location where pots of bean plants were placed in the cage.



Appendix 2. Diagram of a pot of bean plants used in experiment to evaluate competition between *Aphidius ervi* and *Praon pequodorum* in screen cages under controlled environment conditions. For illustration purposes, only two plants are pictured.

Appendix 3. Data from experiments on competition between *Aphidius ervi* and *Praon pequodorum* in screen cages under controlled environment conditions.

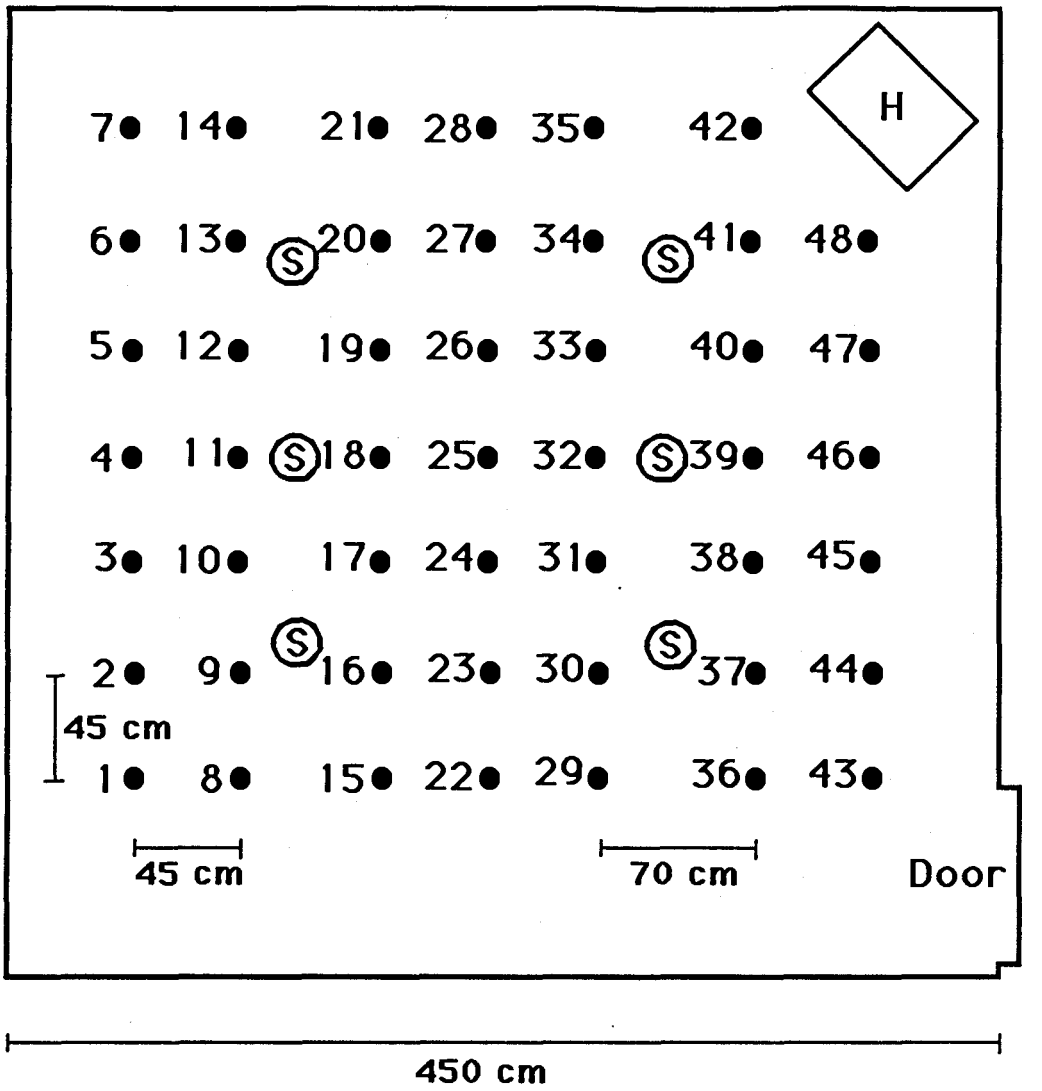
Trial	Relative abundance of *A. ervi* (%)[a]	Population variables[b]						
		Gen	No_{aem}	No_{ppm}	SR_{ae}[a]	SR_{pp}[a]	Mo_{ae}[a]	Mo_{pp}[a]
1	47.59	2	20	43	45.84	28.59	27.23	29.28
	34.02	3	51	40	46.27	42.15	14.63	18.03
	58.74	4	40	66	48.09	33.26	24.90	22.13
	76.10	5	109	34	15.71	36.90	30.27	14.80
	90.00	6	218	15	23.18	19.10	23.35	24.65
4	14.35	2	59	93	32.93	42.61	24.34	24.22
	11.97	3	15	211	28.71	30.00	25.66	27.60
5	38.44	2	52	46	18.14	50.53	18.91	20.00
	68.43	3	44	88	55.66	43.66	-[c]	-[c]
	64.19	4	38	3	45.92	54.74	34.52	45.00
	74.10	5	108	15	39.03	48.59	0	10.02
	90.00	6	89	7	38.92	42.54	28.10	20.70

a. Values are angular transformed.
b. Gen = generation number, No_{aem} = number of *A. ervi* mummies, No_{ppm} = number of *P. pequodorum* mummies, SR_{ae} = sex ratio of *A. ervi*, SR_{pp} = sex ratio of *P. pequodorum*, Mo_{ae} = percent mortality of *A. ervi*, Mo_{pp} = percent mortality of *P. pequodorum*.
c. Percent mortality not measured in this generation.

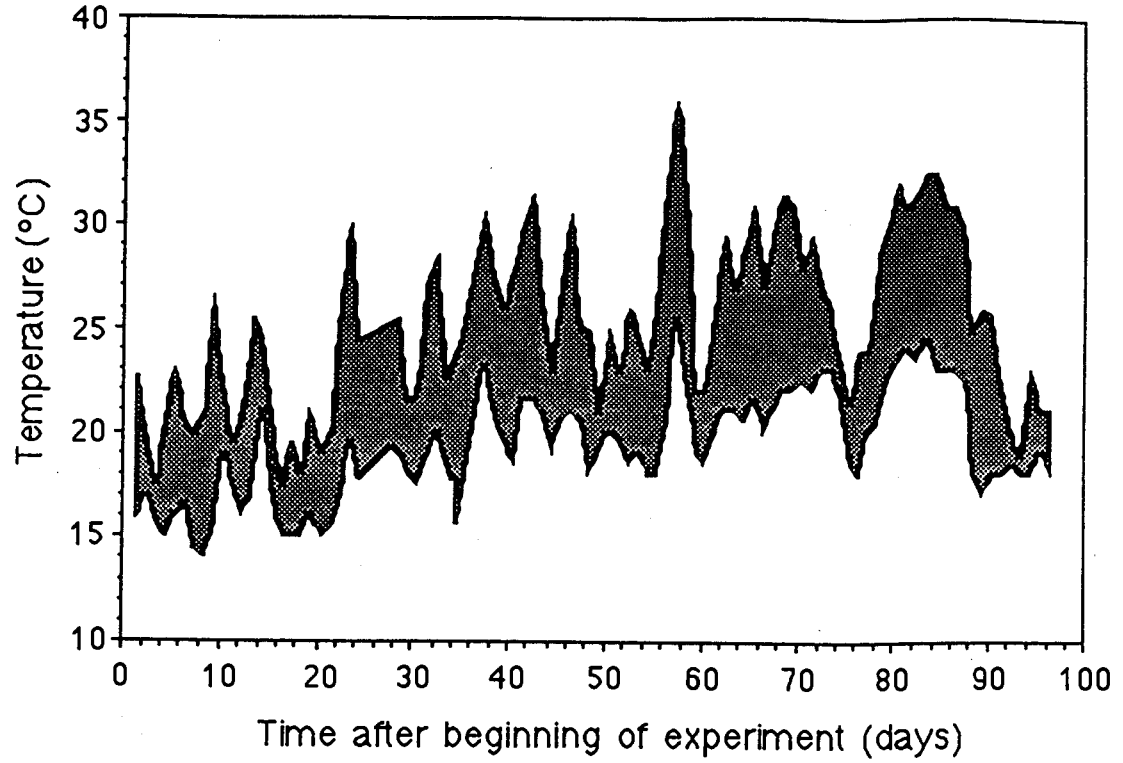
Appendix 4. Data from experiments on competition between *Aphidius ervi* and *Praon pequodorum* in screen cages under controlled environment conditions.

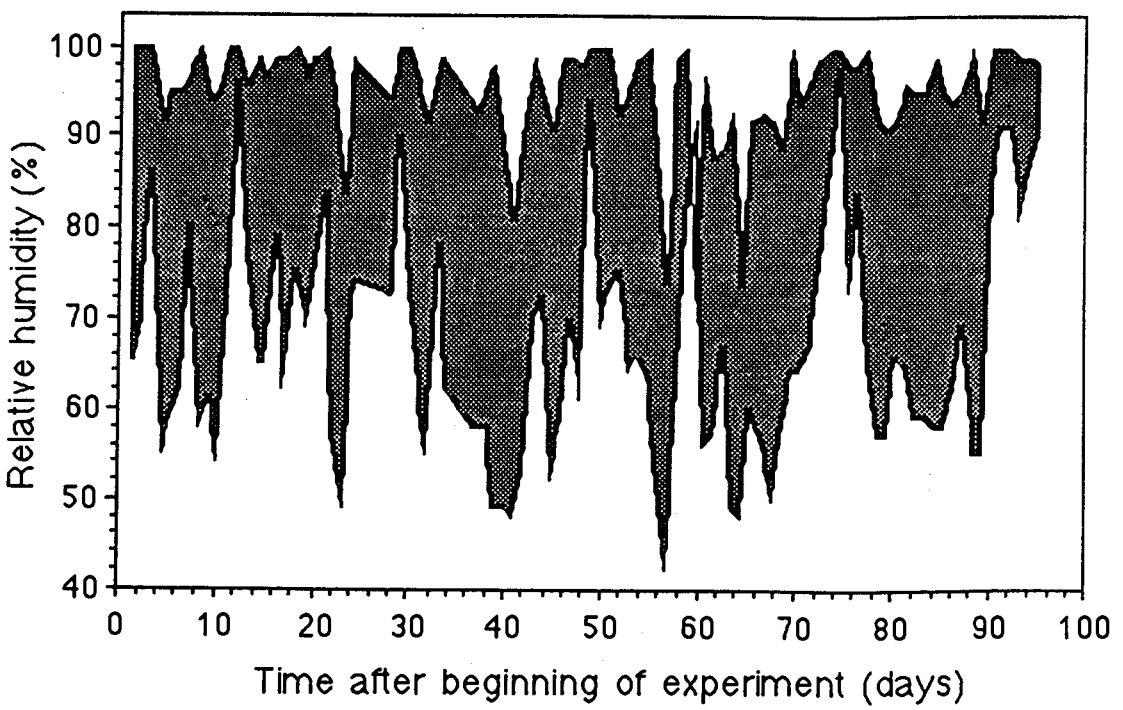
Trial	Relative abundance of *A. ervi*(%)[a]	Population variables[b] Gen	No_{aef}[c]	No_{ppf}[c]	$Ovip_{ae}$	$Ovip_{pp}$
1	31.02	1	19.000	7.000	2.4000	0.8000
	47.59	2	8.139	7.479	0.7647	0.1179
	34.02	3	24.925	16.286	2.6540	0.8846
	58.74	4	18.226	17.035	1.5000	0.1000
	76.10	5	5.960	11.458	1.2160	0
	90.00	6	28.480	1.327	1.4090	0
4	26.89	1	7.000	7.000	1.9740	0.9211
	14.35	2	14.474	35.448	0.6949	1.6950
	11.97	3	2.813	41.429	0.4697	1.2273
5	46.95	1	5.000	5.000	1.0000	0.0286
	38.44	2	4.510	24.209	0.4167	0.5000
	68.43	3	-[d]	-[d]	1.0690	0.2759
	64.19	4	13.313	1.000	3.3330	0.0833
	74.10	5	42.833	8.182	2.8000	0.4000
	90.00	6	27.335	2.800	2.8900	0

a. Values are angular transformed.
b. Gen = generation number, No_{aef} = number of *A. ervi* females, No_{ppf} = number of *P. pequodorum* females, $Ovip_{ae}$ = oviposition activity of *A. ervi* (mean no. larvae/ dissected aphid), $Ovip_{pp}$ = oviposition activity of *P. pequodorum* (mean no. larvae/ dissected aphid).
c. Values in Gen 1 are the numbers of females released, whereas values in subsequent generations are estimated from number of mummies, sex ratio and percent mortality (see text for details).
d. Numbers of females not estimated percent mortality was not recorded for this generation number.

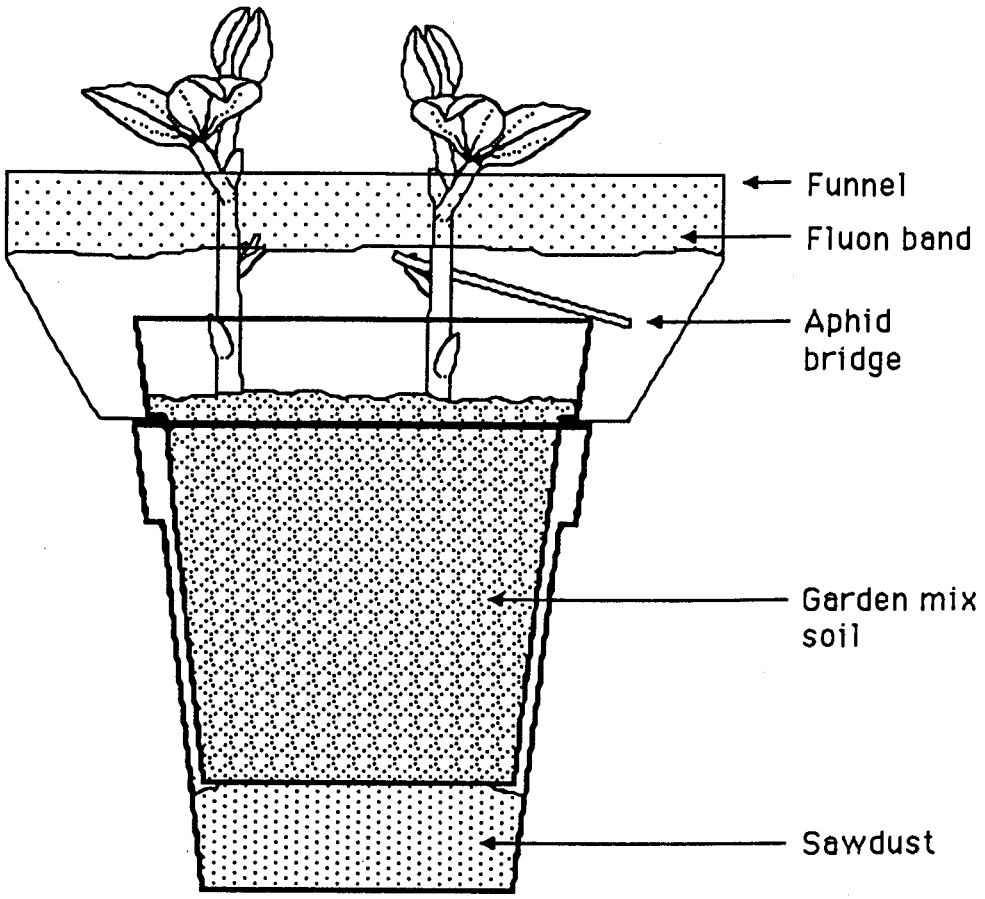


Appendix 5. Floor plan of the screen-house used in experiment to evaluate competition between *Aphidius ervi* and *Praon pequodorum* under semi-natural conditions. Numbered dots represent pots of bean plants. Circles surrounding the letters "S" indicate the locations where sample pots (see Appendix 7) were placed to estimate the oviposition activity of parasitoids. The rectangle enclosing the letter "H" represents the hygrothermograph.





Appendix 6. Daily range of air temperature and relative humidity inside the screen-house used in experiment to evaluate competition between *Aphidius ervi* and *Praon pequodorum* under semi-natural conditions.



Appendix 7. Diagram of sample pot used to estimate the oviposition activity of parasitoids in experiment to evaluate competition between *Aphidius ervi* and *Praon pequodorum* under semi-natural conditions. For illustration purposes, only two plants are shown.

Appendix 8. Numbers of aphids containing given numbers of parasitoid larvae after aphids were exposed to female *Aphidius ervi* and *Praon pequodorum* for 48 hours in screen cages under controlled environment conditions.

a) Five *A. ervi* and five *P. pequodorum* females released in the same cage.

		Number of *A. ervi* larvae			
		0	1	2	3
Number of *P. pequodorum* larvae	0	8	75	16	3
	1	17	52	17	4
	2	1	3	3	-

b) Seven *A. ervi* and seven *P. pequodorum* females released in the same cage.

		Number of *A. ervi* larvae								
		0	1	2	3	4	5	6	7	8
Number of *P. pequodorum* larvae	0	1	11	4	1	1	1	-	-	1
	1	6	23	19	7	3	4	-	-	-
	2	-	6	3	3	3	-	-	-	-
	3	-	-	3	2	-	1	-	-	-
	4	-	-	-	1	-	-	-	-	-

c) Ten *A. ervi* and ten *P. pequodorum* females released in separate cages.

Number of *A. ervi* larvae

0	1	2	3	4	5	6	7
3	62	49	22	15	6	-	2

Number of *P. pequodorum* larvae

0	1	2	3
46	87	10	1

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