Reproductive behaviour of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae)

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CANDIDATE'S DECLARATION

This is to certify that the research carried out in the Doctoral thesis entitled "Reproductive behaviour of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae)" is my own work and that the thesis material has not been used in part or in whole for any other qualification.

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SUPERVISOR'S DECLARATION

This is to certify that the research carried out in the Doctoral thesis entitled "Reproductive behaviour of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae)" was done by Xiong Zhao He in the Institute of Natural Resources, Massey University, Palmerston North, New Zealand. This thesis material has not been used in part or in whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

Prof. Qiao Wang

28 August 2008

Abstract

Aphidius ervi Haliday is a cosmopolitan parasitoid species of several major aphid pests on economically important crops. Prior to this research, little information was available on its reproductive behaviour. Emergence of *A. ervi* peaks during the first few hours of the photophase with males being protandrous. Females become sexually mature earlier than males and oviposit primarily in the photophase. Aphids parasitised in their early instars die before reproduction but those parasitised in later instars produce a limited number of progeny. Females prefer aphids of 3- to 5-d-old over the younger and older aphids for oviposition. Females ovipositing in 4- to 7-d-old aphids have more fitness gains in terms of progeny body size and egg load at emergence. Fertilised eggs are more likely deposited in large hosts and unfertilised eggs in small ones. Large individuals have greater longevity, large males father more progeny, and large females have higher fecundity, parasitism and greater ability in host searching. However, with increasing body size females gain more than males in longevity and fecundity but males gain more than females in the number of female progeny. Males can inseminate up to nine females and they carry about 82% effective sperm at emergence and replenish about 18% sperm during their adult life. Females adjust the oviposition and sex allocation strategies in response to increasing host density with higher number of aphids parasitised at higher host densities and lower proportion of female progeny produced at lower host densities. Males play an active role in mating behaviour. Males having mating experience, and being large or younger, respond to females more quickly and perform better courtships resulting in higher mating success. Males prefer larger and younger females for mating probably because the latter have greater reproductive potential. Males optimize the use of their sperm based on the availability of their sperm and the reproductive status (age) of females. The switching-off of female receptivity of male mating attempt after the mating is a gradual process. Some females accept the second males within 1 minute since the termination of the first mating. The shorter mating period in the second mating suggests that females remate probably due to the gradual process of switching-off of female receptivity rather than the insufficient sperm transformation during the first mating. Males prolong their mating duration in male-biased operational sex ratio to reduce the probability of female remating.

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This thesis is dedicated

to *my loving parents* who showed me the way to succeed in my life

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CHAPTER 1
GENERAL INTRODUCTION

1.1 Introduction

Aphidius ervi Haliday 1834 is an important biological control agent and usually introduced to control aphid species on lucerne, *Medicago sativa* (Leguminosae) (Cameron et al. 1979; Sandow 1981; Mackauer and Kambhampati 1986; Cameron and Walker 1989; Waterhouse and Sands 2001). Lucerne has been cultivated for thousands of years and is the most important forage/grazing legume (Grewal and Williams 2001). Lucerne, with its high nitrogen fixation capacity, is also grown in rotation with wheat to improve the yield and protein content of wheat grain in Australia (Grewal and Williams 2001). It gives high yields of nutritious fodder that often out-yields pasture by 50~100% in New Zealand (McSweeney and Dunbier 1978; Rive 2003). It is grown today on all continents except Antarctica and more than 33 million hectares cultivated in the world (Seigler 2005).

Both blue-green lucerne aphid, *Acyrthosiphon kondoi* Shinji and pea aphid, *Ac. pisum* (Harris) are the major pests on lucerne that reduce the yields and quality (Kain et al. 1977; Kain and Biggs 1980). Kain et al. (1979a) indicate that, in New Zealand, infestation with blue-green lucerne aphids and pea aphids may result in large losses in herbage production (30~60%) and reduction in plant density (18%). In the United States, pea aphid was responsible for lucerne production losses of $60 million in 1963 (Harper et al. 1978). Moreover, the field and glasshouse studies indicate that infestation of blue-green lucerne aphid and pea aphid caused increased coumestrol levels in severely aphid-damaged lucerne which were high enough to impair ewe fecundity (Kain and Biggs 1980).

To control the cosmopolitan blue-green lucerne aphid and pea aphid, three common aphidiids: *A. ervi, A. eadyi* Stary, Gonzalez and Hall, and *A. smithi* Sharma and Subba Rao, were introduced as potential biological control agents in South and North America, Australia and New Zealand (Cameron et al. 1979; Sandow 1981; Mackauer and Kambhampati 1986; Cameron and Walker 1989; Waterhouse and

Sands 2001). *A. eadyi* and *A. smithi* are specialized species of pea aphid and *A. ervi* is polyphagous. Of these three species, *A. ervi* is the most abundant, and widely distributed and adapts to wide range of climates and habitats (González et al. 1978).

In North America, *A. ervi* displaced *A. smithi* as the predominant species even if the latter was introduced first and rapidly became established (Mackauer and Kambhampati 1986). In Australia, *A. ervi*, *A. eadyi*, *A. smithi* and *A. urticae* Haliday were introduced and released specifically for control of pea aphid between 1980 and 1981 in New South Wales and Victoria (Sandow 1981; Milne 1986; Carver 1989). *A. ervi* spread up to 300 km in a year and is now widely distributed in Queensland, New South Wales, Victoria, Western Australia and Tasmania (Sandow 1981; Milne 1986; Carver 1989). *A. smithi* had become established in Tasmania only but not in the mainland Australia, and in Tasmania its population is lower than *A. ervi* (Carver 1989). In New Zealand, five species including three *Aphidius* species (*A. ervi*, *A. eadyi* and *A. smithi*) and *Ephedrus plagiator* (Nees) and *Praon barbatum* Mackauer were imported from California for biological control of aphids on lucerne (Cameron et al. 1979; Cameron and Walker 1989). However, only *A. ervi* and *A. eadyi* successfully established (Cameron and Walker 1989). *A. eadyi* established easily and spread rapidly, but *A. ervi* eventually replaced *A. eadyi* as the predominant parasitoid in the North Island (Cameron and Walker 1989). This displacement was driven through direct and indirect larval interactions and possibly superior searching ability by *A. ervi* females in mixed populations (Chua et al. 1990; McBrien and Mackauer 1990; Bueno et al. 1993).

A. ervi has significantly suppressed lucerne aphid populations in the field. In Australia, the parasitism rate of blue-green lucerne aphid was up to 70% in Queensland, New South Wales, Victoria and West Australia in 1981 (Sandow 1981). Surveys in 1982 and 1983 demonstrated the successful establishment and dispersal of *A. ervi* in blue-green lucerne aphid and pea aphid in the major lucerne-growing areas of New South Wales and also the ability of the parasitoid to built up populations rapidly again after a severe and widespread drought; sampling in southern New South Wales and in costal areas of Australia from 1994 to 1996 revealed that high populations of blue-green lucerne aphid and pea aphid were undoubtedly parasitised by *A. ervi* (Waterhouse and Sands 2001). In New Zealand, since 1979-80, populations

of blue-green lucerne aphid and pea aphid have steadily declined at a number of northern North Island sites. This decline likely resulted from a number of factors, including the establishment of the parasitoids (Cameron et al. 1979), and replacement of susceptible cultivars with aphid resistant lucerne (Dunbier and Easton 1982), together with better management practices (Kain and Trought 1982); however the increase of parasitoid populations indicates that parasitoids have provided successful control of lucerne aphids (Cameron and Walker 1989).

Compared to *A. eadyi* and *A. smithi*, the greater effectiveness of *A. ervi* in biological control of pea aphid and blue-green lucerne aphid may be due to high behavioural adaptation of *A. ervi* to the target aphids and biotypes, and phenological adaptations to the climates of aphid environment (Starý 1988). Moreover, the major aphid host plant, lucerne, is usually grown for many years so that the community system of plant-host and aphid-parasitoid is not disturbed by agricultural activities at the end of each year (Starý 1962). Finally, the high persistence of *A. ervi* within its hosts may be a key factor leading to successful biological control in the disturbed systems (Rauwald and Ives 2001).

Although *A. ervi* has been effective in reducing aphid populations in the high rainfall areas, it is less effective in drier inland areas such as in Australia (Waterhouse and Sands 2001). Aphid populations are still high in some lucerne-growing areas, for example, in Palmerston North, New Zealand, the number of blue-green lucerne aphids can be as high as 120 individuals per stem on the susceptible cultivar, 'Wairau', in spring and summer 2003 (unpublished data), which is four times higher than the critical control threshold (i.e. 30 aphids per stem) (Kain and Trought 1982). Therefore, augmentation of field populations may be an appropriate option which is relevant to mass-rearing.

1.2 Importance of the Study of Parasitoid Reproductive Behaviour

The study of reproductive behaviour is an important key for the understanding of how parasitoids should influence the population dynamics of their hosts and the structure of the insect communities in which they live (van Alphen and Jervis 1996),

and for the determination of what behaviours parasitoids adopt in order to maximize their reproductive success (Anon 2005). In parasitoids, the production of progeny directly reduces the host populations. Therefore, studying how parasitoids optimize their reproductive output should provide means to improve pest control efficacy in biological control programmes.

Until the 1990s, behavioural ecologists largely focused on developing and testing theoretical predictions for the fitness consequences of behaviour but devoted less effort to the studying of behavioural mechanisms employed by the animals in order to optimize their reproductive output (Anon 2005). In the last decade, behavioural ecologists have tried to integrate the study of behavioural mechanisms with the functional approach to study the fitness consequences of behaviour (Anon 2005). In *A. ervi*, many studies have investigated the behavioural mechanisms employed by parasitoids to find hosts (Vinson 1984; Schmidt 1991; Vet and Dicke 1992; Guerrieri et al. 1993, 1997; Turlings et al. 1993; Godfray 1994; Du et al. 1996, 1997; Quicke 1997) and allocate sex of progeny to the hosts (Micha et al. 1992; Battaglia et al. 1993, 1995, 2000; Pennacchio et al. 1994; Du et al. 1997; Weinbrenner and Volkl 2002), but how these mechanisms contribute to fitness or act as constraints on the optimization of reproductive behaviour of parasitoids is still poorly known. The study of parasitoid reproductive behaviour is essential for understanding and modeling life history evolution and behavioural decisions regarding host choice, fecundity, and sex ratio (Godfray 1994; Visser 1994; Cloutier 2000). It is also important in the development of theory in behavioural ecology that incorporates knowledge of the underlying mechanisms of parasitoid behaviour in models that predict maximal fitness (Anon 2005). Knowledge of these can be used to define more efficient biological control technologies of insect pests in agriculture.

1.3 Relevance of This Research

Success in biological control is often dependent on a thorough understanding of the organisms involved both injurious and beneficial species, and their intricate interactions. Few studies have investigated the reproductive behaviour of *A. ervi*, limiting the design, improvement and implementation of biological control strategies.

The study of *A. ervi* reproductive behaviour is relevant because:

(1) Lucerne is an important cosmopolitan legume crop grown for both grazing and hay production in the world, and it can also improve soil quality, which consequently enhances agricultural profitability (Hanson et al. 1988). However, some pests, such as blue-green lucerne aphid and pea aphid have continually caused serious damage resulting in the reduction of fresh or hay production and quality. Although the resistant lucerne cultivars have been introduced (Dunbier and Easton 1982) and better management practices have been developed (Kain and Trought 1982), around a third of lucerne grown in New Zealand is still the cultivar 'Wairau', which is susceptible to all major pests (Rive 2003).

(2) *Aphidius ervi* is a cosmopolitan parasitoid (Marsh 1977) and an important biological control agent of several major aphid species on economically important crops such as legumes and cereals (Starý 1978; Powell 1982). However, its ability in suppressing aphid population decreases in drought prone regions (Waterhouse and Sands 2001). Lucerne is drought resistant (Seigler 2005), it is expected that more lucerne will be grown in drier regions if the global temperature continues to increases due to the 'global greenhouse effect'. There is thus a need to find strategies to improve the success of pest control using *A. ervi*, especially in cropping systems where lucerne is grown for many years.

(3) Development of IPM techniques, including the application of biological control agents, can reduce the amount of chemical used and increase the value of the harvested produce. The study of *A. ervi* reproductive behaviour provides information for the understanding of its biology and the development of environmentally friendly IPM practices. The host habitat location and host searching behaviour of *A. ervi* have been well studied (Vinson 1984; Schmidt 1991; Vet and Dicke 1992; Guerrieri et al. 1993, 1997; Turlings et al. 1993; Godfray 1994; Du et al. 1996, 1997; Quicke 1997). However, little is known about its reproductive behaviour such as host preference, functional response and mating behaviour. Moreover, how these behaviours affect reproductive fitness of parasitoids and population dynamics of both aphids and parasitoids is not clear. Unraveling the factors which govern the reproductive

behaviour and its impact upon the reproductive fitness of this species will provide valuable information for IPM developers in improving mass rearing and biological control strategies.

1.4 Aim and Objectives

In order to provide useful information for the development of biological control strategies and theoretical research, the aim of this research is to study the reproductive biology and mating behaviour of *A. ervi* with the following objectives:

(1) To investigate the basic biology of *A. ervi*;

(2) To determine the factors affecting the reproductive fitness of *A. ervi*;

(3) To investigate the mating behaviour and sexual selection of *A. ervi*.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

This chapter reviews the current knowledge on Aphidiinae species that are relevant to my PhD studies. Special references are given to known biology of *A. ervi*.

2.2 Taxonomy of *A. ervi*

Among genera that attack aphids, *Aphidius* is the largest one in the Aphidiinae with over 70 species described worldwide (Mescheloff and Rosen 1990) and the number of described species continues to increase (Kavallieratos et al. 2004, 2006; Tomanović et al. 2003).

Aphidius ervi Haliday was first named in 1834, and has since been known by many synonyms (Marsh 1977) including:
Aphidius ulmi Marshall, 1896
Aphidius medicaginis Marshall, 1898
Aphidius (*Aphidius*) *pisivorus* Smithi, 1944
Aphidius fumipennis Gyorfi, 1958
Aphidius ervi nigrescens Mackauer, 1962
Aphidius caraganae Starý, 1963
Aphidius mirotarsi Starý, 1963

The classification for this species is:
Order: Hymenoptera
Family: Braconidae
Subfamily: Aphidiinae
Genus: *Aphidius*
Species: *ervi*

2.3 Identification of *A. ervi*

A large number of *Aphidius* species have been recorded for pea aphids and cereal aphids including *A. ervi*, *A. eadyi*, *A. smithi*, *A. picipes* (Nees), *A. pisivorus* Smith, *A. popovi* Starý, *A. nigripes* Ashmead, *A. uzbekistanicus* Luzhetski, *A. rhopalosiphi* De Stefani Perez, *A. matricariae* (Haliday) and *A. colemani* Viereck (Pungerl 1983). As mentioned above, three *Aphidius* species and *E. plagiator* and *P. barbatum* had been imported into New Zealand to control pea aphids and blue-green lucerne aphids, but only *A. ervi* and *A. eadyi* had successfully established (Cameron et al. 1979; Cameron and Walker 1989). Because the breeding colony used in this study started from *A. ervi* emerged from blue-green lucerne aphids collected on lucerne (Section 3.2), I only identified the *A. ervi* and *A. eadyi* and keys for identification of other *Aphidius* species have been reported by Pungerl (1983).

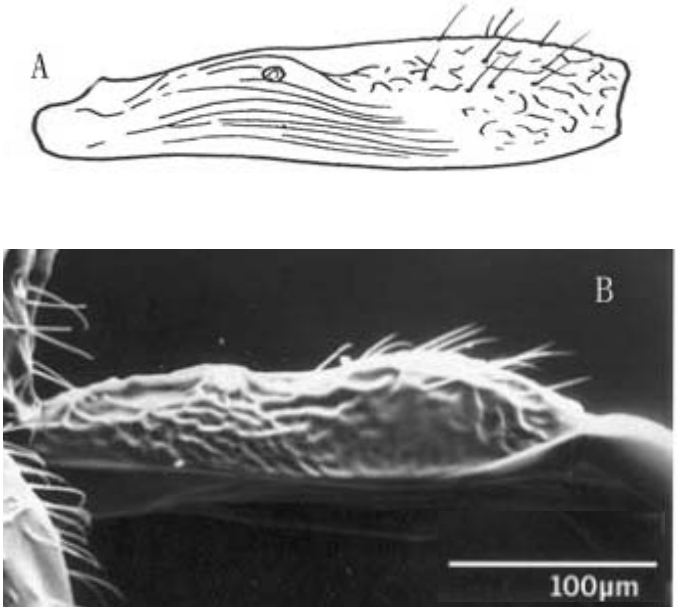

Figure 2.1 Lateral view of tergum of female: (A) *A. eadyi* (from Starý et al. 1980) and (B) *A. ervi* (from Kavallieratos et al. 2001).

Routine examination of the two key-characters may identify *A. ervi* and *A. eadyi*, i.e. number of antennal segments and character on the anterolateral area of petiole (Marsh 1977; Starý et al. 1980; Pungerl 1983). The numbers of antennal segments are 19-22 and 17-19 for *A. ervi* male and female, respectively, and 20-24 and 20-21 for *A. eadyi* male and female, respectively. The anterolateral area of petiole

is costulate, bearing 7-9 ridges in *A. eadyi*, (Figure 2.1A), while the surface of the anterolateral area of petiole is coarsely rugose, with no ridge in *A. ervi* (Figure 2.1B).

2.4 Distribution of *A. ervi*

Aphidius ervi is an aphidiid of Palaearctic origin. *A. ervi* can adapt to different climatic conditions: continental Europe, high plateaus, coastal and desert (González et al. 1978). Now it occurs in North and South America, Europe, North Africa, Middle East, Australia, New Zealand, India, Japan and China (Marsh 1977; Cameron et al. 1979; González et al. 1979; Sandow 1981; Starý and Delfino 1986; Raychaudhuri 1990).

2.5 Hosts and Host Preference of *A. ervi*

Aphidius ervi is a polyphagous parasitoid and has wide host range including: *A. pisum*, *A. kondoi*, *A. caraganae* (Cholodkovsky), *Aphis* sp., *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), *Microlophium evansi* (Theobald), *Myzus certus* (Walker), *M. persicae* (Sulzer) and *Rhopalosiphum padi* (L.) (Marsh 1977).

Aphidius ervi can be reared either on blue-green lucerne aphid or pea aphid. However, host preference of *A. ervi* depends on the previous aphid host (Cameron and Walker 1989). For example, when reared on blue-green lucerne aphid for one year, *A. ervi* continues to prefer blue-green lucerne aphid rather than pea aphid; when reared on pea aphid, *A. ervi* prefers pea aphid in the first generation and performs as well on pea aphid as on blue-green lucerne aphid in the second generation, but its performance on pea aphid declines in the third generation (Cameron and Walker 1989). Thus, *A. ervi* is capable to switch its host preference behaviour by rapid responding to available host types, which enables it to survive as a biological control agent in natural conditions.

2.6 General Biology of *A. ervi*

The life cycle of *A. ervi* includes egg, larva, pupa and adult. It is a solitary endophagous species. At 20°C, eggs hatch in 3 days; larvae last for about 8 days, after which time, a cocoon is formed and the aphid is 'mummified' (unpublished data). The parasitoid pupae last for about 5 days in aphid mummies before adults emerge. Adults may survive and oviposit for about 10 days. The low temperature thresholds for development is 3.9, 2.6 and 4.5ºC for *A. ervi*, blue-green lucerne aphid and pea aphid, respectively, thus it is well synchronized with their hosts in cool spring temperature (Cameron and Walker 1989). *A. ervi* overwinters as diapausing final instar larvae in its host aphids and its diapause is induced by short day length (vaz Nunes and Hardie 1996; Christiansen-Weniger and Hardie 1997).

2.6.1 Eggs

Mature eggs in ovaries of *A. ervi* are elongate and oval in shape with one or both ends being more or less tapering (Spencer 1926), and 50 × 20 μm in size (Figure 2.2A). During oviposition eggs are placed by parasitoids in the adipose tissue of aphids, usually with part of the egg projecting a short distance into the body cavity. Soon after oviposition the egg absorbs liquid from the aphid by osmosis, and increases enormously in size, becoming lemon shape and as large as 130 × 60 μm (Figures 2.2B-F).

2.6.2 Larvae

Aphidiine wasps are endoparasitoids that undergo larval development inside a living host. Three instars based on larval morphology have been proposed (Schlinger and Hall 1960; Starý 1966; Calvert and van den Bosch 1972; O'Donnell 1987; Pennacchio and Digilio 1990) and confirmed for *A. ervi* by counting the exuviae of *in vitro* reared larvae (Pennacchio and Digilio 1990). The first instar larva is mandibulate-caudate with sickle-shaped mandibles, the second instar larva is hymenopteriform and amandibulate, and the third instar larva has well developed mandibles (Pennacchio and Digilio 1990). After hatching, larvae feed primarily by

surface absorbing and ingesting host hemolymph, and begin to feed on host tissues and embryos using mouthparts 7 days after oviposition (Polaszek 1986; Pennacchio and Digilio 1990).

The first, second and third instar larvae are recorded 56, 90 and 110 h after oviposition, respectively at 26°C (Pennacchio and Digilio 1990). The development of *A. ervi* larvae at 20°C is shown in Figure 2.3.

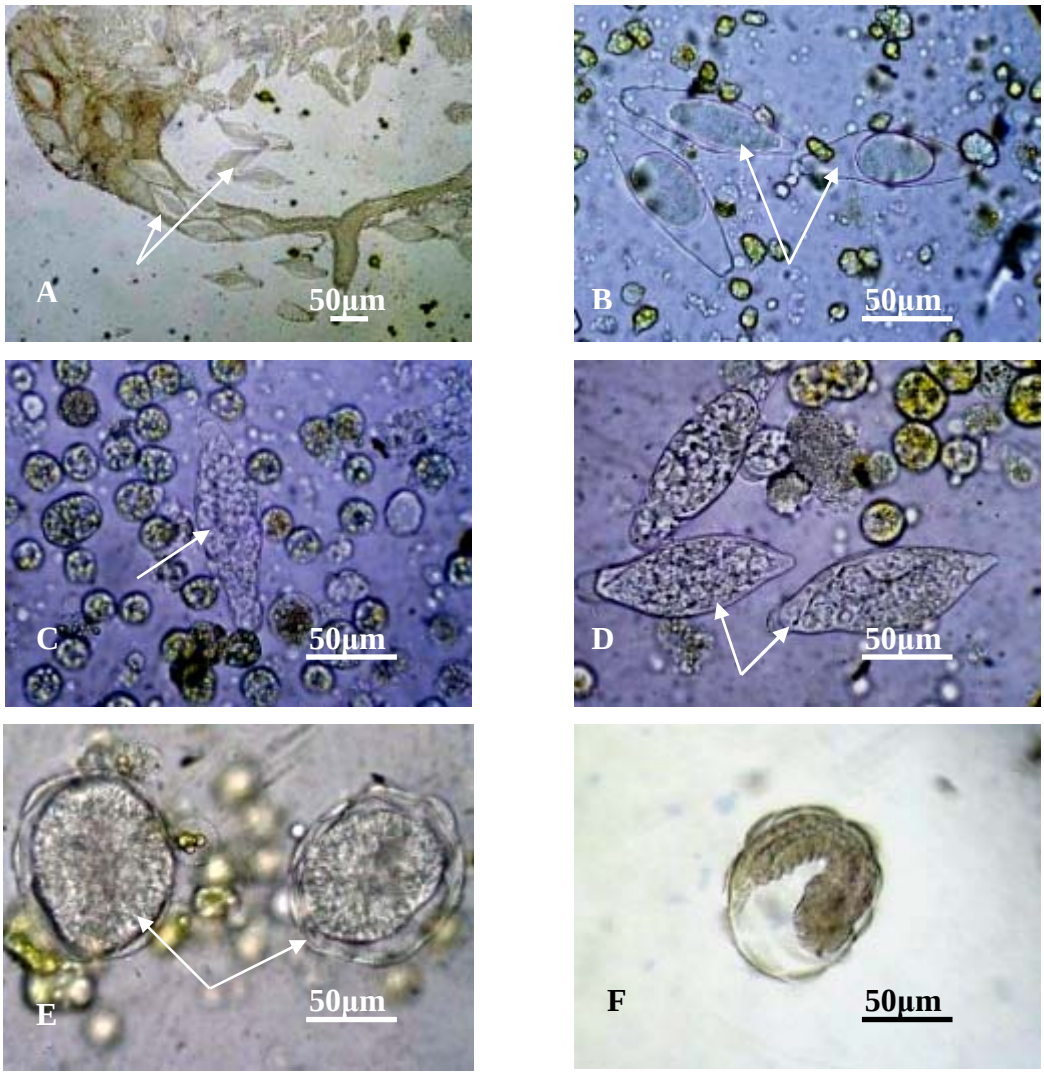

Figure 2.2 Development of *A. ervi* eggs: mature eggs in ovaries (A), and eggs 1 h (B), 12 h (C), 24 h (D), 48 h (E) and 72 h (F) after oviposition. All pictures were taken in the present study.

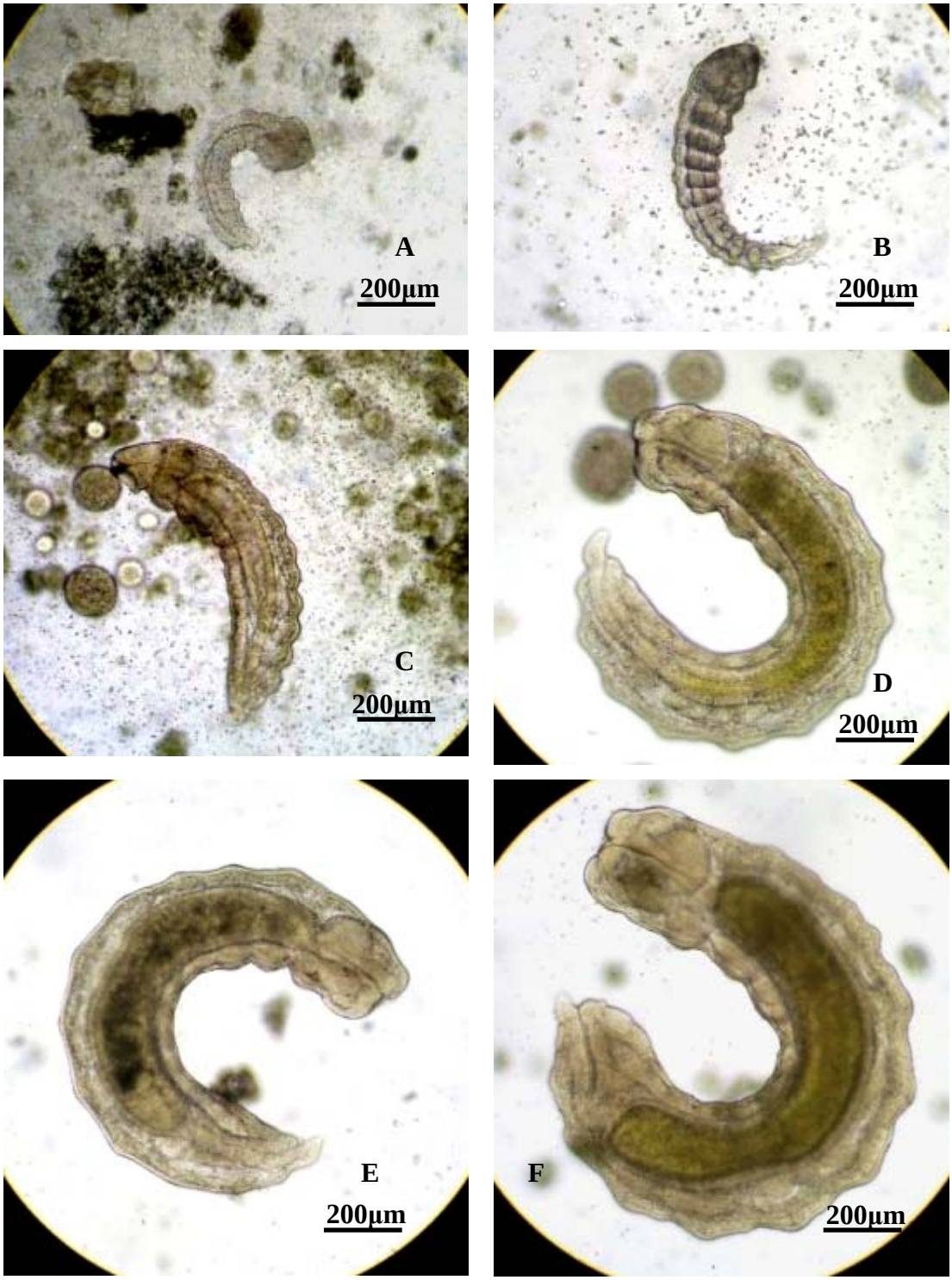

Figure 2.3 Development of *A. ervi* larvae: newly hatched larva (3 days after oviposition) (A), and larvae of 1 day old (B), 2 day old (C), 3 day old (D), 4 day old (E) and 5 day old (F). All pictures were taken in the present study.

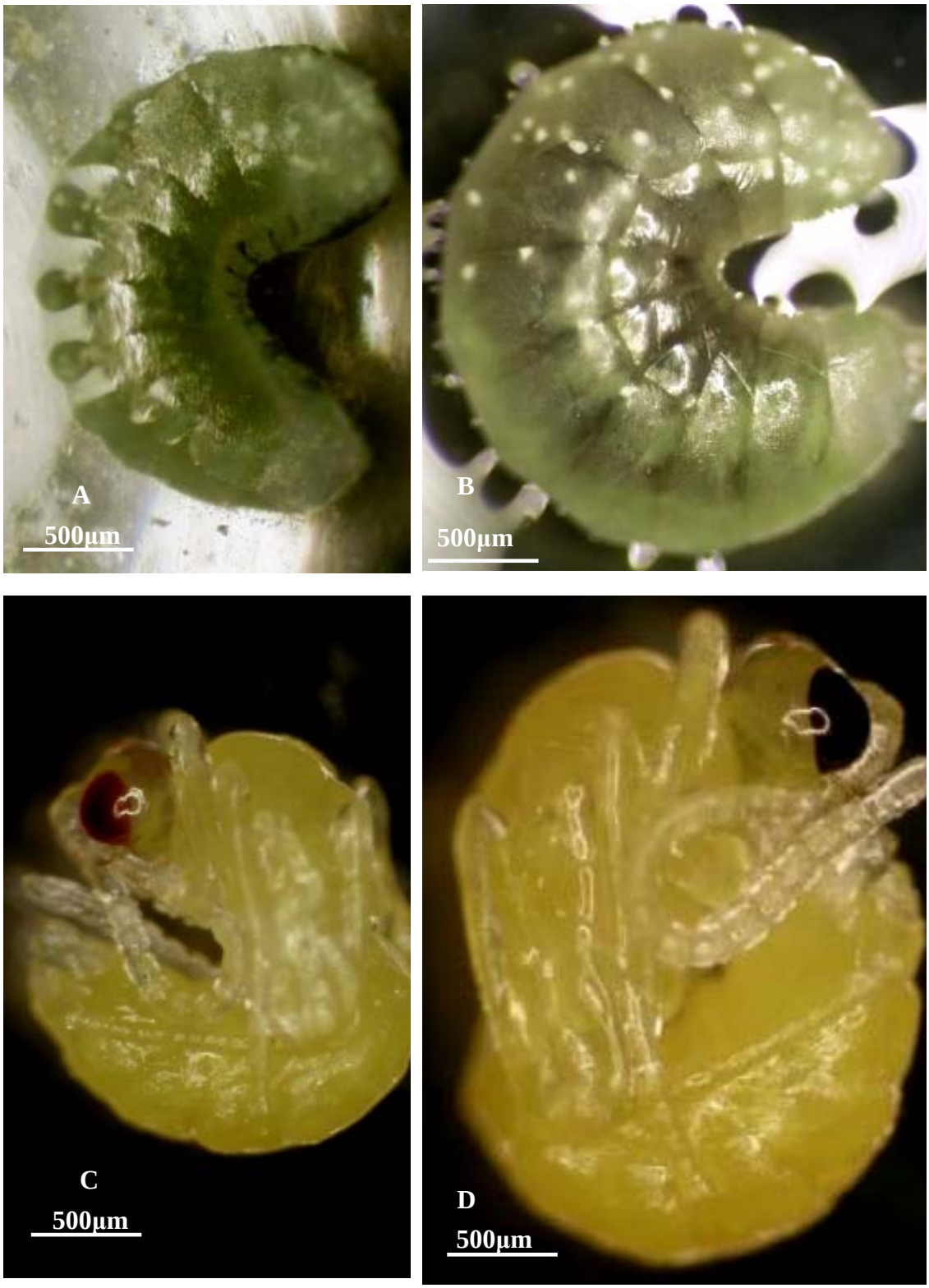

Figure 2.4 Pupation of *A. ervi*: mature larvae [9 days (A) and 10 days (B) after oviposition] and pupae [11 days (C) and 12 days (D) after oviposition]. All pictures were taken in the present study.

Figure 2.5 *A. ervi* pre-adult (A) (13-14 days after oviposition), aphid mummy (B) and adults (C). All pictures were taken in the present study.

2.6.3 Pupae

The mature larva (Figures 2.4A and B) makes a hole at lower side of the aphid's skin, fixes the aphid by the secretion of its salivary glands to the leaf surface, and then pupates inside the mummified aphid (Starý 1962). The pupa is initially whitish, gradually turning yellow (Figures 2.4C and D) and black (Figure 2.5A) before adult eclosing from aphid mummy.

2.6.4 Adults

The eclosed adult cuts out a circular hole in the dorsal abdominal part of the host just above the siphons, and then emerges (Figure 2.5B). The period of emergence usually lasts several minutes (Starý 1962).

The newly emerged parasitoid is soft and in only about five minutes its wings expend and body becomes compact (Spencer 1926). During this period, the parasitoid sits on the plant, moves its legs and antennae and cleans itself intensively. In about 10 minutes after emergence, it is able to fly. A certain period for the maturation of eggs seems necessary (Spencer 1926). *A. ervi* adult is a long (4~5 mm), slender, and black wasp (Figure 2.5C).

2.6.5 Host Searching and Oviposition of *A. ervi*

In general, the foraging behaviour of hymenopteran species is divided into three mechanistic steps: (1) host habitat location, (2) host location and (3) host acceptance (Doutt 1959; Vinson 1976). Chemical and physical cues appear to play a major role at almost every level of the foraging behaviour (Vinson 1984; Schmidt 1991; Vet and Dicke 1992; Turlings et al. 1993; Godfray 1994; Quicke 1997). Success of host searching depends upon the execution of appropriate behavioural responses to an array of cues which are available during foraging.

Host habitat location. Plant volatiles emanating from the host's food and host odors have been shown to be important cues in host habitat location at long distances

for a number of hymenopteran parasitoids. In a wind tunnel bioassay, naïve *A. ervi* females responded poorly to undamaged bean plants and to pea aphid isolated from the plant, but they showed strong oriented flight responses to aphid-infested plants and to aphid-damaged plants from which the aphids had been removed (Guerrieri et al. 1993; Du et al. 1996). When offered a choice, more parasitoids flew toward and landed on the host-infested or host-damaged plants than on undamaged plants (Du et al. 1996). These results suggest that *A. ervi* females use host-induced plant volatiles as host habitat location cues.

The compounds of volatile extracts from pea aphid infested plants that could be involved in parasitoid attraction, are identified as 6-methy1-5-hepten-2-one, linalool, (*E*)-*β*-ocimene, (*Z*)-3-hexeny acetate, (*Z*)-3-hexen-1-ol, and (*E*)-*β*-farnesene, with *A. ervi* appearing to use the more specific, 6-methy1-5-hepten-2-one, which is not induced in plants infested by the non-hosts, such as black bean aphid, *Aphis fabae* Scopoli (Du et al. 1998). Thus, 6-methy1-5-hepten-2-one is potentially one of the volatile components that allow *A. ervi* to distinguish between plants infested with hosts and non-hosts (Du et al. 1998), and its ability has been demonstrated in the wind tunnel study (Du et al. 1996).

The parasitoid experience enhances searching ability in at least 20 species (Turlings et al. 1993). In the wind tunnel study, responses of *A. ervi* females to volatiles emanating from undamaged, host-infested and damaged or host-damaged plants are enhanced by previously exposing the females to the plants infested by aphids (Du et al. 1997; Guerrieri et al. 1997). Thus the plasticity of parasitoid foraging behaviour may provide ways of enhancing the efficiency of *A. ervi* in pest management (Guerrieri et al. 1997).

Moreover, long range attraction of *A. ervi* toward monochromatic light in the green region of the spectrum (514 nm) has been reported (Goff and Nault 1984), which suggests that this parasitoid employs a visual response to green vegetation during the host habitat location phase of host selection. The pea aphid sex pheromones as a potential host habitat location cues have also been demonstrated for *A. ervi* in a wind tunnel study (Glinwood et al. 1999).

Host location and acceptance. In a short range, host location and acceptance by *A. ervi* are influenced by the physical cues. *A. ervi* females show strong attack responses toward green pea aphid even without physical contact, indicating the use of visual cues during host location (Battaglia et al. 1995). *A. ervi* females react to yellow pigment with repeated oviposition attack responses, but they do not react to green pigment (Battaglia et al. 2000). The spectrum of reflected light from the yellow pigments is very similar to that from the 'green' natural pea aphid, with a high proportion of the total radiation energy being emitted in the yellow-orange wavebands (580~660 nm) (Battaglia et al. 2000).

The host location and acceptance by *A. ervi* are also regulated by semiochemical cues. It has been shown that the cornicle secretion, cuticula and honeydew of pea aphid act as contact kairomones and elicit an intense oviposition attack response by *A. ervi* females, which appears to be innate (Battaglia et al. 1993; Pennacchio et al. 1994; Du et al. 1997). *A. ervi* females do not attack wet pea aphid that are washed previously with water, but one hour later this phenomenon disappears and *A. ervi* females attack washed aphids to the same degree as dry ones (Weinbrenner and Volkl 2002), confirming the chemical cues on the cuticula of aphids as contact kairomones.

In the field, *A. ervi* females may encounter parasitised and unparasitised hosts and the parasitised hosts may have been attacked by conspecific or heterospecific parasitoids. Host discrimination (i.e. the ability to distinguish parasitised and unparasitised hosts) is a common phenomenon among Hymenopteran parasitoids (van Lenteren 1981). In *A. ervi*-blue-green lucerne aphid system, newly parasitised hosts appear to be susceptible for superparasitism but parasitoids tend to avoid superparasitism six hours after the hosts are attacked (Micha et al. 1992). This suggests that *A. ervi* females do not mark their hosts with an oviposition-deterring pheromone to limit short term superparasitism, and the trend to avoid superparastism at longer intervals is probably attributed to changes occurring in the host as a response by the aphid to parasitism or to changes induced by the presence of a parasitoid egg within the host (Micha et al. 1992). *A. ervi* females generally avoid ovipositing in a pea aphid which has been parasitised by another species, such as *Aphelinus asychis* Walker, but they can survive in the hosts superparasitised by *A. asychis*, killing the

competing larvae by physical and possibly physiological suppression (Bai and Mackauer 1991).

2.7 Reproductive Biology of Aphidiinae

2.7.1 Reproductive System

The female reproductive system of Aphidiinae usually comprises the ovipositor, ovaries, oviducts, vagina, spermatheca and associated glands, and the venom gland (with its associated reservoir) (Quicke 1997). The ovarial imaginal bud gives rise to the lateral oviducts and the posterior ends of the lateral oviducts unite to form the common oviduct, sometimes referred to as the vagina. There is only one pair of meroistic ovarioles in *Aphidius* species (Quicke 1997). Mature eggs move from the ovarioles and pass through the lateral oviduct toward the common oviduct (see Figure 2.6 for *A. ervi*) and then the ovipositor. The spermathecal complex consists of a small, nearly spherical reservoir which opens into a narrow spermathecal duct which opens into the anterior end of the common oviduct; sperm release for fertilization is stimulated by movement of an egg into the oviduct (Quicke 1997). The venom gland may insert on to the venom reservoir which opens either into the distal part of the vagina or into the egg canal of the ovipositor via the dorsal ovipositor valve (Quicke 1997). *A. ervi*'s venom is injected into the body of aphids when ovipositing, and is an imported regulatory factor of the target host ovaries and responsible for pathological syndrome which results in developmental arrest (Digilio et al. 1998). The female reproductive system of *A. ervi* is shown in Figure 2.6.

The male reproductive system in Hymenoptera comprises paired testes, vas deferens, accessory glands and ejaculatory duct which leads to the aedeagus of the external genitalia (Sanger and King 1971; Quicke 1997) and contents of the accessory glands mix with sperm from the vas deferens or seminal vesicle before ejaculation (Quicke 1997). An example of the reproductive system in male Hymenoptera is shown in Figure 2.7.

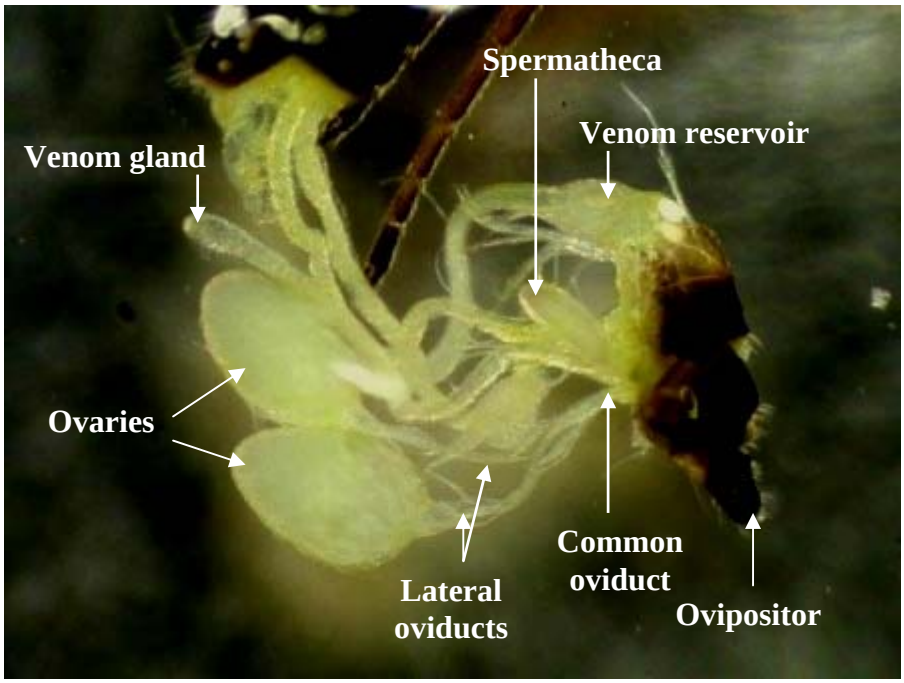

Figure 2.6 Female reproductive system in *A. ervi*. This picture was taken in the present study.

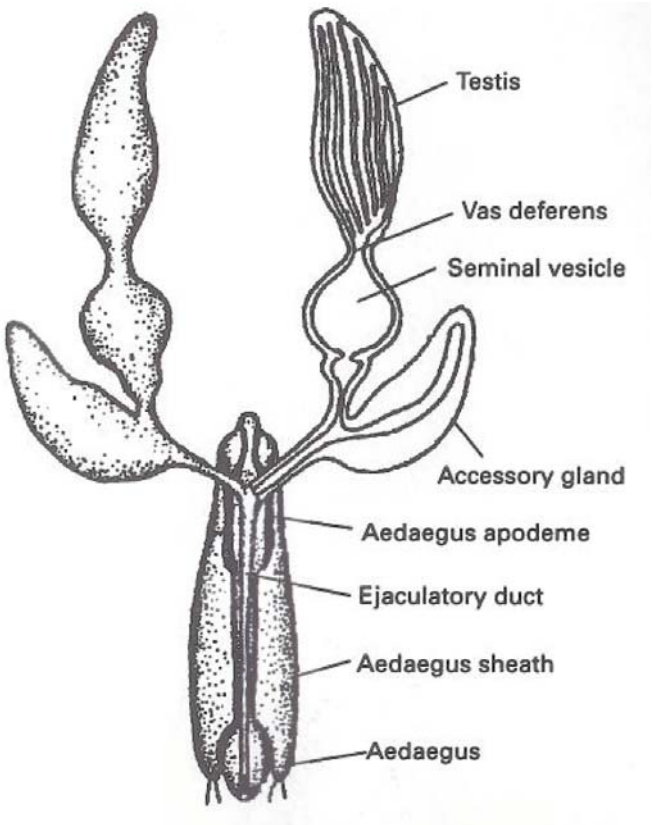

Figure 2.7 Male reproductive system in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) (from Sanger and King 1971).

2.7.2 Mating Behaviour

The primary mating behaviour has been described by Spencer (1926), Mackauer (1969), Battaglia et al. (2002) and McClure et al. (2007). Mating in *A. ervi* is mediated by sex pheromones produced by females. As found in another *Aphidius* species, *A. nigripes* (McNeil and Brodeur 1995), virgin females of *A. ervi* produce a long-distance sex pheromone that stimulates upwind flight and then produce a close-range pheromone that elicits courtship behaviour by males (McClure et al. 2007). After they detect the presence of females within a short distance of seven to ten millimeters (Spencer 1926), males perform courtship behaviour such as wing fanning, approaching and mounting (Battaglia et al. 2002; McClure et al. 2007). After a male mounts a female, he crawls forward and moves his antennae rapidly from side to side and touches those of the female. When the female accepts the male, the latter moves backward, curves his abdomen, and inserts his aedeagus into her genitalia. The female terminates the mating by walking away from the copulation site (McClure et al. 2007).

2.7.3 Sex Allocation and Sex Ratio

In Hymenoptera, unmated females lay unfertilised haploid eggs that develop into males, and mated females can lay either unfertilised eggs that develop into males or fertilised diploid ones that develop into females. Because of the haplo-diploid system of sex determination in Hymenoptera, sex ratio in parasitoid wasps can vary considerably. In the laboratory mass-rearing of parasitoids, male-biased sex ratios are undesirable because males produced in excess of those required for ensuring that all females are inseminated are useless for biological control. Thus the study of sex allocation in parasitoids is an important area of reproduction not only for those interested in evolutionary ecology, but also for practitioners of biological control (Luck 1990).

Natural selection acts upon oviposition behaviour to sex allocation that is adaptive in relation to different conditions. When only one female colonises a patch, she should produce only enough sons to fertilise all her daughters (Hamilton 1967). However, sex allocation of a searching female may be affected by the density of

female population. When several females colonise a patch, each female needs to produce sufficient sons to compete with the sons of other females for mating, the evolutionarily stable sex ratio for a parasitoid population should switch to be male-biased (Hamilton 1967). This is one reason why the mass-rearing of parasitoids often does not result in desired female-biased sex ratios.

Moreover, sex allocation is affected by host quality. Hosts provide the only resource for the development of immature stages of parasitoids, and they vary in the amount of resource they contain (e.g. vary in size). Charnov et al. (1981) and Charnov (1982) suggest that parasitoids lay fertilized eggs in larger hosts resulting in larger female progeny. This may be that females have more to gain from developing in large host than do males, namely that major components of female fitness, in particular fecundity, are more strongly correlated with their body size than major components of male fitness, in particular mating ability (van den Assem et al. 1989).

2.8 Factors Affecting Reproductive Fitness of Parasitoids

2.8.1 Host Quality

The hosts encountered by parasitoids will often vary in their quality as food for their larva development, in the time needed for their attack and parasitism, and possibly in the risks of their mortality during oviposition. Variation in host quality often depends on the age of the host. Most hosts are immature insects and grow in size as they age; larger hosts are often better food sources for parasitoid larvae (Charnov et al. 1981; Liu 1985), though they may be better defended, both physically and physiologically. According to the optimal diet model (Stephens and Krebs 1986), parasitoids should attack the best hosts and this is assumed to maximize the gain of a quantity or quality such as the number or size of offspring. Thus, a parasitoid may show a preference for a particular host type available in the environment (Godfray 1994). In koinobiont parasitoids, where the hosts continue developing for a certain period of time after parasitisation, host selection may not reflect current host quality but may be based on an assessment of future growth rates and resources available for the developing larvae (Rivero 2000). For some solitary species of parasitoids, hosts

selected for oviposition are determined by host size (Kouamé and Mackauer 1991). Many studies demonstrate that host size preference by parasitoids affect their progeny fitness, such as the body size (Liu 1985; Lampson et al. 1996), progeny sex ratio (Godfray 1994; Napoleon and King 1999; Ueno 1999), and egg load at emergence (Liu 1985; Visser 1994; Mills and Kuhlmann 2000).

2.8.2 Host Density

The impact of a parasitoid on its host population depends upon its ability to find and parasitise hosts and to increase the number of its offspring when needed (Mackauer 1983; Waage and Hassell 1982). This is largely determined by parasitoid's functional response to host density (density-dependent parasitisation) and density-dependent sex ratio (Hassell and Waage 1984).

The functional response is defined as the number of hosts parasitised per parasitoid per unit of time as a function of host density and is one of the essential ingredients in modeling any host interaction (Hassell 1986). It is important to identify the response form in population models, from which prescriptions for augmentative biological control can be developed (Mills and Lacan 2004). Three types of functional response of parasitoids are classified by Holling (1959): Type I, where there is a linear increase to a maximum in the number of hosts parasitised as host density increases; Type II, where the response increases non-linearly towards a maximum value; and Type III, where the response is sigmoid, again approaching an upper asymptote.

In analytical host-parasitoid models, changes in the density-dependent sex ratio influence the level of equilibrium populations as well as the stability of the host-parasitoid relationships (Hassell and Waage 1984), and in turn determine the success or failure of biological control (Waage and Hassell 1982). Both functional response and density-dependent sex ratio together affect the dynamic interaction between parasitoids and pests, and determine parasitoid population size of future generations.

2.8.3 Parasitoid Body Size

Parasitoid quality control is important to ensure the success of a biological control programme. One straightforward indicator of parasitoid quality is size, as there is a positive correction between body size or weight and one or more fitness variables for many parasitoids (Jervis and Copland 1996). Body size of insects has usually been considered to be a key determinant factor for many physiological and fitness characters (Thornhill and Alcock 1983; Honék 1993; Cloutier et al. 2000; Jiménez-Pérez and Wang 2004). For example, the reproductive fitness of females in terms of searching rate, longevity, and fecundity, and ability to parasitise hosts, is often positively correlated with their body size (van den Assem et al. 1989; Honék 1993; Godfray 1994; Visser 1994; Kazmer and Luck 1995; Cloutier et al. 2000; Sagarra et al. 2001; Arakawa et al 2004). Large females are able to regenerate eggs faster when required (Cloutier et al. 2000). Large males usually have greater ability to inseminate and compete for mates (van den Assem et al. 1989; Kazmer and Luck 1995) or have better genes and more sperm supply (van den Assem et al. 1989). Therefore, knowledge of these biological parameters is important for the implementation of an efficient mass-rearing system as it leads to better understanding of the progeny production and sex ratio. It should also enhance the efficiency of field releases by ensuring the quality of the released parasitoids. Moreover, the fitness consequences of size and its correlates, especially the supply of eggs or sperm and adult longevity, are important in population dynamics and essential to the understanding and modelling of life history evolution and behavioural decisions (Visser 1994; Cloutier et al. 2000).

2.8.4 Mating History of Parasitoids

In hymenopteran species, no evidence is found that females obtain a nutritional contribution from males during copulation (Madel et al. 1990; Godfray 1994; Fauvergue et al. 1998). Thus, mating affects parasitoid reproductive fitness mainly in terms of egg fertilization. In a review of mating behaviour among parasitoids, Gordh and Debach (1978) found that multiple mating by females occurred in only five out of thirty-four species, while multiple mating by males occurred in all

cases. Ridley (1993) has collected information from the literature on the female mating behaviour of ninety-nine species of parasitoid wasps, of which nearly 80% were reported to mate only once; furthermore, there was a strong tendency for gregarious females to mate several times and for solitary females to be monandrous. Therefore, in aphidiide species, mating history affecting reproductive fitness may only be relevant to males, and male mating history or age may affect reproductive fitness by the supply of sperm during copulation.

2.9 Sexual Selection

Sexual selection is one of the most active areas in studying behavioural ecology because it directly influences the reproductive fitness of insects. Individuals select their partners because potential mates vary in quality, quantity and availability. Sexual selection may occur through intrasexual selection where males or females compete for mates or through intersexual selection where females or males choose their mates with certain characteristics (Jennions and Petrie 1997; Panhuis et al. 2001).

For aphidiide species, females are monandrous and males are polygynous. Therefore, there is no issue about sperm competition. For females, premating mate choice is extremely important especially in species with very female-biased sex ratio; they should be very choosy theoretically and mate with ‘best’ possible mates. Age and body size may be the major characteristics that affect the sexual selection. It is expected that females should choose large and young males to obtain more sperm, and males should select large and young females to fertilize more and better eggs.

CHAPTER 3
GENERAL BIOLOGY OF *APHIDIUS ERVI*

3.1 Introduction

This chapter described the general methodology applied throughout this research, investigated the general biology of *A. ervi* in emergence, sex maturation and oviposition patterns of *A. evi* and then determined the effect of parasitism on host reproductive potential.

3.2 General Methodology

The materials, procedures, environmental conditions and definitions detailed in this section were used throughout the thesis.

3.2.1 Materials

Breeding colony: A breeding colony started from *A. ervi* emerged from blue-green lucerne aphid, collected on lucerne in Palmerston North, New Zealand in December 2002. Both blue-green lucerne aphid and pea aphid are hosts of *A. ervi*, and lucerne is the host plant of both species; however, because of the difficulty of growing lucerne in greenhouse or laboratory, pea aphid was used as host of *A. ervi* and broad bean, *Vicia faba* L. cv. Pride, was used as host plant of pea aphid. The *A. ervi* colony was reared on pea aphid for five generations before used in experiments.

Breeding cage: *A. ervi* and pea aphid were maintained separately in aluminium framed cages (64 cm in length × 45 cm in width × 40 cm in height) with fine metal screen (0.2 mm in length × 0.2 in mm in width) on the back and both sides and perspex on the top and front and aluminium alloy on the bottom (Figure 3.1).

Figure 3.1 Breeding cage used for rearing *A. ervi* and pea aphid.

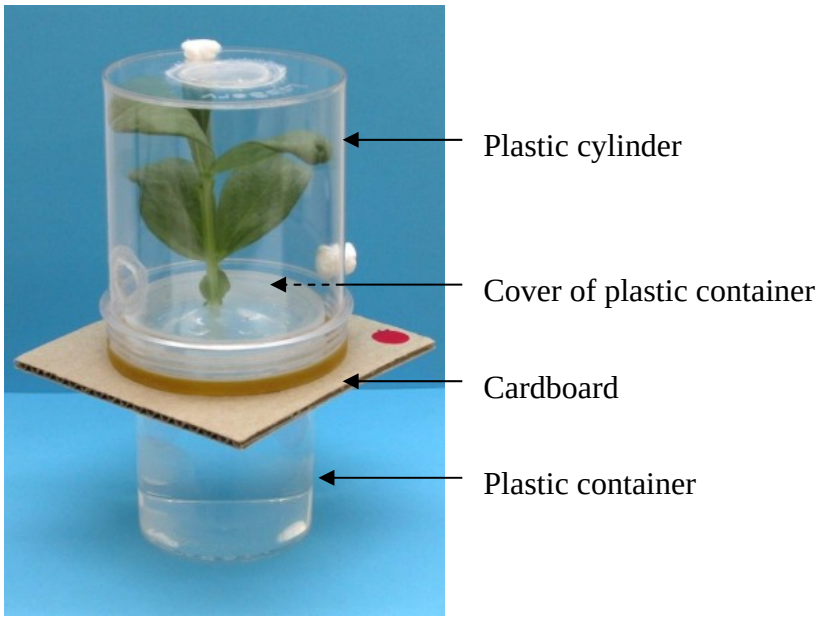

Figure 3.2 Experimental cylinder.

Experimental cylinders: All experiments were carried out in transparent plastic cylinders (8.5 cm in diameter × 10.5 cm in height) with gauze-covered holes, one in the top (5 cm in diameter) and two (2 cm in diameter) in opposite sides for ventilation (Figure 3.2). A plastic container (6.5 cm in diameter × 8.5 cm in height) was firstly inserted into a cardboard (10.5 cm in length × 10.5 cm in width) via hole (6.5 cm in diameter) cut in the centre before the container was filled with tap water and covered. A broad bean cutting was placed in the plastic container via a hole (0.6 cm in diameter) cut in the centre of a cover. The plastic cylinder was then placed on and sustained by

the cardboard (Figure 3.2). The broad bean cutting was replaced when necessary. Honey solution (10%) was supplied for parasitoids as food daily in a cotton wool wick (1 cm in length), inserted through a hole (0.6 cm in diameter) in the top of the cylinder (Figure 3.2).

Microscope: A stereomicroscope (Leica MZ12, German) equipped with a micrometer eyepiece was used for dissecting parasitised aphids and measuring body size of newly emerged adults. A compound microscopy (Olympus, GH, Japan) equipped with transmitted light and a micrometer eyepiece was employed for counting the number of eggs in newly emerged *A. ervi* females.

3.2.2 Procedures

Eggs laid: To determine the number of eggs laid by *A. ervi* in a parasitised aphid, aphids were dissected in a droplet 70% alcohol 4 d after parasitisation under the stereomicroscope and the number of larvae in each aphid was counted. The number of parasitoid larvae recorded from dissecting was assumed equal to the number of eggs laid (Bueno et al. 1993).

Egg load: To determine the egg load of *A. ervi* females in ovaries at emergence, females were killed by freezing at -20°C and dissected in a droplet of 70% alcohol on a slide under the stereomicroscope. One droplet of acid fuchsin was added to the alcohol for staining of the ovaries. After 3 ~ 5 min, the ovaries were covered with a slide cover and spread by gently pressing the slide cover. The number of eggs in the ovaries was counted and recorded under a compound microscope.

Emergence: *A. ervi* adults used for experiments emerged from mummies that were individually maintained in glass vials (1.5 cm in diameter, 5.0 cm in height). Mummies from each experiment were removed from bean plants and maintained in Petri dishes (5.5 cm in diameter × 1.3 cm in height) for emerging, and emerged adults were sexed.

Sex identification: The females could be distinguished from males by the sharp abdomen tip.

3.2.3 Environmental Conditions

The colonies were maintained and experiments were carried out in bioassay rooms at 20 ± 1°C and RH 60-70% with a photoperiod of 16:8 h L:D. Lighting was provided by high frequency broad-spectrum biolux tubes (Osram, Germany).

3.2.4 Definitions

Number of parasitism: number of aphids parasitised by female *A. ervi*.

Parasitism rate: percentage of aphids parasitised by female *A. ervi.*

Superparasitism: when two or more eggs were laid in an aphid.

Fecundity: the total number of eggs laid detected by dissecting.

Fertility rate: the proportion of female progeny.

Reproductive fitness: female parasitoids - fecundity, fertility, longevity, number and body size of progeny, and egg load of newly emerged female progeny; male parasitoids – longevity, number of mates inseminated and progeny fathered, and body size of progeny.

3.2.5 Statistical Analysis and Reported Values

All statistical analysis were set at $P < 0.05$ and carried out using SAS STAT (SAS Institute 2006). Unless stated otherwise, all reported values are means ± SE.

3.3 Emergence, Sexual Maturation and Oviposition of *A. ervi*

3.3.1 Introduction

Insect emergence events are usually rhythmic (Saunders 1982). In the parasitic hymenopterans, such rhythmicity is often synchronised with mating (Gordh and DeBach 1976; Nadel and Luck 1985) and oviposition (Armstrong et al. 1996; Couch et al. 1997) activities for an optimal reproductive fitness.

Daily activity patterns have been studied in detail in some parasitic hymenopterans (Vogt and Nechols 1991; Armstrong et al. 1996; Couch et al. 1997). Quicke (1997) suggested that for many parasitoid species, most oviposition occurs in the morning, for example, the squash bug egg parasitoid, *Gryon pennsylvanicum* (Ashmead) (Vogt and Nechols 1991). However, in some other species, such as the *Sitona* weevil parasitoid, *Microctonus aethiopoides* Loan, oviposition may occur during light and dark with the circadian oviposition activity corresponding with its host activity (i.e. feeding and oviposition) (Armstrong et al. 1996; Couch et al. 1997). Knowledge of parasitoids' emergence, mating and oviposition patterns is vital to an understanding of the ecology and evolution of their reproductive strategies, which in turn contributes to the development and implementation of biological control programmes.

Michaud and Mackauer (1994) reported that *A. ervi* could successfully oviposit in pea aphid in both photophase and scotophase, but the circadian emergence and oviposition patterns were still unknown prior to this study. To provide information for the development of mass-rearing and field releasing techniques and better understanding of biological control ecology of *A. ervi*, the circadian patterns of emergence and oviposition, and sexual maturation in *A. ervi* were investigated.

3.3.2 Materials and Methods

3.3.2.1 Experimental Parasitoids and Hosts

Parasitoids used for experiments emerged from mummies that were parasitised in the third instar (3 d old), and the third instar pea aphids were used as hosts in all experiments.

3.3.2.2 Emergence

To observe the 24-h emergence patterns of *A. ervi*, two bioassay rooms were set up. The photophase in one room was set from 0800-2400 hours (normal-light regime) and in the other room the photophase was between 1800-1000 hours (reverse-light regime). To obtain parasitised aphids, a mated female parasitoid (<12 h old) was introduced into a Petri dish (5.5 cm in diameter × 1.3 cm in height) containing 25 third instar aphids for a period of 5 h. Fifty-eight females were used for this study.

Fifty parasitised aphids were reared on the bean plant in a transparent plastic cylinder (Figure 3.2). Fifteen and nine cylinders were maintained in the normal- and reverse-light regimes, respectively. Parasitoid emergence was observed from 675 mummies in the photophase in the normal-light regime and from 378 mummies in the scotophase in the reverse-light regime. The emergence incidence was recorded hourly and emerged adults were sexed. Developmental time from eggs to adults of both sexes was also recorded.

3.3.2.3 Sexual Maturation

Because most matings occurred during the photophase (unpublished data), all experiments on sexual maturation were carried out during the photophase. To detect the sexual maturation period of adult parasitoids, two experiments were set up, each with 7 treatments. In the first experiment 12-h-old virgin females were paired with 0 (newly emerged), 2, 4, 6, 8, 10 and 12-h-old virgin males, and in the second

experiment 12-h-old virgin males were paired with 0 (newly emerged), 2, 4, 6, 8, 10 and 12-h-old virgin females.

A virgin male was paired with a virgin female in a clear glass vial (1.5 cm in diameter, 5.0 cm in height) with a 0.5 cm mesh covered hole in lids. Twenty pairs were established for each treatment. The sexual behaviour of both sexes in a 30-minute period was observed and the number of courtship displays (male wing fanning) and matings (insemination) was recorded.

3.3.2.4 Oviposition

To determine the oviposition patterns of *A. ervi* on a 24 h basis, an experiment was carried out with 20 *A. ervi* females in the normal-light regime and another 20 *A. ervi* females in the reverse-light regime. Each mated female (<12 h old) was introduced into a Petri dish containing 20 healthy aphids on a broad bean leaf and allowed to stay for 2 h (first oviposition bout). She was then transferred to another Petri dish with 20 healthy aphids and allowed to stay for 2 h (second oviposition bout). This was repeated until 8 and 4 oviposition bouts were completed in the photophase and scotophase, respectively.

All aphids from each oviposition bout (in a single Petri dish) were transferred to and reared in a plastic cylinder (Figure 3.2). Because females might lay more than one egg in a host aphids, to determine the number of eggs laid, 10 aphids from each oviposition bout were dissected 4 days after parasitisation and the number of larvae in each aphid was counted under the stereomicroscope. The remaining parasitised aphids were reared until mummification. The number of parasitisms in each oviposition bout was recorded as the number of aphids parasitised.

3.3.2.5 Statistical Analysis

A chi-square test was used to determine the difference in emergence incidence between the photophase and scotophase. The rejection level was when $\chi^2 > \chi^2_{1,0.05} =$

3.84. The Marascuilo procedure of the nonparametric analysis (Daniel 1990) was used to assess the sexual maturation. The rejection level was when $U_0^{'} > \chi^2_{6,0.05} = 12.59$. All other data were normally distributed and analysed using ANOVA followed by a Tukey's studentised range test. Data of proportion of female offspring were subject to arcsine transformation before ANOVA, but untransformed means are presented.

3.3.3 Results

3.3.3.1 Emergence

The developmental time from oviposition to emergence was significantly shorter for males (mean ± SE, 13.94 ± 0.03 d) than that for females (14.31 ± 0.03 d) (ANOVA: F = 99.60; df = 5,541; P < 0.0001).

The hourly emergence rate (mean ± SE) was significantly higher in the photophase (5.99 ± 0.94%) than in the scotophase (0.74 ± 0.32%) (ANOVA: F = 14.94; df = 1,22; P < 0.001). About 95% of parasitoids emerged in the photophase in both light regimes. On a 24-h basis, the male emergence peaked 2 h after light-on and the female emergence peaked between 3-6 h after light-on (Figure 3.3).

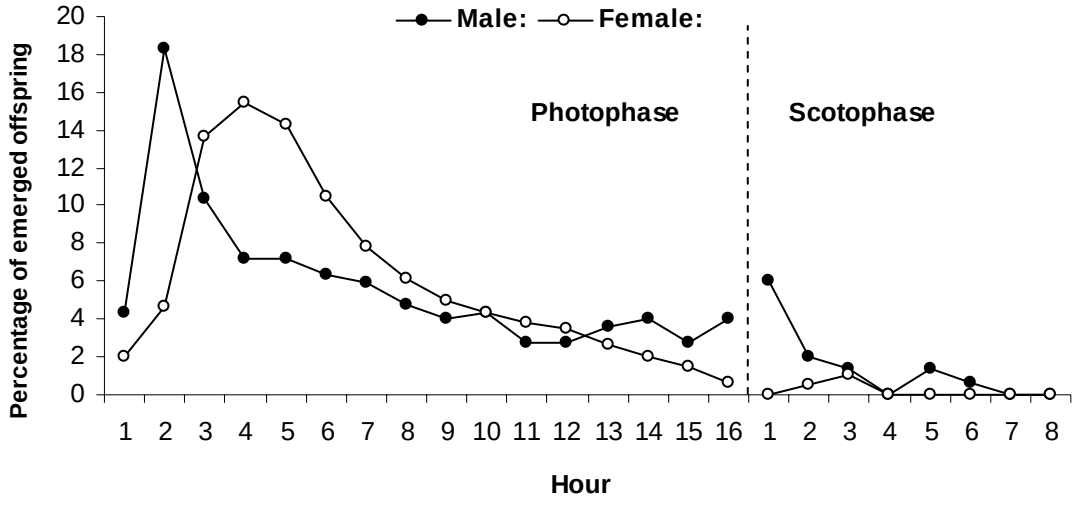

Figure 3.3 The percentage of female or male *A. ervi* offspring emerging throughout photophase or scotophase.

3.3.3.2 Sexual Maturation

Newly emerged males were able to perform their courtship display to 12-h-old females but failed to mate until they were 4-h-old (Figure 3.4). Both courtship and mating increased with age (h); however, 10- and 12-h-old males were significantly more likely to court females than ≤ 4-h-old males ($U_0^{'}$ = 105.22 > $\chi^2_{6,0.05}$; df = 6; P < 0.0001); mating success of 12-h-old males was also significantly higher than that of ≤ 4-h-old ($U_0^{'}$ = 80.01 > $\chi^2_{6,0.05}$; df = 6; P < 0.0001) (Figure 3.4).

Newly emerged females were able to entice the courtship display by 12-h-old males and successfully mate (Figure 3.5). Twelve-h-old females were significantly more likely to respond to males' courtship display than ≤ 4-h-old females ($U_0^{'}$ = 32.35 > $\chi^2_{6,0.05}$, df = 6, P < 0.0001); mating success of 12-h-old females was significantly higher than that of ≤ 2-h-old females ($U_0^{'}$ = 14.97 > $\chi^2_{6,0.05}$; df = 6; P < 0.0001).

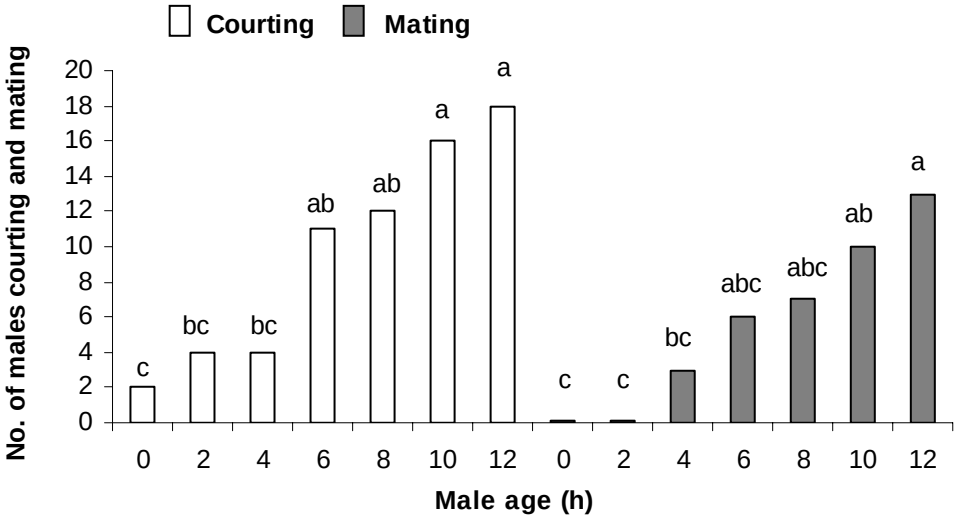

Figure 3.4 The number of *A. ervi* males courting or mating at 0, 2, 4, 6, 8, 10 or 12 h after emergence. Within the same category (Courting or Mating) columns with the same letters are not significantly different (P > 0.05).

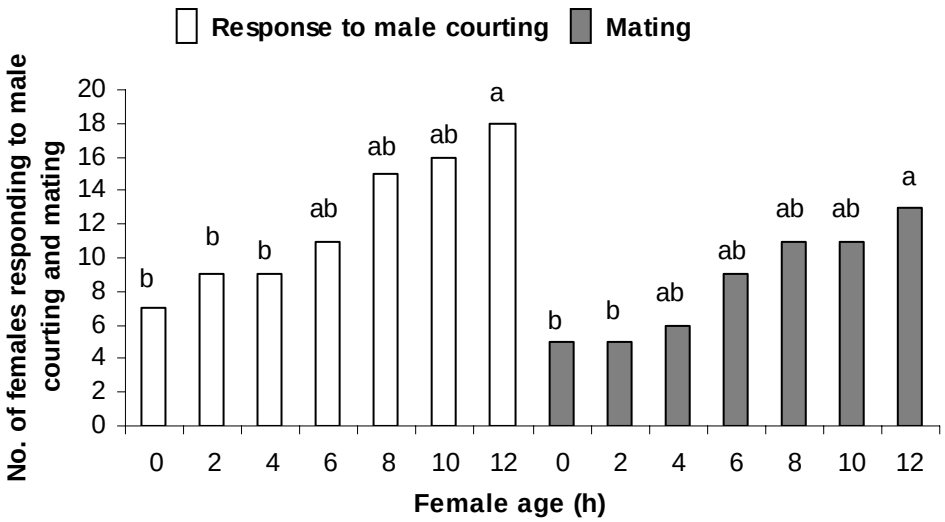

Figure 3.5 The number of *A. ervi* females responding to male courting or mating at 0, 2, 4, 6, 8, 10 or 12 h after emergence. Within the same category (Response to male courting or Mating) columns with the same letters are not significantly different ($P > 0.05$).

3.3.3.3 Oviposition

Females oviposited in both photophase and scotophase. In the photophase, the mean (± SE) number of eggs laid (11.73 ± 2.73) and parasitism (8.6 ± 1.03) per oviposition bout were significantly greater than that in the scotophase (4.18 ± 0.88 eggs and 3.43 ± 0.69 parasitism, respectively) (ANOVA: $F = 7.87$ and 24.19 for number of eggs laid and parasitism, respectively; $df = 1,22$; $P < 0.01$). In the photophase, the numbers of eggs laid and parasitism were significantly higher in the first oviposition bout (ANOVA: $F = 40.95$ and 12.64 for number of eggs laid and parasitism, respectively; $df = 7,72$; $P < 0.0001$) (Figure 3.6). However, no significant difference in number of eggs laid or parasitism between oviposition bouts was detected in the scotophase ($F = 2.39$ and 2.77 for number of eggs laid and parasitism, respectively; $df = 3,36$; $P > 0.05$) (Figure 3.6).

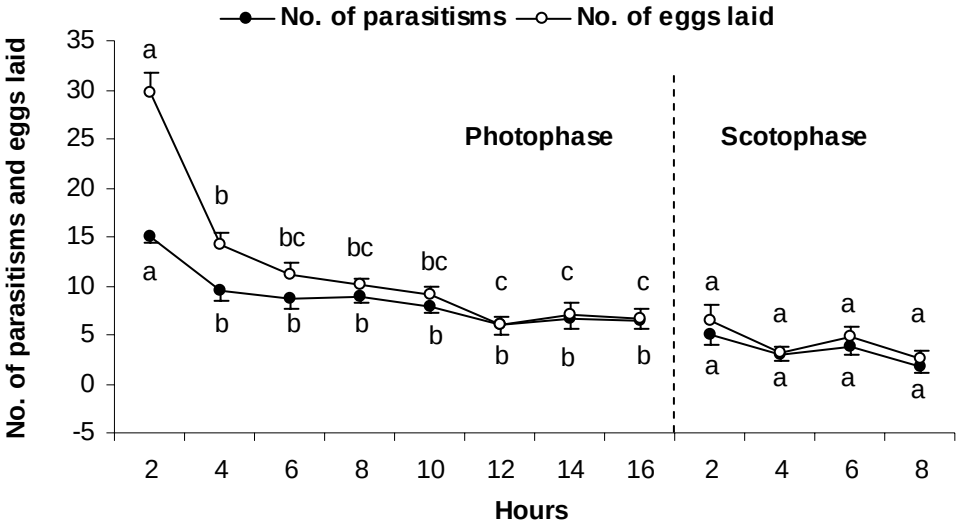

Figure 3.6 The number of parasitisms and eggs laid by *A. ervi* females throughout photophase and scotophase. Within the same category (Photophase or Scotophase), means (± SE) followed by the same letters within each line are not significantly different (P > 0.05).

3.3.4 Discussion

Among parasitoid insects, adult emergence restricted to certain times of the day is known in several species, including *Trichogramma minutum* Riley (Corrigan et al. 1995), *Telenomus busseolae* Gahan (Fantinou et al. 1998) and *Encarsia formosa* Gahan (van Lenteren et al. 1992). The results of the present study demonstrate that *A. ervi* males and females have an emergence peak during the first few hours of the photophase (Figure 3.3). This suggests that the onset of light may act as a signal that synchronises the rhythmic function of *A. ervi* adult emergence.

This study has shown that *A. ervi* is a protandrous species with males emerging ca 9 h earlier than females. Singer (1982) argued that selection for protandry can only occur when generations are discrete. *A. ervi* does not appear to match that hypothesis because generations usually overlap in the field and females are readily available. However, *A. ervi* females mate only once during their lifespan, limiting the chance for males to encounter virgin females. Quicke (1997) suggested that the protandry is common in many parasitoids because a late-emerging male is

likely to encounter already mated females and he is genetically doomed, as he cannot get any matings.

It has been reported that for some parasitic hymenopterans, a period of sexual maturation is necessary, for example, the bean weevil parasitoid, *Chryseida bennetti* Burks (Perez-Lachaud and Campan 1994) and the tortrix moth parasitoid, *Ascogaster reticulatus* Watanabe (Kainoh 1986). In the present study, newly emerged *A. ervi* females were able to mate with males but males need at least 4 h to become sexually mature. Therefore, the early emergence of *A. ervi* males may be a selective strategy for higher reproductive fitness. For example, early emerged males have a better chance to encounter virgin females (Nadel and Luck 1985, 1992), inseminate a greater number of the females they encounter (Waage and Ng 1984), and reduce the risk of females' death before oviposition (Fagerström and Wiklund 1982). Moreover, since virgin females start to lay eggs within 30 min after emergence (unpublished data), early emerged males are able to mate with females before their oviposition.

Diel periodicities of insect activity are often determined by a combination of endogenous and exogenous rhythms (Beck 1980). The decreasing oviposition and parasitism of *A. ervi* during the photophase may be the result of a decreasing load of mature eggs, as reported in the sycamore aphid parasitoid, *Monoctonus pseudoplatani* (Marshall) (Collins and Dixon 1986). Furthermore, visual cues play an important role in host finding and attacking by *A. ervi* (Michaud and Mackauer 1994). In this study, *A. ervi* females laid fewer eggs and attacked fewer aphids during the scotophase even though they had sufficient eggs. This suggests that the oviposition pattern of *A. ervi* is determined by an exogenous factor, the light regime.

In conclusion, light appears to entrain emergence and oviposition rhythms of *A. ervi*. Emergence of the adults in the morning probably coincides with more favourable conditions, such as the light, in which parasitoids may increase the chance for host habitat location, host searching and oviposition (Battaglia et al. 1995, 2000). The findings of this study have implications for laboratory mass rearing and field release of *A. ervi*. For example, newly emerged parasitoids should be stored for 12 h for copulation to occur before release, and the parasitoids should be released the following morning to achieve the higher reproductive output.

3.4 Effect of Parasitism by *A. ervi* on Development and Reproduction of Pea Aphid

3.4.1 Introduction

Many studies have reported that hymenopteran endoparasitoids can reduce the reproductive potential of aphids (Campbell and Mackauer 1975; Liu and Hughes 1984; Mackauer and Kambhampati 1984; Sequeira and Mackauer 1988; Tang and Yokomi 1996; He et al. 2003; Lin and Ives 2003). The majority of these studies indicate that aphids parasitised in their early instars die before reproduction but those parasitised at later stages of development may reach the adult stage and produce a limited number of progeny before mummification. Most aphid populations consist of multiple stages of development. Therefore, knowledge of the effect of parasitism on development, reproduction and population growth of aphids of different ages is critical to the success of biological control (Tsai and Wang 2002; Zhang and Hassan 2003).

Several authors (Pennacchio et al. 1995; Digilio et al. 1998; Rahbé et al. 2002) have studied biochemical changes in pea aphid caused by *A. ervi*. However, little is known about how parasitism by *A. ervi* affects the population of the pea aphid, making it difficult to evaluate the biological control efficiency of this parasitoid. This section investigated the effect of parasitism by *A. ervi* on the development, reproduction and population growth of pea aphid at different stages of development.

3.4.2 Materials and Methods

3.4.2.1 Development and Reproduction

The effect of parasitism by *A. ervi* on the growth and reproduction of pea aphids at different stages of development (1~10 d old = 1st instar to adult, Table 3.1) was investigated. To obtain parasitised aphids of the same age class, a mated female was released into a Petri dish (8.5 cm in diameter × 1.3 cm in height) containing 10 aphids of the same age. Aphids that received a single oviposition sting from the female parasitoid were removed and replaced with unparasitised individuals of the same age until 15 parasitised aphids were collected. Parasitised aphids were placed

individually onto a bean plant cutting which was held in a transparent plastic cylinder (Figure 3.2). There were 10 treatments (age classes) and 15 replicates (number of parasitised aphids) for each treatment in this study. Survival, development and reproduction of parasitised aphids were monitored at 24 h intervals. Any offspring from these aphids were counted and removed. In addition, the survival and reproductive periods of aphids were also recorded. Fifteen unparasitised aphids (start at 1 day old) reared individually in cylinders served as controls and their survival and reproduction were monitored as mentioned above.

3.4.2.2 Population Growth

The daily survival rate and reproduction of aphids were compiled into a life table to assess the population growth according to the method of Jervis et al. (2005). The intrinsic rate of increase (female/female/day; r_m) was estimated by solving the Lotka-Euler equation ($\sum e^{-r_m x} l_x m_x = 1$). Other calculations included the net reproductive rate (females/females/generation; $R_0 = \sum l_x m_x$), mean generation time (days; $T = \log_e(R_0)/r_m$) and doubling time (days; $DT = \log_e(2)/r_m$). In these calculations x is the pivotal age, l_x is the proportion of the females surviving to age x, and m_x is the number of offspring produced per female at age x. For each treatment and the control, a jackknife method (Caswell 2001) was used to estimate the means and standard errors of the above life table parameters by partitioning the 15 parasitised aphids into 5 groups of 3 individuals.

3.4.2.3 Statistical Analysis

Linear regression analysis was used to identify the relationships between aphid age at the point of parasitism, reproductive period and the number of progeny produced. A logistic regression was applied to analyse the relationships between aphid age (x) at the point of parasitism and r_m [i.e. $r_m = c/(1+\exp(a+b*\ln(x)))$], where the constant c represents the maximum possible value of r_m; and a and b are constants to fit the curve. All other data were normally distributed and analysed using ANOVA followed by a Tukey's studentised range test.

3.4.3 Results

3.4.3.1 Development and Survival

Aphids parasitised at 1 and 2 d old (1st and 2nd instar) became mummified in the 4th instar. However, aphids parasitised at 3 to 6 d old (3rd and 4th instar) continued development to the adult stage. For all ages, parasitised aphids died about 7 days after being parasitised and their longevity was significantly shorter than unparasitised aphids (ANOVA: $F = 419.15$; $df = 10,154$; $P < 0.0001$) (Table 3.1).

Table 3.1 Effect of parasitism by *A. ervi* on survival (days), reproductive period (days) and number of progeny of pea aphid.

Aphid age at parasitisation (days)	Longevity (days)	Reproductive period (days)	No. of progeny
1 (1st instar)	8.13 ± 0.09 j	---	---
2 (2nd instar)	9.13 ± 0.09 ij	---	---
3 (3rd instar)	10.20 ± 0.11 hi	---	---
4 (3rd instar)	11.13 ± 0.09 gh	2.07 ± 0.15 g	4.93 ± 0.43 g
5 (4th instar)	12.07 ± 0.07 fg	2.33 ± 0.16 fg	10.07 ± 0.90 g
6 (4th instar)	13.13 ± 0.09 ef	3.13 ± 0.13 f	19.40 ± 1.13 f
7 (adult)	14.07 ± 0.07 de	4.20 ± 0.18 e	30.47 ± 1.48 e
8 (adult)	15.13 ± 0.09 cd	5.20 ± 0.11 d	39.07 ± 1.49 d
9 (adult)	16.20 ± 0.11 c	6.33 ± 0.19 c	52.33 ± 0.88 c
10 (adult)	17.47 ± 0.17 b	7.60 ± 0.19 b	62.60 ± 1.92 b
Control	27.87 ± 0.82 a	15.60 ± 0.38 a	111.20 ± 3.17 a

Means (± SE) followed by the same letters in columns are not significantly different ($P > 0.05$).

3.4.3.2 Reproduction

Only those aphids parasitised when ≥ 4 d old (late 3rd instar) produced progeny. Most of these aphids started reproduction from 8 d old. Parasitised aphids had a significantly shorter reproductive period and produced significantly fewer progeny

than healthy aphids (ANOVA: F = 483.15 and 452.23 for reproductive period and number of progeny, respectively; df = 7,112; P < 0.0001) (Table 3.1). For the parasitised aphids, the reproductive period and the number of progeny produced increased significantly with the age at parasitisation (Linear regression: F = 845.95 and 155.17 for reproductive period and number of progeny, respectively; df = 1,103; P < 0.0001) (Figures 3.7 and 3.8, respectively).

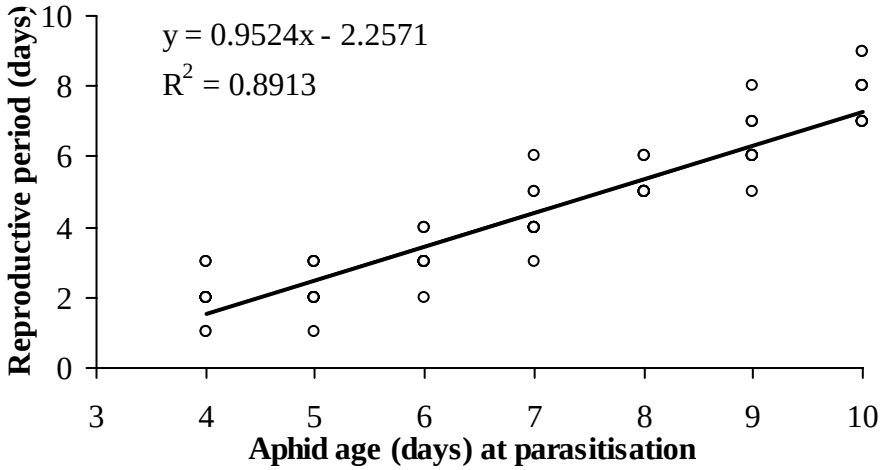

Figure 3.7 Relationship between host age at parasitisation and reproductive period.

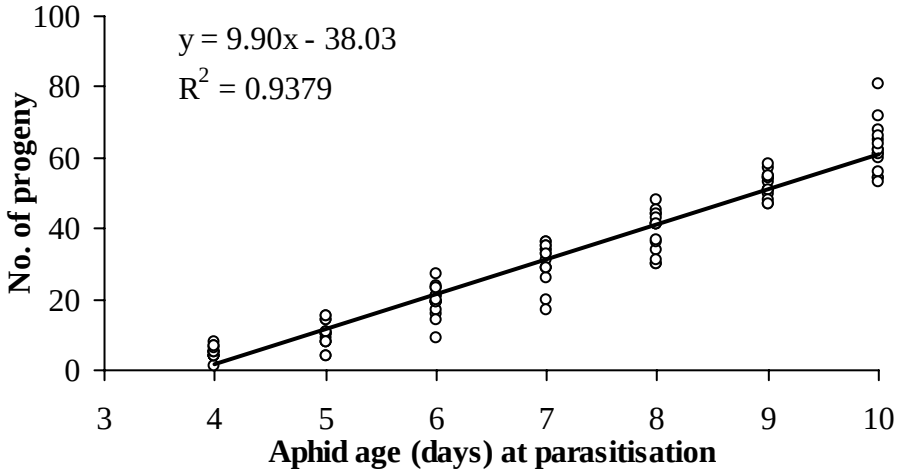

Figure 3.8 Relationship between host age at parasitisation and reproduction of progeny.

3.4.3.3 Population Growth

Parasitism by *A. ervi* significantly affected the population growth of pea aphids, reflected in changes in the intrinsic rate of increase (r_m), net reproductive rate (R_0), generation time (T), and doubling time (DT) (Table 3.2). Aphids parasitised as immature stages (≤ 6 d old) had a significantly lower r_m and R_0, shorter T, and longer DT than those parasitised as adults and healthy aphids (ANOVA: F = 206.33, 520.21, 202.18 and 46.63 for r_m, R_0, T and DT, respectively; df = 7,32; P < 0.0001). The r_m increased rapidly with the increase of aphids' age when they were parasitised and reached a plateau at 9 and 10 d old (Logistic regression: F = 688.84; df = 2,33; P < 0.0001) (Figure 3.9).

Table 3.2 Effect of parasitism by *A. ervi* on life table parameters of pea aphid.

Aphid age at parasitization (days)	r_m	R_0	T	DT
4 (3rd instar)	0.1799 ± 0.0083 e	4.93 ± 0.39 g	8.71 ± 0.09 d	3.95 ± 0.21 a
5 (4th instar)	0.2573 ± 0.0050 d	9.13 ± 1.17 g	8.86 ± 0.07 d	2.94 ± 0.35 b
6 (4th instar)	0.3398 ± 0.0075 c	19.40 ± 1.18 f	8.69 ± 0.02 d	2.05 ± 0.05 c
7 (adult)	0.3705 ± 0.0056 b	30.47 ± 0.85 e	9.21 ± 0.07 c	1.87 ± 0.03 d
8 (adult)	0.3924 ± 0.0072 ab	39.07 ± 1.53 d	9.33 ± 0.07 c	1.77 ± 0.03 d
9 (adult)	0.3972 ± 0.0031 a	52.07 ± 0.67 c	9.95 ± 0.05 b	1.74 ± 0.01 d
10 (adult)	0.3979 ± 0.0033 a	62.60 ± 2.52 b	10.29 ± 0.06 b	1.73 ± 0.01 d
Control	0.4018 ± 0.0023 a	113.00 ± 2.51 a	11.89 ± 0.13 a	1.74 ± 0.02 d

Means (± SE) followed by the same letters in columns are not significantly different (P > 0.05).

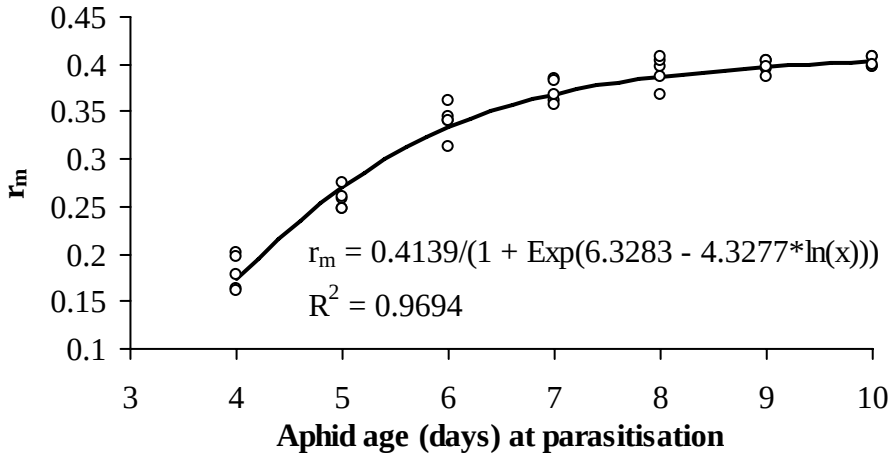

Figure 3.9 Relationship between host age at parasitisation and r_m.

3.4.4 Discussion

Results of this study show that, in comparison with the unparasitised pea aphid controls, parasitism by *A. ervi* significantly reduced pea aphids' survival period, reproductive potential and population growth. However, when compared to *Praon pequodorum* Viereck (Sequeira and Mackauer 1988), another parasitoid of pea aphid, *A. ervi* has greater potential to suppress aphid population because those parasitised by *P. pequodorum* produce 20 to 30 more progeny than those parasitised by *A. ervi*. The effect of parasitism by *A. ervi* on pea aphid reproduction is similar to that by *A. eadyi* (He et al. 2003).

The efficiency of parasitoids in the control of aphids depends on their ability to suppress aphids' population growth. Aphids parasitised by *A. ervi* continued to feed and grow until they were killed by developing parasitoid larvae. Because the time from being parasitised to mummification is about 7 days for all parasitised aphids, their survival and reproduction depend on the time when they are parasitised. Aphids ≤ 3 d old at parasitisation died at the fourth instar or early adult stage. In contrast, those parasitised when ≥ 4 d old were able to reach the adult stage and reproduce. This may be because the aphid embryos in the later instar and adult stages have developed a resistant cuticle by the time of oviposition by the parasitoid (Polaszek 1986) and have thus escaped consumption by the developing parasitoid larvae.

Results also indicate that development, survival, reproduction and population growth of parasitised aphids vary with their age at the time of parasitism. It appears that attacking young hosts will be more effective in suppressing aphid populations than attacking the adult aphids. According to Schowalter (2000), early in the season, growing populations have a high proportion of aphids in young age classes. Thus, the application of *A. ervi* early in the season could significantly suppress populations of pea aphid in the field, inhibiting its dispersal and population build-up later in the season.

CHAPTER 4
FACTORS AFFECTING REPRODUCTIVE FITNESS OF *APHIDIUS ERVI*

4.1 Introduction

The effectiveness of potential biological control agents greatly depends on their fecundity, longevity and sex allocation strategy which are affected by host quality (i.e. age or size) and density, and parasitoid body size and age of both sexes (Jervis et al. 2005). Understanding how parasitoids optimise their reproductive output in response to these factors can improve pest control efficacy in biological control programmes. Thus, this chapter investigated how these factors affect the reproductive fitness of *A. ervi*.

4.2 Host Age Preference and its Effect on Reproductive Fitness of *A. ervi*

4.2.1 Introduction

Many studies have demonstrated that parasitism can influence development, fecundity, and population growth of the host aphids (Tsai and Wang 2002; He et al. 2003; Lin and Ives 2003). Generally, aphids parasitised in early instars are mummified before reaching reproductive maturity, whereas those parasitised in late instars are able to reach adult stage and produce progeny. Therefore, parasitoids' impact on the aphid population growth largely depends on the pattern of the host age selected for parasitisation (Hågvar and Hofsvang 1991; Tsai and Wang 2002) and host age preference is critical to the success of biological control of aphids (Tsai and Wang 2002).

Foraging parasitoids usually encounter hosts of different ages or sizes and have opportunities to select the most suitable hosts to maximize their reproductive fitness. For some solitary species of parasitoids, hosts selected for oviposition are determined by host size (Kouamé and Mackauer 1991) because large hosts contain

more resources for parasitoid progeny development than small hosts (Charnov et al. 1981; Liu 1985). Many studies suggest that host size preference by parasitoids affects progeny fitness of parasitoids, such as the body size (Liu 1985; Lampson et al. 1996) and egg load at emergence (Liu 1985; Mills and Kuhlmann 2000). Host age may also affect sex allocation of parasitoids (Godfray 1994). According to Charnov et al. (1981) and Charnov (1982), parasitoids can make efficient use of the size variation in the hosts encountered by allocating fertilized diploid eggs to large hosts and unfertilized eggs to small ones.

Previous studies have demonstrated that the impact of *A. ervi* on population growth of the pea aphid largely depends on the host age at parasitism (Section 3.4), and that body size of pea aphid is positively correlated with its age (Sequeira and Mackauer 1992). However, so far little is known about the host age preference by *A. ervi*. Knowledge of host preference would lead to a better understanding of the population dynamics of the host and parasitoid (Nechols and Kikuchi 1985). Therefore, to provide useful information for the development of biology control strategies, this section investigated the host age preference by *A. ervi* and its effect on the reproductive fitness and sex allocation.

4.2.2 Materials and Methods

4.2.2.1 Experimental Insects

Parasitoid adults used for the experiments emerged from pea aphid mummies parasitised at the third instar (3 d old).

4.2.2.2 Preference Behaviour

To observe the host age preference behaviour, 30 mated females (< 12 h old) were individually released into a closed Petri dish (5.5 cm in diameter × 1.3 cm in height) containing seven healthy aphids of different age from 1 to 7 d old. The oviposition behaviour was observed for 10 minutes and recorded using Panasonic SVHS Camcorder (MS-4) (Panasonic, Japan) (see Section 5.2.1). Behavioural

interactions between hosts and parasitoids were defined as following (Gerling et al 1990; Chau and Mackauer 2001):

(a) Encounter – a female met an aphid;
(b) Non-response – a female walked away from an aphid after encounter;
(c) Non-response rate – percentage of non-response per encounter;
(d) Attack attempt – a female exposed her ovipositor and tried to attack an aphid;
(e) Sting – a female inserted her ovipositor into an aphid regardless of successful oviposition or not;
(f) Sting rate – percentage of sting per attack attempt;
(g) Oviposition – egg(s) laid into an aphid detected by dissecting 4 days later;
(h) Oviposition rate – percentage of laying egg(s) per sting;
(i) Escape – an aphid walked away to avoid being attacked by a female;
(j) Kick – an aphid rised its legs and swayed its abdomen abruptly to prevent from being attacked by a female;
(k) Searching time – the period of a female walking and searching for hosts;
(l) Resting time – the period of a female staying for more than 5 seconds to clean her antennae and legs after performing attack(s);
(m) Handling time – the period between encounter and attack by a female regardless of successful oviposition or not.

Aphids from each dish were separated soon after observation and individually placed on a bean plant cutting. These aphids were dissected four days after oviposition to detect the number of eggs laid.

4.2.2.3 Effect of Age Preference on Reproductive Fitness

A positive relationship was detected between aphids' age (1 to 7 d old) and their body size (i.e. body weight, mg) (RA: body weight = - 0.0976 + 0.2917age, R^2 = 0.7365, $F_{1,58}$ = 162.09, $p < 0.0001$). To determine host age preference by *A. ervi* in relation to host size and its effect on *A. ervi* reproduction, mated females were supplied with host aphids of seven different ages from 1 to 7 d old. Ten females (replicates) were tested in this experiment. For each replicate, one mated female

parasitoid (< 12 h old) was introduced into an above-mentioned plastic cylinder (Figure 3.2) with 105 healthy aphids (15 aphids of each age class) feeding on a bean plant cutting. The female was allowed to stay in the cylinder for 24 h, and then moved to another cylinder with 105 healthy aphids of the same age structure, etc. until she died. After the removal of the female parasitoid, aphids of different age classes were separated and transferred to an uninfested bean plant in a cylinder.

To estimate the number of eggs laid, five aphids of each age class were randomly selected from each cylinder 4 days after the removal of the female parasitoid, and dissected in 70% alcohol under a stereomicroscope. The number of eggs laid in an aphid was recorded (Bueno et al. 1993). The remaining aphids were reared until mummification. The emerged progeny were counted and sexed.

To determine the effect of host age (preference) on reproductive fitness of *A. ervi*, the head width (a measure of body size) of 30 newly emerged progeny of each sex randomly selected from each host age class was measured using the above stereomicroscope. The egg load of parasitoid females at emergence was determined by dissecting 30 newly emerged females randomly selected from each host age class. They were dissected in 70% alcohol on a slide under the above stereomicroscope. One drop of acid fuchsin was added to the alcohol and allowed to stand for 3 to 5 min for staining. The number of eggs in the ovaries was counted under a compound microscope.

4.2.2.4 Statistical Analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Data of egg load were not normally distributed even after transformation and thus analysed using the nonparametric Kruskal-Wallis test followed by Dunn’s procedure for multiple comparisons (Zar 1999). Other data were normally distributed and analysed using ANOVA followed by a Tukey's studentized range test. Data of proportion of female offspring were arcsine transformed prior to analysis. The Paired t-test was applied to analyse the relationship between the number of encounters and

attack attempts. The relationship between body size and egg load of newly emerged females was analysed using regression analysis (RA).

4.2.3 Results

4.2.3.1 Preference Behaviour

Analysed data of host age preference and oviposition behaviour are summarised in Table 4.1. In most cases, searching females switched from random walking to attack position when the host was within a range of 1 cm. Females usually attacked hosts without antennal examination. They were significantly more likely to encounter and attack 5- to 7-d-old aphids than 1- and 2-d-old aphids (Kruskal-Wallis test: H = 49.88 and 58.06 > $\chi^2_{6,0.05}$ = 12.59, for encounter and attack attempt, respectively; df = 6,203; P < 0.0001) (Table 4.1).

Encounter did not always elicit attack, the mean number of attack attempts (3.71 ± 0.72) was significantly lower than that of encounters (5.47 ± 0.95) (t-test for paired two samples: t = 7.12; df = 6; P < 0.001). A parasitoid might not respond to a host and the host might escape from attacking by walking away. The number of non-response was not significantly different between hosts of different age (Kruskal-Wallis test: H = 1.82 < $\chi^2_{6,0.05}$; df = 6,203; P > 0.05), but the probability of parasitoids that did not respond a 1-d-old aphid upon encounter was significantly greater than 6- and 7-d-old aphids (Kruskal-Wallis test: H = 18.59 > $\chi^2_{6,0.05}$; df = 6,189; P < 0.01). Six- and 7-d-old aphids were significantly more likely than 1- to 4-d-old aphids to escape from parasitoids' attack (Kruskal-Wallis test: H = 54.67 > $\chi^2_{6,0.05}$; df = 6,203; P < 0.0001) (Table 4.1).

Older aphids had a greater ability to defend themselves by kicking during attack by a parasitoid (Kruskal-Wallis test: H = 89.55 > $\chi^2_{6,0.05}$; df = 6,181; P < 0.0001). Parasitoids stung significantly more older aphids (5 to 7 d old) than younger ones (1 and 2 d old) (Kruskal-Wallis test: H = 24.35 > $\chi^2_{6,0.05}$; df = 6,203; P < 0.0001); however, the sting rate was significantly higher in younger aphids (1 to 4 d old) than

in older ones (6 and 7 d old) (Kruskal-Wallis test: H = 56.18 > $\chi^2_{6,0.05}$; df = 6,178; P < 0.0001). The fact that the number of stings was greater that the number of eggs laid indicated that a female might deposit no egg in a host when stung, and the oviposition rate was significantly higher in 1- and 2-d-old aphids than in 7-d-old aphids (Kruskal-Wallis test: H = 18.95 > $\chi^2_{6,0.05}$; df = 6,186; P < 0.0001). However, among hosts of different ages, the number of eggs laid was significantly higher in 4- and 5-d-old aphids (Kruskal-Wallis test: H = 25.15 > $\chi^2_{6,0.05}$; df = 6,203; P < 0.0001) (Table 4.1).

Parasitoids allocated significantly longer time in host searching (Mean ± SE: 6.19 ± 0.35 mins) than in resting (3.01 ± 0.37) and the latter was significantly longer than handling time (0.80 ± 0.07) (Kruskal-Wallis test: H = 58.00 > $\chi^2_{2,0.05}$; df = 2,87; P < 0.0001). Parasitoids had significant longer handling time when attacking the 5- to 7-d-old aphids (Kruskal-Wallis test: H = 85.67 > $\chi^2_{6,0.05}$; df = 6,203; P < 0.0001) (Table 4.1).

Table 4.1 Oviposition behaviour of *A. ervi* females in response to host ages.

	1 d old	2 d old	3 d old	4 d old	5 d old	6 d old	7 d old
No. of encounters	2.83 ± 0.37 c	2.60 ± 0.39 c	3.76 ± 0.38 bc	3.83 ± 0.41 bc	5.50 ± 0.61 ab	6.03 ± 0.59 ab	9.03 ± 1.23 a
No. of attack attempts	1.30 ± 0.22 c	1.47 ± 0.25 c	2.97 ± 0.36 bc	2.90 ± 0.37 bc	3.90 ± 0.41 ab	4.50 ± 0.67 ab	7.97 ± 1.41 a
No. of escapes	0.10 ± 0.07 c	0.00 ± 0.00 c	0.07 ± 0.05 c	0.17 ± 0.07 bc	0.77 ± 0.21 ab	1.23 ± 0.24 a	1.97 ± 0.34 a
No. of kicks	0.35 ± 0.12 d	0.64 ± 0.17 cd	0.86 ± 0.17 cd	1.81 ± 0.28 bc	2.52 ± 0.30 ab	3.17 ± 0.35 ab	5.21 ± 0.78 a
No. of non-responses	1.33 ± 0.26 a	1.33 ± 0.34 a	1.07 ± 0.17 a	1.30 ± 0.24 a	0.97 ± 0.19 a	0.93 ± 0.21 a	1.07 ± 0.22 a
Non-response rate	0.44 ± 0.07 a	0.38 ± 0.07 a	0.29 ± 0.04 ab	0.31 ± 0.06 ab	0.17 ± 0.03 b	0.16 ± 0.04 b	0.14 ± 0.03 b
No. of stings	1.30 ± 0.21 b	1.33 ± 0.21 b	2.50 ± 0.31 ab	2.13 ± 0.31 ab	2.83 ± 0.30 a	2.80 ± 0.28 a	3.20 ± 0.61 a
Sting rate	0.97 ± 0.02 a	0.95 ± 0.03 a	0.89 ± 0.04 a	0.79 ± 0.05 ab	0.75 ± 0.05 ab	0.63 ± 0.05 bc	0.45 ± 0.05 c
No. of eggs laid	0.93 ± 0.16 c	1.13 ± 0.13 bc	1.77 ± 0.23 ab	2.00 ± 0.21 a	1.90 ± 0.24 a	1.43 ± 0.21 ab	1.13 ± 0.20 bc
Oviposition rate	0.81 ± 0.07 a	0.82 ± 0.06 a	0.69 ± 0.07 ab	0.70 ± 0.06 ab	0.63 ± 0.06 ab	0.58 ± 0.07 ab	0.46 ± 0.07 b
Handling time (sec.)	2.27 ± 0.54 c	1.50 ± 0.26 c	4.53 ± 0.61 bc	4.67 ± 0.65 bc	6.63 ± 0.67 ab	11.07 ± 1.74 ab	17.33 ± 2.81 a

Means (± SE) followed by the same letters within rows were not significantly different (P > 0.05).

Table 4.2 Mean (± SE) head width (mm) of newly emerged *A. ervi* when parasitised hosts of different ages.

	1 d old	2 d old	3 d old	4 d old	5 d old	6 d old	7 d old
Male	0.535 ± 0.0034 d	0.556 ± 0.0032 c	0.580 ± 0.0031 b	0.601 ± 0.0037 a	0.599 ± 0.0045 a	0.596 ± 0.0049 ab	0.598 ± 0.0051 a
Female	0.585 ± 0.0027 d	0.613 ± 0.0032 c	0.642 ± 0.0031 b	0.660 ± 0.0037 a	0.656 ± 0.0029 ab	0.646 ± 0.0054 ab	0.649 ± 0.0059 ab

Means (± SE) followed by the same letters within rows were not significantly different (P > 0.05).

4.2.3.2 Effect of Age Preference on Reproductive Fitness

Aphidius ervi females significantly preferred aphids that were 3 to 5 d old for oviposition with significantly higher number of aphids parasitised (Figure 4.1) and eggs laid (Figure 4.2) (ANOVA: F = 35.5 and 9.95 for the number of aphids parasitised and eggs laid, respectively; df = 6,63; P < 0.0001).

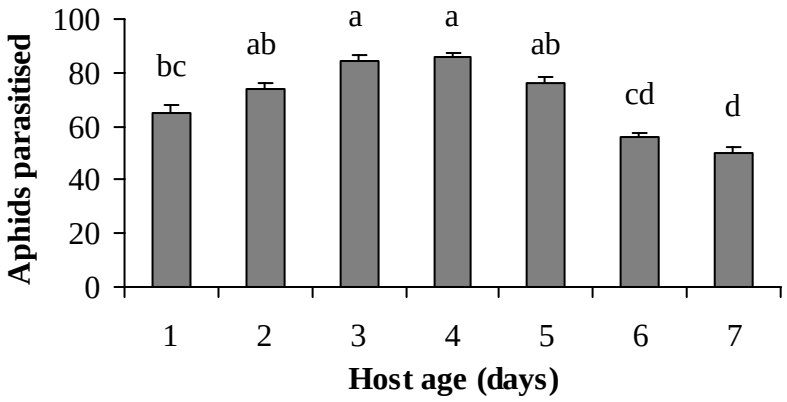

Figure 4.1 Mean (± SE) number of aphids parasitised per *A. ervi* female in relation to host age. Columns with the same letters are not significantly different (P > 0.05).

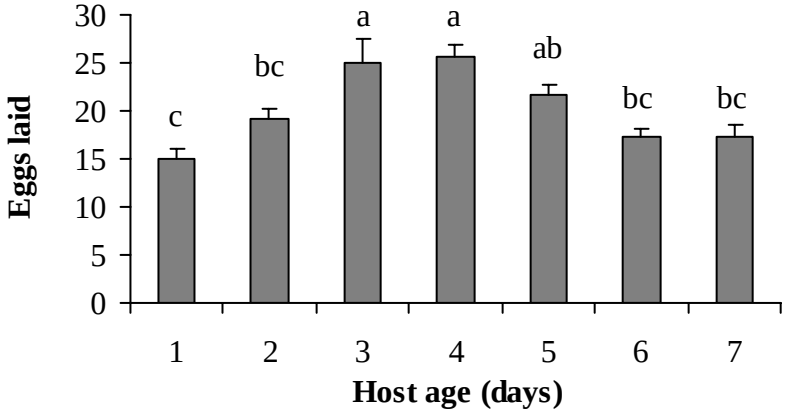

Figure 4.2 Mean (± SE) number of eggs laid per *A. ervi* female (estimated from dissection) in relation to host age. Columns with the same letters are not significantly different (P > 0.05).

Host age had significant effect on proportion of female progeny produced (ANOVA: F = 35.4; df = 6,63; P < 0.0001), which was highest in 6-d-old aphids and lowest in 1-d-old aphids (Figure 4.3).

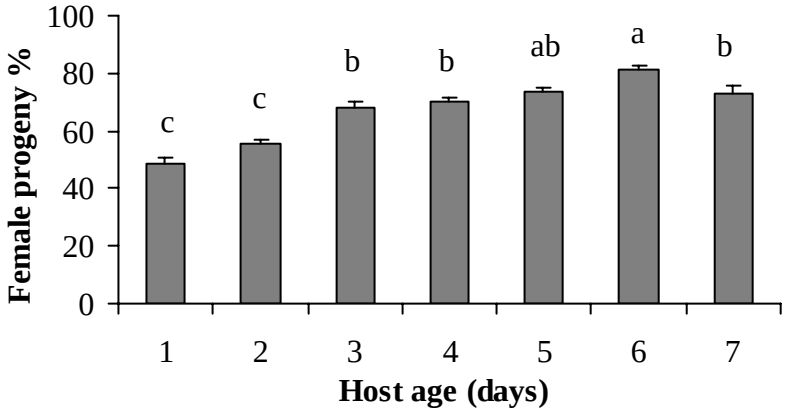

Figure 4.3 Mean (± SE) proportion of female progeny emerged from hosts of different age classes. Columns with the same letters are not significantly different (P > 0.05).

The head width of newly emerged adults of both sexes significantly increased with the increasing host age (from 1 to 4 d old) at the time of parasitisation (ANOVA: F = 40.18 and 45.80 for male and female, respectively; df = 6,203; P < 0.0001) (Table 4.2). Regardless of the host age when parasitised, females were significantly larger than males (ANOVA: F = 130.91, 161.95, 200.13, 125.96, 114.15, 46.56 and 42.18 for 1- to 7-d-old aphids, respectively; df = 1,58; P < 0.01) (Table 4.2). The egg load of newly emerged females was also significantly higher when parasitoids attacked aphids that were 3 to 7 d old (ANOVA: F = 49.29; df = 6,203; P < 0.0001) (Figure 4.4). There was a positive linear relationship between the body size and egg load of newly emerged females (RA: F = 220.13; df = 1,208; P < 0.0001) (Figure 4.5).

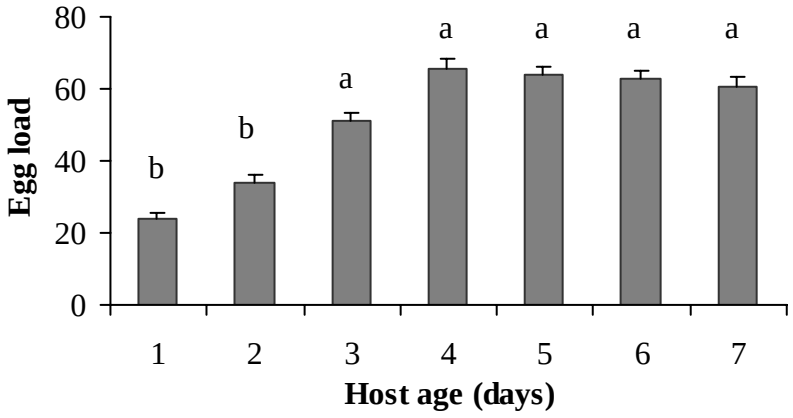

Figure 4.4 Mean (± SE) number of egg load per female progeny emerged from hosts of different age classes. Columns with the same letters are not significantly different ($P > 0.05$).

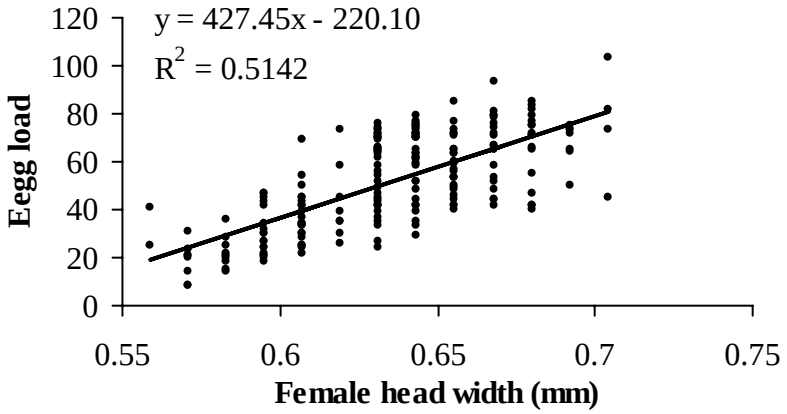

Figure 4.5 Relationship between body size and number of egg load per newly emerged female of *A. ervi*.

4.2.4 Discussion

Aphidius ervi females attacked pea aphids of different ages. Once aphids are detected, hymenopteran parasitoids are usually able to evaluate host species and quality by antennal examination (Desneux et al. 2004; Mehrnejad and Copland 2006), allowing them to verify the presence of the physiological conditions necessary for the development and growth of their progeny. However, *A. ervi* females usually performed attack position without any physical contact. It has been reported that the visual cues (colour and shape of pea aphid) play important roles in the host recognition and acceptance in *A. ervi* (Battaglia et al. 1995, 2000). The behaviour

switching from random searching to attack position suggests that *A. ervi* females recognise hosts by visual and/or odour cues before physical contact when the host is within a range of 1 cm.

Although it was found that the parasitoid was interested in attacking larger hosts with more attack attempts, the larger aphids were more capable of physically defending themselves from parasitisation (i.e. 'escape' when encountered and 'kicking' when attacked). Therefore, larger hosts would cost more to the *A. ervi* females in oviposition (i.e. lower sting and oviposition rates and longer handling time). Similar cases are also reported in *Monoctonus paulensis* (Ashmead) attacking pea aphid (Chau and Mackauer 2001) and in *Psyllaephagus pistaciae* Ferrière parasitising the common pistachio psylla *Agonoscena pistaciae* Burckhardt and Lauterer (Mehrnejad and Copland 2006).

The immature pea aphids grow in size as they develop to adult stage and larger aphids have more food resource for *A. ervi* larvae (Li et al. 2002; Section 3.4). According to the theory of optimal host acceptance in parasitoids, which is based on the model of optimal diet in predators (Stephens and Krebs 1986), *A. ervi* females should prefer larger aphids for oviposition. However, the present results indicate that *A. ervi* preferred aphids that were 3 to 5 d old to younger (1 to 3 d old) and older ones (6 and 7 d old) for oviposition. Therefore, the food resource at the point of oviposition is not the only factor that determines the oviposition decision by parasitoids, and they may select hosts according to the optimal tradeoff between food supply and oviposition costs.

The results in this study also show that host age preference by *A. ervi* is related to its progeny's reproductive fitness. The body size of progeny and egg load of newly emerged females were similar when aphids were attacked at 4 to 7 d old, suggesting that the quantity and quality of food resource for the parasitoid progeny are similar in aphids of this age range.

The increase in proportion of female progeny of *A. ervi* with host size supports the host size-dependent sex allocation theory in parasitic Hymenoptera (Charnov et al. 1981; Charnov 1982). There is also field evidence that the host age plays a role in sex

ratios, as reported for *A. ervi* parasitising pea aphid, where a male-biased population emerged from small aphids in the early season (Sequeira and Mackauer 1993).

Although the body size of parasitoids emerged from aphids parasitised at 4 d old was similar to that emerged from aphids parasitised at 6 d old, the proportion of female offspring was significantly lower when parasitoids selected 4-d-old aphids for oviposition than that when parasitoids selected 6-d-old aphids. Sex ratio data suggest that the body size of hosts at the point of oviposition is the major factor affecting the sex allocation by *A. ervi*.

The findings of this study have implications for laboratory mass rearing and field release of *A. ervi*. For example, aphids that are 3 to 5 d old appear to be the most appropriate hosts in the mass-rearing program because they gave the best fitness return for the parasitoids.

4.3 Effect of Host Density on Reproductive Fitness of *A. ervi*

4.3.1 Introduction

The impact of a parasitoid on its host population greatly depends upon its ability to find and parasitise hosts and to increase offspring numbers in response to increasing host density (Waage and Hassell 1982; Mackauer 1983). It is important to identify the form of the parasitoid response to prey density in population modeling, from which biological control programmes can be developed (Mills and Lacan 2004). In analytical host-parasitoid models, changes in the density-dependent sex ratio of parasitoids influence the level of host population equilibrium and the stability of the host-parasitoid relationships, thus affecting the success of biological control (Waage and Hassell 1982; Hassell and Waage 1984).

Ives et al. (1999) studied some aspects of the functional response of *A. ervi* on pea aphid, with emphasis on the parasitoid's behavioural decisions and their influence on the parasitism rate. In this section, the relationship between host density and reproductive fitness of *A. ervi* was investigated by determining how host density affected parasitoid reproductive potential and sex allocation. The aim was to provide further information for the assessment and improvement of *A. ervi*'s effectiveness in biological control.

4.3.2 Materials and Methods

4.3.2.1 Experimental Insects

All parasitoids used for experiments emerged from mummies that were parasitised in the third instar (3 d old), and the third instar pea aphids were used as hosts in all experiments.

4.3.2.2 Experiment

To determine whether and to what extent host density affected reproduction of *A. ervi*, six densities of aphids (15, 25, 50, 75, 100 and 125 aphids/cylinder/parasitoid female/day) were tested, with 10 replicates for each density. One mated *A. ervi* female

(< 12 h old) was introduced into an experimental cylinder with aphids feeding on a bean plant cutting. The female was allowed to stay in the cylinder for 24 h, and then moved to another cylinder with the same number of healthy third instar aphids, etc. until she died.

Because superparasitism was common under laboratory conditions, the oviposition potential of *A. ervi* was estimated by counting both the number of eggs laid (fecundity) and aphids parasitised (parasitism). To estimate the daily number of eggs laid in a parasitised aphid, five aphids from each cylinder at densities of 15 and 25 aphids, 10 aphids at density of 50 aphids, and 20 aphids at densities of 75, 100 and 125 aphids were randomly selected from each cylinder 4 days after the removal of the female parasitoid. These selected aphids were dissected in 70% alcohol under the stereomicroscope. The numbers of parasitoid larvae recorded from dissecting were assumed equal to the number of eggs laid (Bueno et al. 1993). The remaining aphids were reared until mummification. The number of eggs laid was assumed equal to the total number of aphids parasitised (i.e. the sum of mummies and aphids parasitised detected by dissecting) × the average number of eggs laid in a parasitised aphid detected by dissecting. The emerged offspring were counted and sexed.

4.3.2.3 Statistical Analysis

Goodness-of-fit test was used to test whether the data were normally distributed. Effect of aphid density on the number of parasitoid eggs laid was assessed by ANOVA and means separated by Tukey's studentised range test. Data for percent female progeny, parasitism rate and eggs per parasitised aphid were not normally distributed after transformation and were thus analysed using the nonparametric Kruskal-Wallis test. Means were subsequently separated by Dunn's procedure for multiple comparisons (Zar 1999). A significance level of $P < 0.05$ was used for all tests. In this study, more than 80% of eggs were laid within the first 8 days, thus all data presented were from the first 8 days.

4.3.3 Results

Mean total number of aphids parasitised and eggs laid (actual fecundity) during *A. ervi* females' life time increased with the increase of host density (Kruskal-Wallis test for parasitism: F = 68.39, df = 5,64, P < 0.0001; ANOVA for eggs laid: F = 14.65, df = 5,64, P < 0.0001) (Figure 4.6). Fecundity reached a plateau at host densities of 75/cylinder and above (Figure 4.6). The number of eggs laid in each parasitised aphid decreased with the increase in host density from 15 to 75/cylinder, after which no further decrease occurred [Kruskal-Wallis test: H = 51.96 > $\chi^2_{5,0.05}$ (= 11.07); P < 0.0001] (Figure 4.7). The daily parasitism rate decreased when the host density increased to 50/cylinder (Kruskal-Wallis test: H = 44.59 > $\chi^2_{5,0.05}$; P < 0.0001) (Figure 4.8). The proportion of female progeny was greatest at host densities of 50 and 75/cylinder, and least at extremes of low and high host densities (Kruskal-Wallis test: H = 35.30 > $\chi^2_{5,0.05}$; P < 0.0001) (Figure 4.9).

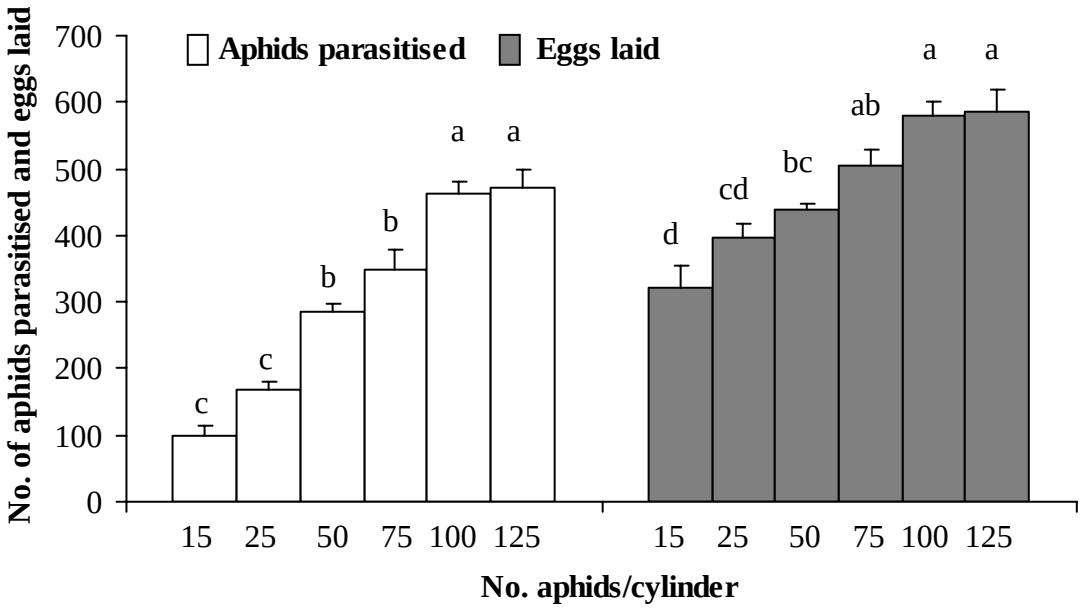

Figure 4.6 Mean (± SE) number of aphids parasitised and eggs laid by *A. ervi* at different host densities (15, 25, 50, 75, 100 and 125 aphids/cylinder/day/female). Within the same category (Aphids parasitised or Eggs laid) columns with the same letters are not significantly different (P > 0.05).

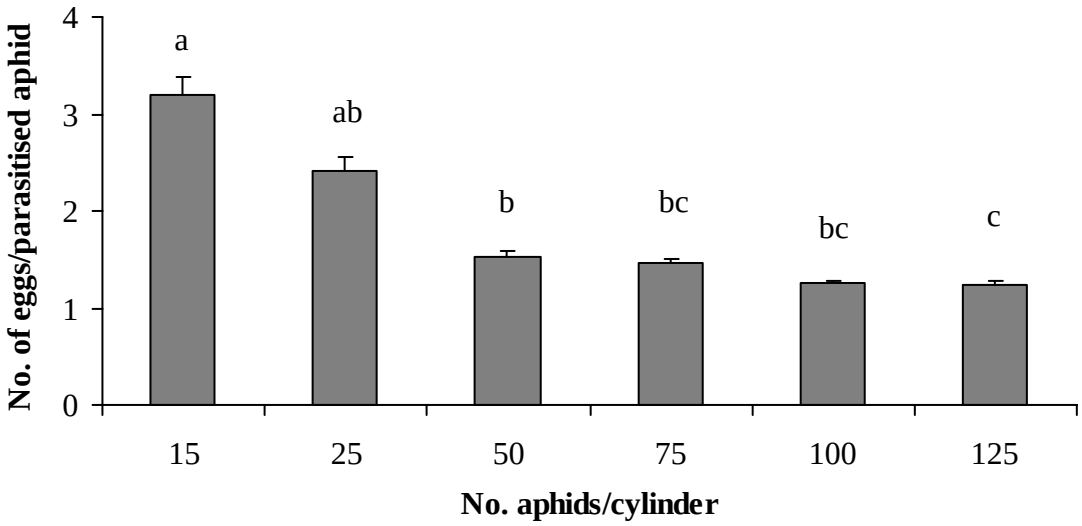

Figure 4.7 Mean (± SE) number of eggs per parasitised aphid laid by *A. ervi* at different host densities. Columns with the same letters are not significantly different (P > 0.05).

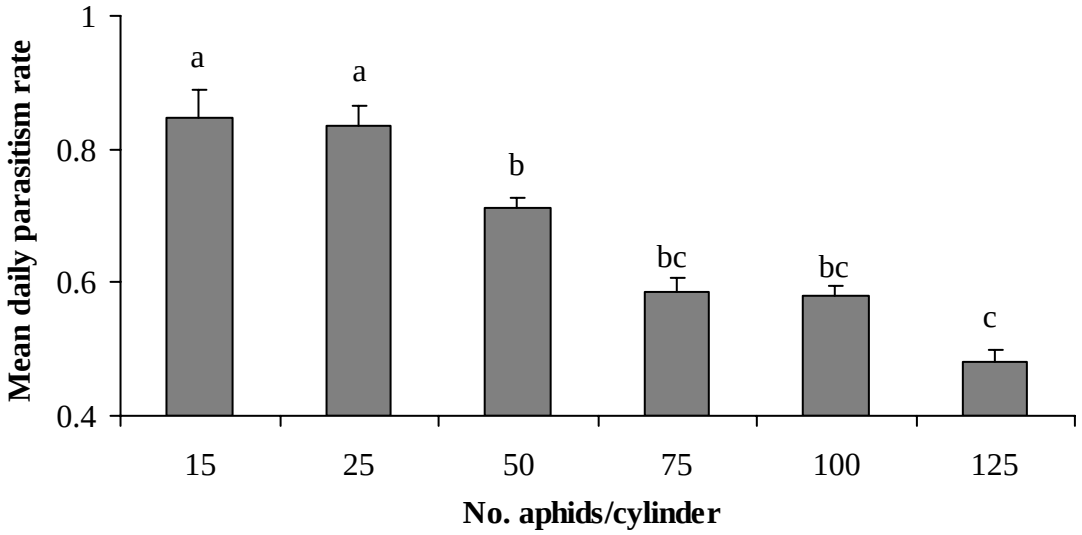

Figure 4.8 Mean (± SE) daily parasitism rate of *A. ervi* at different host densities. Columns with the same letters are not significantly different (P > 0.05).

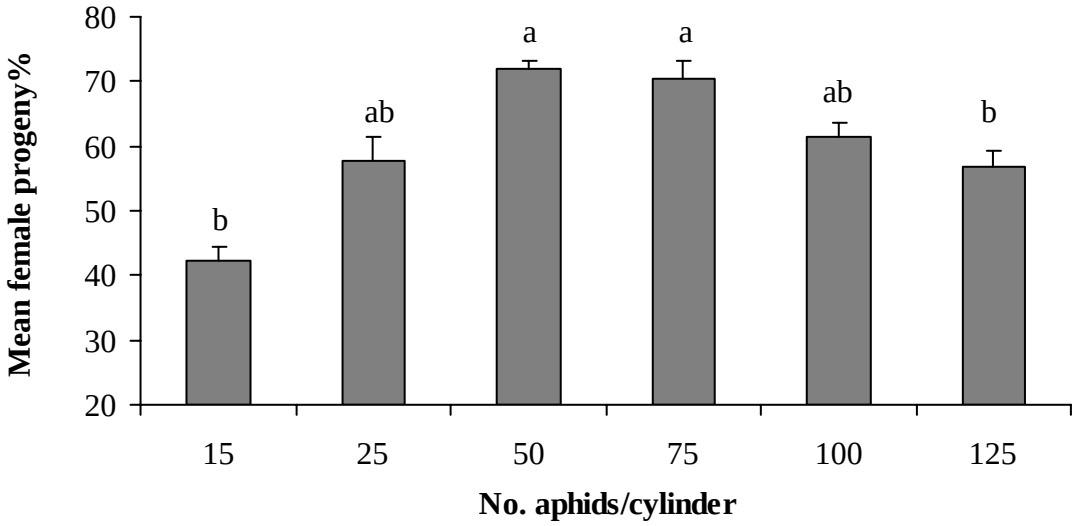

Figure 4.9 Mean (± SE) proportion of female progeny of *A. ervi* at different host densities. Columns with the same letters are not significantly different (P > 0.05).

4.3.4 Discussion

High fecundity and parasitism rates are considered necessary for parasitoids to be able to respond rapidly to the increases in pest density (Waage and Hassell 1982; Waage 1990). The results of the present study indicate that *A. ervi* can adjust oviposition strategy in response to increasing host density through increasing parasitism and decreasing superparasitism (Figures 4.6 and 4.7). These data suggest that this parasitoid has higher potential to suppress the aphid population when the latter increases. However, when the host population was 50/cylinder, mean daily parasitism rate significantly decreased (Figure 4.8). The behaviour of *A. ervi* in this experiment is consistent with the classic Holling type II functional response (Holling 1959), i.e. the number of aphids parasitised increased with an increase in aphid density but at a progressively decreasing rate. A type II functional response was also found in some other *Aphidius* species, such as the *A. smithi* (Mackauer 1983) and *A. sonchi* Marshall (Liu 1985). Results of this study suggest that a host density equivalent to 50 aphids/cylinder is the highest critical density where the parasitoid could maximise its control efficiency. This information could be important when considering host-parasitoid density ratio in biological control programmes (Hassell and Waage 1984). For example, to maximise biological control efficiency of pea aphids, parasitoids could be released at a rate of about one female parasitoid/50 aphids.

The proportion of female progeny developed from fertilised eggs quickly and increased significantly with the increase of host density from 15 to 50 aphids/cylinder, after which it gradually declined. This suggests that the highest potential proportion of female progeny that *A. ervi* can produce is about 70% and sperm limit occurs when host density reaches 50 to 75 aphids/cylinder. Offspring sex ratio of other *Aphidius* species, such as *A. smithi* (Mackauer 1983) and *A. sonchi* (Liu 1985), has also been found to vary with host density. In hymenopteran parasitoids, males can mate several times while females mate only once. It is thus to the female's benefit that she produces the minimum number of males possible. However, when hosts occur at low density, a highly female-biased sex ratio should reduce the ability of parasitoid persistence (Tripathi and Singh 1991), leading to extinction of the host population and eventually the parasitoid population. This suggests that the female parasitoids can adjust the sex allocation strategy in response to the host density. A similar case was found in *Aphelinus mali* Haldeman, which attacks the woolly apple aphid (Mueller et al. 1992).

4.4 Effect of Body Size on Reproductive Fitness of *A. ervi*

4.4. 1 Introduction

Body size of insects has usually been considered to be a key trait potentially affecting fitness in many ways, both directly and indirectly through correlated physiological parameters (Cloutier et al. 2000; Jiménez-Pérez and Wang 2004). Many studies have provided strong support for the 'size-dependent fitness' model, i.e., large individuals often have physiological and behavioural advantages (Visser 1994; Cloutier et al. 2000). For example, in hymenopteran parasitoids the reproductive fitness of females in terms of fecundity, parasitism and longevity is often positively correlated with their body size (Sagarra et al. 2001; Arakawa et al. 2004); large males usually have greater ability to inseminate and compete for mates (Kazmer and Luck 1995) or have better genes and more sperm supply (van den Assem et al. 1989). Therefore, the fitness consequences of body size and its correlates, especially the supply of eggs or sperm and adult longevity, are important in population dynamics and essential for understanding and modeling the life history evolution and behavioural decisions.

On the basis of generally accepted 'size-dependent fitness', Charnov's 'variation in fitness' model predicts that parasitoids lay fertilized eggs in larger hosts resulting in female progeny, and unfertilized eggs in small hosts giving rise to male progeny, and female fitness increases more rapidly with body size than does male fitness (Charnov et al. 1981; Charnov 1982). However, most studies on size-fitness relationships focus on females, neglecting males (King 1987; Visser 1994). Studies on the relationship between body size of male parasitoids and reproductive fitness usually concentrate on male mate-searching behaviour, competitive ability and copulation capacity (van den Assem et al. 1989; Kazmer and Luck 1995), and rarely address the effect of male size on the other fitness parameters such as the number of resulting female progeny (Ode et al. 1996). To my knowledge, Heinz (1991) was the first author to consider the effect of male body size on the reproductive fitness in a wasp species, *Diglyphus begini* (Ashmead). He compared the male and female reproductive success in relation to their body size, and suggested that females gained more in terms of the number of progeny with increasing body size than did males.

However, like many parasitic wasps, *D. begini* is a species of haplodiploid sex determination. Heinz's results had thus overestimated the effect of female body size on reproductive fitness because the number of fathers' progeny (only female progeny) was always fewer than that of mothers' progeny (both female and male progeny).

A positive relationship was found between *A. ervi* body weigh and pea aphid size at parasitisation (Sequeira and Mackauer 1992). However, no study has addressed whether and how body size of both *A. ervi* males and females affects their reproductive fitness. The fitness of parasitoids of different sizes needs to be known when calculating the value of particular hosts to foraging female parasitoids, which is important in modeling host selection strategies (Charnov and Stephens 1988; Visser 1994). Moreover, body size-fitness relationships are also relevant to mass-rearing programmes, as body size is commonly monitored as an indicator of parasitoids' quality (Jervis and Copland 1996; Sagarra et al. 2001).

In the laboratory *A. ervi* females mated only once while males could inseminate up to 8 females if the mated male was offered a virgin female daily; the first 6 inseminated females produced similar number and proportion of female progeny (Section 4.5). Similar results were also reported for another parasitoid species, *Trichogramma evanescens* Westwood (Jacob and Boivin 2004). This information has two implications. On the one hand, the greater longevity of adult wasps may be important not only for females to lay more eggs (Cloutier et al. 2000) but also for males to inseminate more females. On the other hand, a male in the field may not be able to inseminate as many females as in the laboratory because there are simply not enough virgin females available (i.e. the proportion of female in the field population is about 66%) (Sequeira and Mackauer 1993); therefore, the first or first few matings may be the most important for the male's reproductive success. In this study, the data from the first insemination were recorded and analyzed only.

This section reports the relationship between the body size of both sexes and reproductive fitness in *A. ervi*, with two objectives: (1) to determine how body size affected reproductive fitness in terms of fecundity, parasitism, number and proportion of female progeny produced, searching efficiency, and longevity, and (2) to evaluate whether body size of both sexes affected reproductive fitness in similar way.

4.4.2 Materials and Methods

4.4.2.1 Measurement of Body Size

On the basis of previous work reported by various authors (e.g. Cloutier et al. 2000; Arakawa et al. 2004; Paine et al. 2004), head width of adults was used as the index of body size for *A. ervi*.

The body size of emerging *A. ervi* wasps is host size-dependent. In this experiment, the mean head width (mean ± SE) of parasitoids emerged from aphids parasitised at the first instar (male, 0.547 ± 0.003 mm; female, 0.588 ± 0.004 mm) was significantly smaller than that from aphids parasitised at the fourth instar (male, 0.612 ± 0.004 mm; female, 0.663 ± 0.003 mm) (ANOVA: $F = 184.14$ and 218.55 for male and female, respectively; df = 1,58; $P < 0.0001$). The former was defined as "small" (S) and the latter as "large" (L) in this section.

4.4.2.2 Experiments

To determine whether and to what extent parasitoids' body size affected their reproductive fitness, four treatments were set up with fifteen replicates per treatment: small female (SF) × small male (SM), large female (LF) × large male (LM), SF × LM and LF × SM.

For each replicate, one mated *A. ervi* female (< 12 h old) was introduced into a plastic cylinder with 50 healthy third instar aphids (3 d old) feeding on a bean plant cutting. The female was allowed to stay in the cylinder for 24 h, and then moved to another cylinder with 50 healthy third instar aphids, etc. until she died. Data on the daily basis and all data collected during females' lifetime were recorded and included in the analysis. The total number of aphids used in the experiment was 8350, 8800, 10550 and 10600 for treatments SF × SM, SF × LM, LF × SM, and LF × LM, respectively. The once-mated males were numbered in correspondence with the females they mated with and reared individually in glass vials (1.5 cm in diameter, 5.0

cm in height) with a 0.5 cm mesh-covered hole in lids, and supplied with 10% honey solution in a cotton wool ball (0.5 cm in diameter) daily.

To estimate the number of eggs laid, 10 aphids from each cylinder were randomly selected 4 days after the removal of the female parasitoid and then dissected in 70% alcohol under the stereomicroscope. In total 1680, 1640, 2000 and 2030 aphids were dissected for above four treatments, respectively. The number of parasitoid larvae recorded from dissecting was assumed equal to the number of eggs laid (Bueno et al. 1993) or fecundity. The remaining aphids were reared until mummification. The number of parasitism is the sum of the number of parasitised aphids detected by both dissecting and mummies. The number and proportion of female progeny were counted and calculated from emerged progeny. Longevity of both sexes was also recorded for all treatments.

4.4.2.3 Statistical Analysis

A goodness-of-fit test indicated that data were normally distributed and thus analyzed using ANOVA followed by a Tukey's studentized range (HSD) test. The data on the proportion of female progeny were arcsine transformed prior to analysis.

A central composite design (CCD), i.e., response surface (Box and Draper 1987) was used to analyze the differential size-dependent fitness gain of sexes in terms of the number and proportion female progeny produced. The relationship between parasitoid body size and reproductive fitness is given by the polynomial equation: fitness = $\mathrm{Exp}(\beta_0 + \beta_1 x_f + \beta_2 x_m + \beta_{11} x_f^{\,2} + \beta_{22} x_m^{\,2} + \beta_{12} x_f x_m)$, where β_0, β_1, β_2, β_{12}, β_{11} and β_{22} are model parameters, and x_f and x_m are female and male body size, respectively. Only significant terms, after running the full regression models, were kept in the final models. A log likelihood ratio test (McCullagh and Nelder 1989) was then applied to determine whether body size of sexes had different effect on the number and proportion female progeny produced. The slopes of the regression lines of size-longevity relationships of sexes were also analyzed using an analysis of covariance (ANCOVA).

4.4.3 Results

4.4.3.1 Male Reproductive Performance in Relation to Body Size

Male body size had no significant effect on fecundity (Figure 4.10A) and parasitism (Figure 4.10B). However, females mated to large males produced significantly more female progeny (Figure 4.10C) and had significantly higher proportion of female progeny (Figure 4.10D) than those mated to small males. Moreover, females mated to large males produced female-biased progeny for significantly longer period than those mated to small males (Figure 4.10E).

4.4.3.2 Female Reproductive Performance in Relation to Body Size

Large females had significantly higher fecundity (Figure 4.10A), parasitised significantly more aphids (Figure 4.10B), and produced significantly greater number of female progeny (Figure 4.10C) than small females. The proportion of female progeny was significantly higher for small females than that for large females (Figure 4.11D). However, female body size did not affect how long females sustained the production of female-biased progeny (Figure 4.10E).

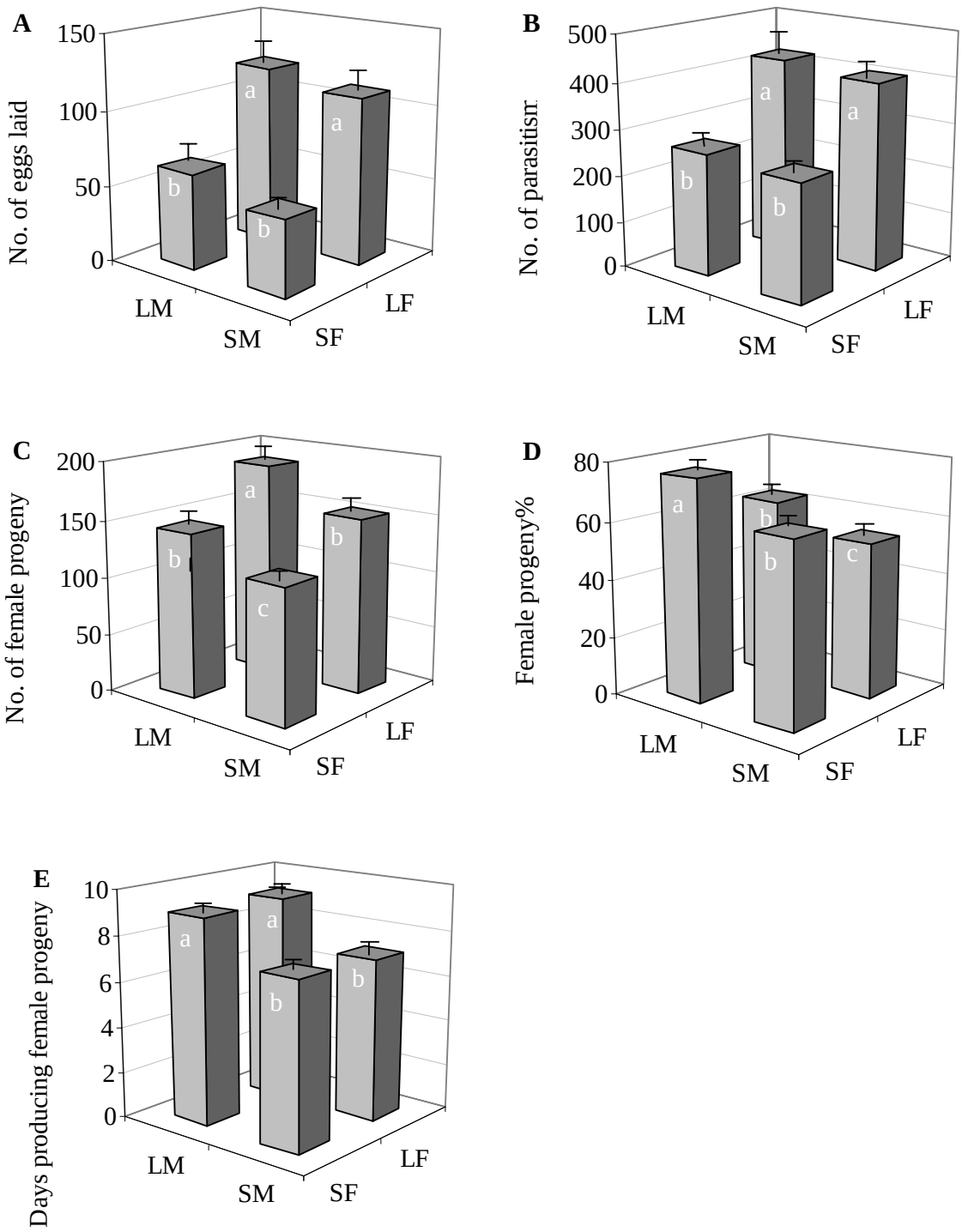

Figure 4.10 Effect of body size of both sexes on the number of eggs laid (A), number of parasitism (B), number of female progeny (C), proportion of female progeny (D) and days of proportion of female progeny > 50% (E). Columns with the same letters were not significantly different ($P > 0.05$). For A to D: $F = 43.43$, 36.12, 13.69, and 29.68 for A, B, C and D, respectively; df = 3,56; $P < 0.0001$. For E: $F = 3.88$; df = 3,56; $P < 0.05$.

4.4.3.3 Relative Fitness Gain of Males and Females in Relation to Body Size

The number of female progeny significantly increased with the body size of both sexes (Figure 4.11A) but log likelihood ratio test shows that males had significantly more gain than females in the number of female progeny with the increasing body size (χ^2 = 234.15; df = 2; P < 0.0001). Unlike males, the female size had negative effect on the proportion of female progeny (Figure 4.11B). Although large males and females lived significantly longer than small males and females (ANOVA: F = 4.35, df = 3,56, P < 0.01 for female; F = 3.15, df = 3,56, P < 0.05 for male) (Table 4.3), females gained disproportionately greater longevity with the increase of body size than did males (ANCOVA: F = 19.17; df = 2,117; P < 0.0001) (Figure 4.12).

Table 4.3 Effect of body size (head width, mm) on longevity (days) in *A. ervi*.

	SF × SM	SF × LM	LF × SM	LF × LM
Female	11.13 ± 0.74 b	11.73 ± 0.63 b	14.07 ± 0.74 a	14.13 ± 0.86 a
Male	12.13 ± 0.63 b	13.87 ± 0.52 ab	13.67 ± 0.45 ab	14.40 ± 0.45 a

Means (± SE) followed by the same letters in rows were not significantly different (P > 0.05).

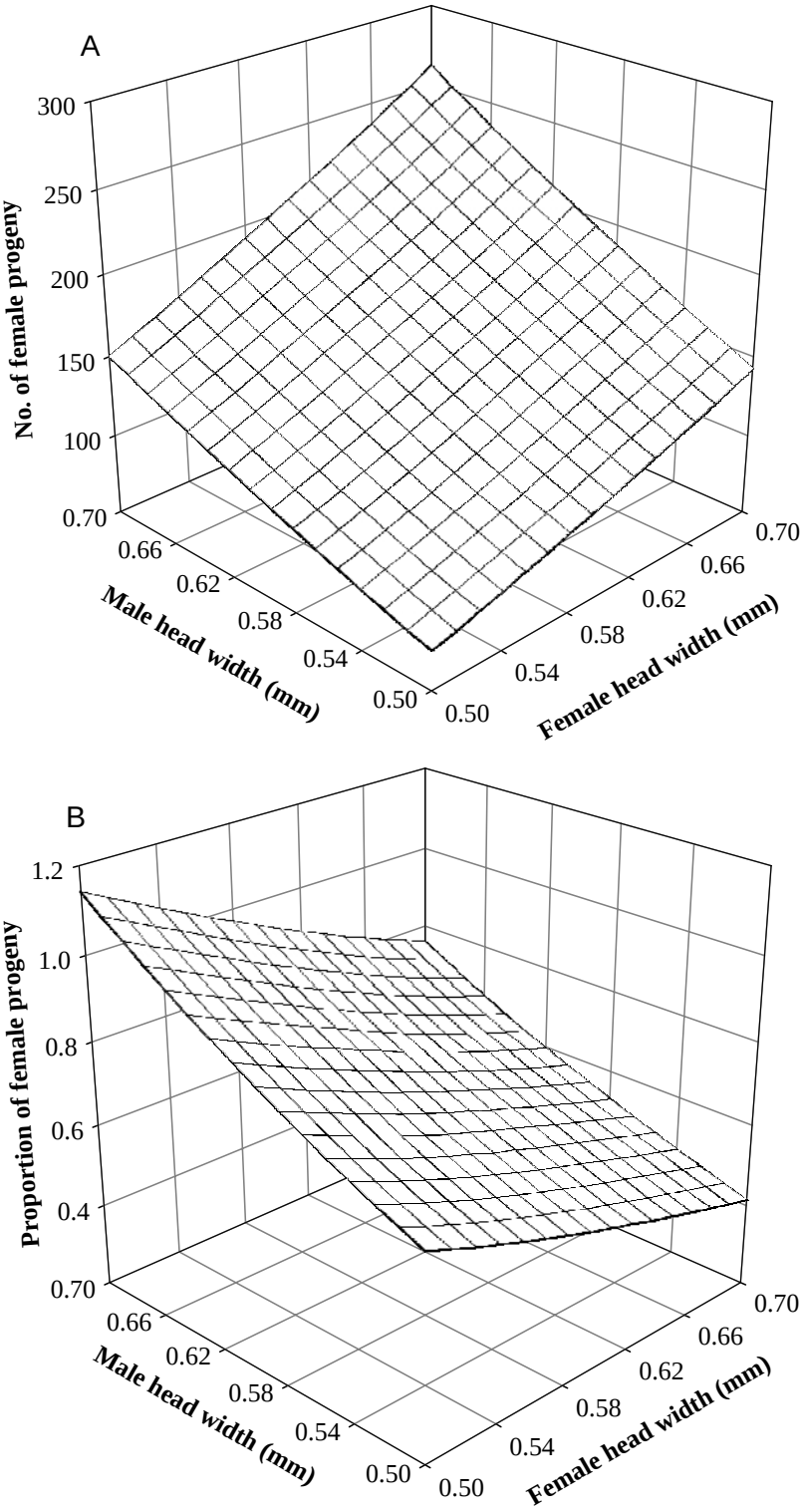

Figure 4.11 Effect of female (x_f) and male (x_m) body size on fertility [A: y = exp(1.26 + 2.97x_f + 3.23x_m) (R^2 = 0.5222; F = 31.15; df = 2,57; P < 0.0001)] and fertility rate [B: y = exp(-0.67 - 2.25x_f + 2.77x_m) (R^2 = 0.6328; F = 48.96; df = 2,57; P < 0.0001)] in *A. ervi*.

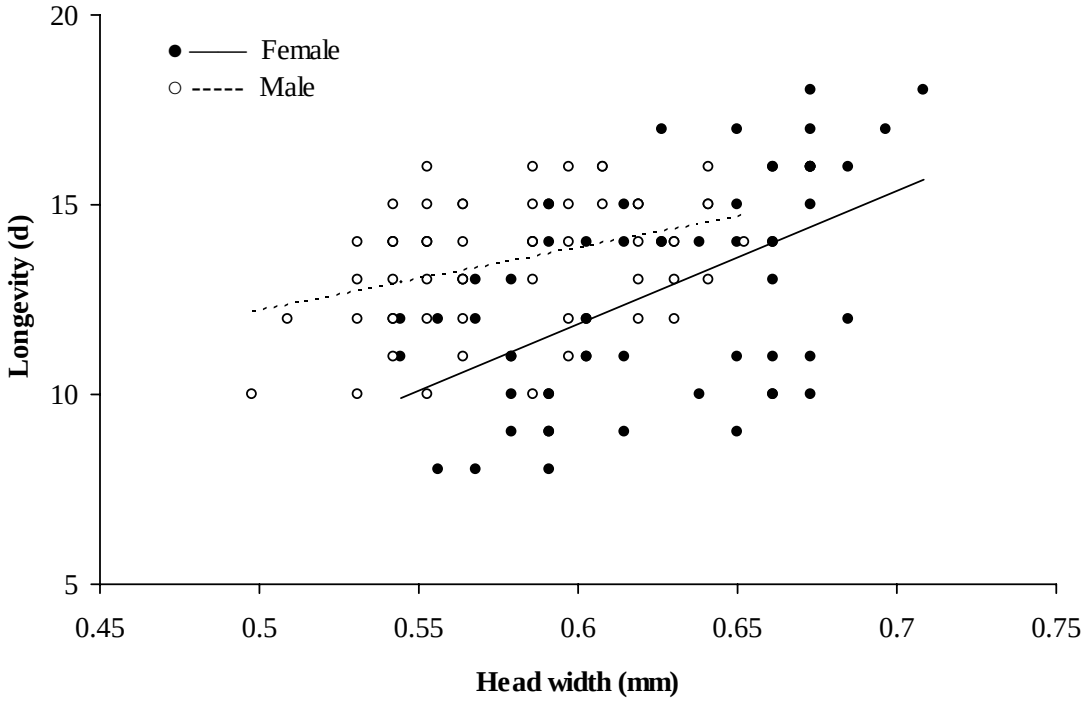

Figure 4.12 Relationship between body size and longevity of *A. ervi.* For female: y = 35.05*x* – 9.16 (R^2 = 0.2895; F = 23.63; df = 1,58; P < 0.0001); for male: y = 16.54*x* + 3.93 (R^2 = 0.1452; F = 9.85; df = 1,58; P < 0.01).

4.4.4 Discussion

The body size of *A. ervi* progeny is influenced by the host stage or age at parasitisation (Sequeira and Mackauer 1992). Like many hymenopteran species (Honék 1993; Godfray 1994; Visser 1994; Sagarra et al. 2001; Arakawa et al. 2004), the reproductive fitness of *A. ervi* females and males depends on their body size.

The increase in the number of eggs laid with the increasing female body size in *A. ervi* may result from increasing longevity and greater ability to generate eggs when needed, as detected in *A. nigripes* (Cloutier et al. 2000). A higher number of eggs laid by large females may also translate into a higher parasitism due to their greater longevity. However, unlike many other insects where nutrients contributed by males through copulation are used by females for egg production (Huignard 1983; Simmons and Gwynns 1993; Jiménez-Pérez and Wang 2004), large *A. ervi* males do not increase the number of eggs laid by females. This suggests that *A. ervi* females do

not obtain a nutritional contribution from males during copulation as suggested by Godfray (1994), Fauvergue et al. (1998), Cheng et al. (2003) and Khanh et al. (2005).

With the increase of body size, males gained more in the number of daughters and less in the longevity than did females. It is indicated that males concentrate their resource gain (body size) more on sperm production and insemination than on longevity, suggesting that the benefit from longevity is less certain than inseminating virgin females they can find.

The higher number of female progeny produced by larger females may be due to the greater storage capacity of the spermatheca and more production of eggs in those females (Lauzière er al. 2000). As found in other hymenopteran species, for example, the encyrtid parasitoid, *Anagyrus kamali* Moursi (Sagarra et al. 2001), the proportion of female progeny of *A. ervi* is higher for small females than that for large females. This may be because small females live shorter and deposit fewer eggs than large females and never become sperm limited. Furthermore, females mated with large males laid female-biased eggs for a longer period of time, suggesting that large males supply more sperm and thus sustain a female-biased population longer than small males.

The fitness data of *A. ervi* are generally in agreement with the main premise of Charnov's 'variation in fitness' model, which concerns the differential size-fitness relationship in males vs. females (Charnov et al. 1981; Charnov 1982). However, this study demonstrates that body size of *A. ervi* females and males affects reproduction in different ways, i.e. female size has major effect on fecundity, and male size has more impact on paternity. The asymmetrical size-fitness between sexes may be because reproductive success of parasitoids directly relies on females' potential fecundity and their ability to search and parasitise hosts whereas the success of male reproduction absolutely relies on, and is mediated, by females. In addition, virgin females still reproduce with the absence of males. Therefore, the different reproductive strategies of different sexes may result in the asymmetrical size-dependent fitness.

In conclusion, the body size of both sexes contributes to population growth and sex ratio distribution in *A. ervi*. Results of the asymmetric size-dependent fitness

in different sexes provide us useful information when we manage to predict parasitoid population dynamics and practice effective mass-rearing and release programmes.

4.5 Effect of Mate Age at Mating and Male Mating History on Reproduction of *A. ervi*

4.5.1 Introduction

Hymenopteran parasitoids are usually arrhenotokously parthenogenetic, where fertilized eggs produce diploid females and unfertilized eggs produce haploid males; virgin females give birth to only sons while inseminated females produce both sons and daughters (Quicke 1997). As a result, the reproductive success of females is mainly affected by egg production patterns and fertilization (Godfray 1994; Boivin et al. 2005) and that of males mainly by the number of daughters they father (Quicke 1997; Roitberg et al. 2001; Jacob and Boivin 2004). For the parasitoid species with monandrous females and polygamous males, the net reproductive gain of females from mating may depend on the age of sexes at mating and male mating history, and that of males on the age of sexes at mating, the number of females they inseminate, and oviposition history of their mates. The ways and extent of these effects may vary considerably between sexes as well as between species, depending on their life history strategies (Pandey et al. 1983; Srivastava and Singh 1995; King 2000; Jacob and Boivin 2004; Damiens and Boivin 2005).

The understanding of reproductive strategies of hymenopteran parasitoids is ultimately important for the success of biological control (Gordh and DeBach 1976; Nadel and Luck 1985; Godfray 1994; King 2000; Roitberg et al. 2001; Jacob and Boivin 2004; Damiens and Boivin 2005). In pro-ovigenic species, females have all their egg complement mature on emergence while in synovigenic females they mature eggs during adult stage (Flanders 1950; Quicke 1997; Boivin et al. 2005). However, most species probably fall in between these two extremes, and are termed pro-synovigenic, i.e. females carry some mature eggs on emergence but continue to produce and mature more eggs throughout adult lifespan (Quicke 1997; Jervis et al. 2001, 2003). Both pro-ovigenic and synovigenic traits are found in an encyrtid parasitoid, *Coccidoxenoides peregrinus* (Timberlake) (Ceballo and Walter 2004), suggesting that female parasitoid life history strategies may be far more complicated than what we understand today. Prospermatogenic males have fully developed their

testes (which have fixed number of sperm) by the time they reach maturity, whereas synspermatogenic males produce sperm during adult stage (Gerling and Legner 1968; Gordh and DeBach 1976; Nadel and Luck 1985; Jacob and Boivin 2004; Boivin et al. 2005). Similar to females, many species appear to be moderately synspermatogenic, i.e. males emerge with some mature sperm, and can continue to produce and mature sperm throughout their lifespan (Boivin et al. 2005). Prospermatogeny is expected to be advantageous for gregarious wasps where mating takes place mostly at emergence, and for the solitary species synspermatogeny should be advantageous as it enables males to disperse first and then locate mates (Boivin et al. 2005). Boivin et al. (2005) argue that species that are prospermatogenic should also be pro-ovigenic and *vice versa* because the constraints linked to life history parameters are likely to act on gamete production in both sexes.

Hymenopteran parasitoids may develop gregariously, solitarily or quasi-gregariously. Gregarious parasitoid species favor sibmating on the natal patch and local mate competition (Mackauer and Völkl 2002), and usually produce strongly female-biased broods, e.g. only one or two males in each brood (Hardy et al. 1998). Solitary parasitoid species, in contrast, favor dispersal from natal patch and outbreeding (Nadel and Luck 1992), and their offspring sex ratio is usually not strongly biased (Fisher 1930). If the hosts of a solitary parasitoid species are gregarious such as aphids or coccids, quasi-gregarious broods of the parasitoid offspring can be produced (van den Assem et al. 1980), where partial sibmating and local mate competition may occur (Mackauer and Völkl 2002). Furthermore, in quasi-gregarious parasitoid species, sex ratio is often moderately female-biased and unfertilized eggs are usually laid earlier than fertilized eggs (Hardy 1992).

Aphidius ervi is an endophagous, solitary and koinobiont parasitoid. The field sex ratio is ca. 1 male:1.9 females (Sequeira and Mackauer 1993). Males are polygynous (Starý 1962), and females monandrous (Mackauer 1969). Under the conditions similar to the present study, *A. ervi* adults can live for 11-14 d (Section 4.4); the female carries about 60 mature eggs on emergence (Section 4.2) but can parasitize more than 300 aphids in her lifespan (Section 4.3), indicating that *A. ervi* females are pro-synovigenic with an ovigeny index of ca. 0.2 (calculated according to Boivin et al.

2005). On the basis of the above property of *A. ervi*, it should be a quasi-gregarious species, i.e. a solitary parasitoid species attacking gregarious hosts.

In this study, I postulate that, in *A. ervi*, (1) the age of males and females at mating has a differential effect on fecundity and daughter production because the number of matings varies between sexes, and (2) males are synspermatogenic or pro-synspermatogenic due to the quasi-gregarious and polygynous property of this species. These hypotheses were tested through investigating how the age at mating and male mating history affected reproductive outputs. Knowledge of these reproductive strategy parameters is essential to the understanding of life history evolution and manipulation of daughter production for mass-reared and field populations of *A. ervi*.

4.5.2 Materials and Methods

4.5.2.1 Experimental Insects

Parasitoids used for experiments emerged from mummies that were parasitised in the third instar (3 d old), and the third instar pea aphids were used as hosts in all experiments.

4.5.2.3 Experiments

To examine whether mate age at mating affected *A. ervi* female reproductive output and sex allocation, nine treatments were set up, i.e. virgin males and virgin females mated at nine combinations of three age classes: 1, 3 and 5 d old. There were ten replicates for each treatment. This experiment was carried out in transparent plastic cylinders (Figure 3.2).

A male and a female were maintained in a glass vial (1.5 cm in diameter, 5.0 cm in height with a 0.5 cm mesh-covered hole in lids) for mating. After mating, the female was introduced into a plastic cylinder with 50 healthy third instar aphids feeding on a bean plant cutting. The female was allowed to stay in the cylinder for 24 h, and then moved to another cylinder with 50 healthy third instar aphids, etc. until

she died. Females mated at 3 and 5 d old were also individually provided with 50 health aphids daily for oviposition before and after mating. Honey solution (10%) was supplied daily as food for parasitoids. For all females, the number of aphids parasitised was recorded and newly emerged offspring were sexed. The number of progeny produced by females after they mated was applied to calculate the proportion of daughters produced. Ten virgin females were used as control.

To determine how male mating history affected female offspring production of *A. ervi*, two experiments were set up: (1) hourly mating and (2) daily mating. In the hourly mating experiment, a male was offered with a 1-d-old virgin female hourly in an above mentioned glass vial. Immediately after each mating, the mated males were removed, and individually kept in another glass vial. The number of females that a male mated with during one day (16 h photophase) was recorded. In the daily mating treatment, a male was offered a 1-d-old virgin female once a day during his life time. The number of females that a male could mate with was recorded as mentioned above. The males were supplied with 10% honey solution as food by dropping on a cotton wool ball (0.5 cm in diameter). This experiment was conduced during photophase and ten males were tested for each experiment.

Soon after mating, each female in both hourly and daily mating experiment was provided with 50 healthy third instar aphids daily as mentioned above, until she died. Ten percent honey solution was also supplied as food for females as mentioned above. The number of aphids parasitised and proportion of female offspring produced were recorded.

4.5.2.4 Statistical Analysis

Goodness-of-fit test was used to test the distribution of data. The data on the number of parasitism were normally distributed and analysed using ANOVA, followed by Tukey's studentised range test. The data on proportion of female offspring were not normally distributed even after arcsin transformation and thus analyzed using the nonparametric Kruskal-Wallis test followed by Dunn's procedure for multiple comparisons (Zar 1999).

A central composite design (CCD), i.e., response surface (Box and Draper 1987) was used to analyze the effect of mate age at mating on proportion of female offspring produced. The relationship between mate age and proportion of female offspring produced is given by the polynomial equation: female offspring% = $\exp(\beta_0 + \beta_1 x_f + \beta_2 x_m + \beta_{11} x_f^2 + \beta_{22} x_m^2 + \beta_{12} x_f x_m)$, where β_0, β_1, β_2, β_{12}, β_{11} and β_{22} are model parameters, and x_f and x_m are female and male age, respectively. Only significant terms, after running the full regression models, were kept in the final models. A log likelihood ratio test (McCullagh and Nelder 1989) was then applied to determine whether age of sexes had different effect on the proportion female offspring.

The relationship between the proportion of female offspring produced and number of mates a male mated with was analysed using regression analysis (RA), in both hourly and daily mating experiments. ANCOVA was used to analyse the slopes.

4.5.3 Results

Age of mates at mating and male mating history had no significant effect on the number of aphids parasitised (from 326.8 ± 11.6 to 352.6 ± 9.4) which was similar to that for virgin females (341.4 ± 5.1) (ANOVA: $F = 0.18$; $df = 26,183$; $P > 0.05$).

The proportion of female offspring produced significantly decreased with the increasing age of both sexes at mating (CCD: $F = 7.14$; $df = 2,87$; $P < 0.0001$; $R^2 = 0.1410$) (Figure 4.13A). However, female age had significantly more effect on the proportion of female offspring produced than did male age (likelihood rate test: $\chi_2^2 = 96.18$; $P < 0.0001$) (Figure 4.13B). When mated with 5-d-old females, 40, 20 and 30% 1-, 3- and 5-d-old males did not transferred sperm to their mates, respectively.

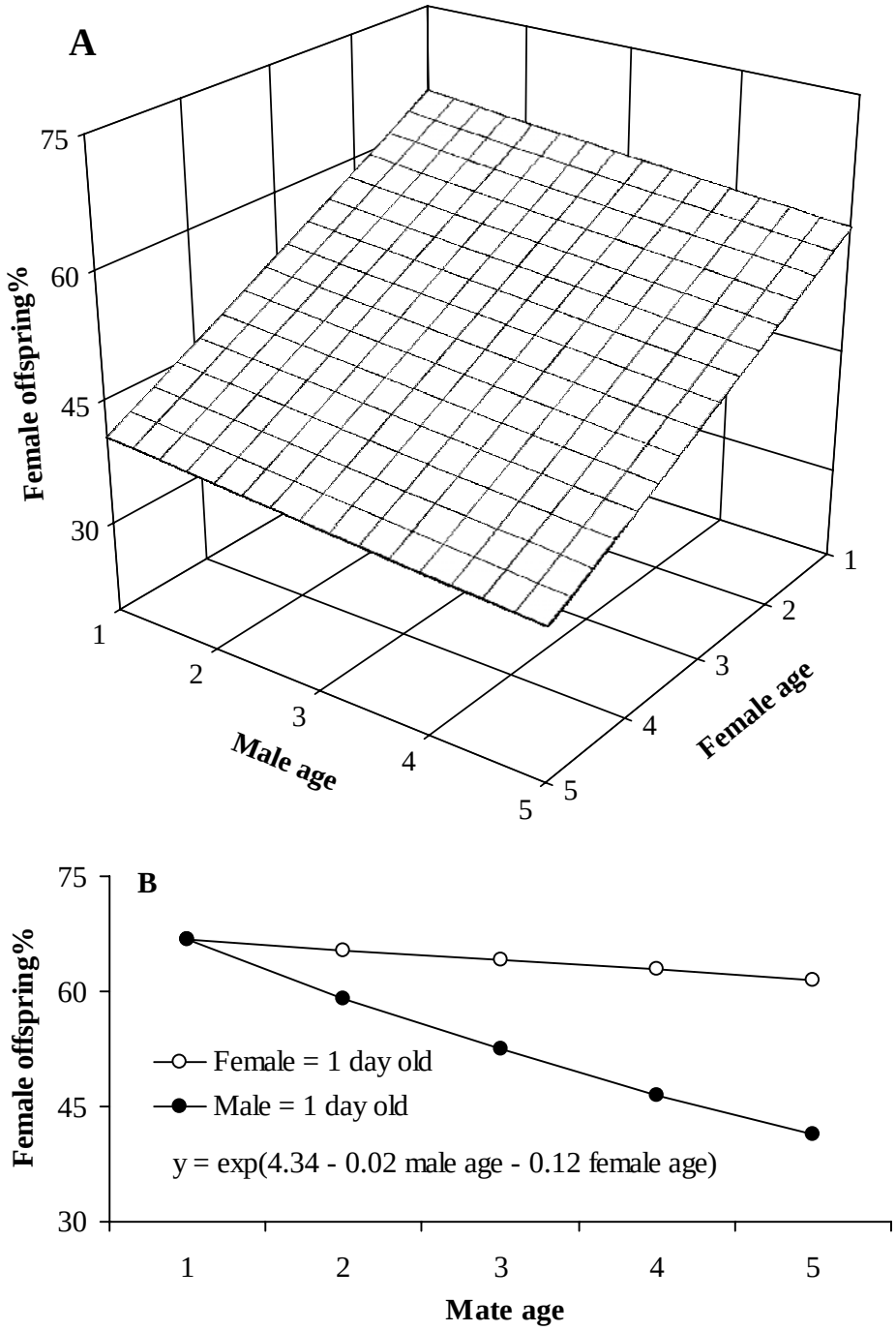

Figure 4.13 Effect of mate age (days) at mating on female offspring production of *A. ervi*: (A) proportion of female offspring produced in pairs of different age combinations of sexes (n = 90); (B) predicted proportion of female offspring produced by males and females of different ages.

The proportion of female offspring produced started to become significantly lower at fourth and seventh mating of a male in hourly and daily experiments, respectively (Kruskal-Wallis test: H = 59.75 > $\chi^2_{8,0.05}$ = 15.51 for hourly experiment and H = 21.78 > $\chi^2_{7,0.05}$ = 14.07 for daily experiment; P < 0.0001) (Figure 4.14). Furthermore, regression analysis shows that in both hourly and daily experiments, the proportion of female offspring produced significantly decreased with the increasing number of matings in males (RA: F = 132.62 and df = 1.59 for hourly experiment, and F = 34.77 and df = 1,47 for daily experiment; P < 0.0001) (Figure 4.15), while it decreased significantly faster in hourly than in daily experiments (ANCOVA: F = 77.57; df = 2,107; P < 0.0001).

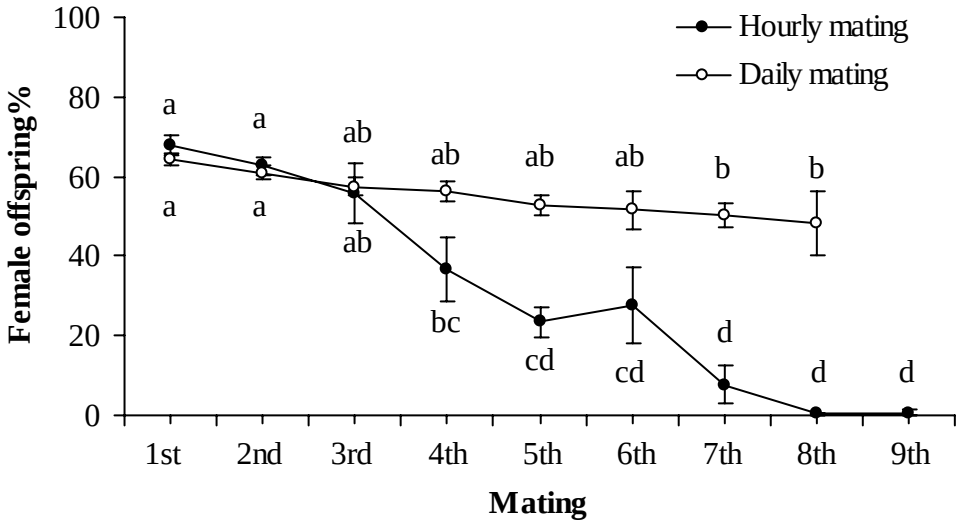

Figure 4.14 Proportion of female offspring of *A. ervi* males after successive matings. Means (± SE) followed by the same letters within each line are not significantly different (P > 0.05).

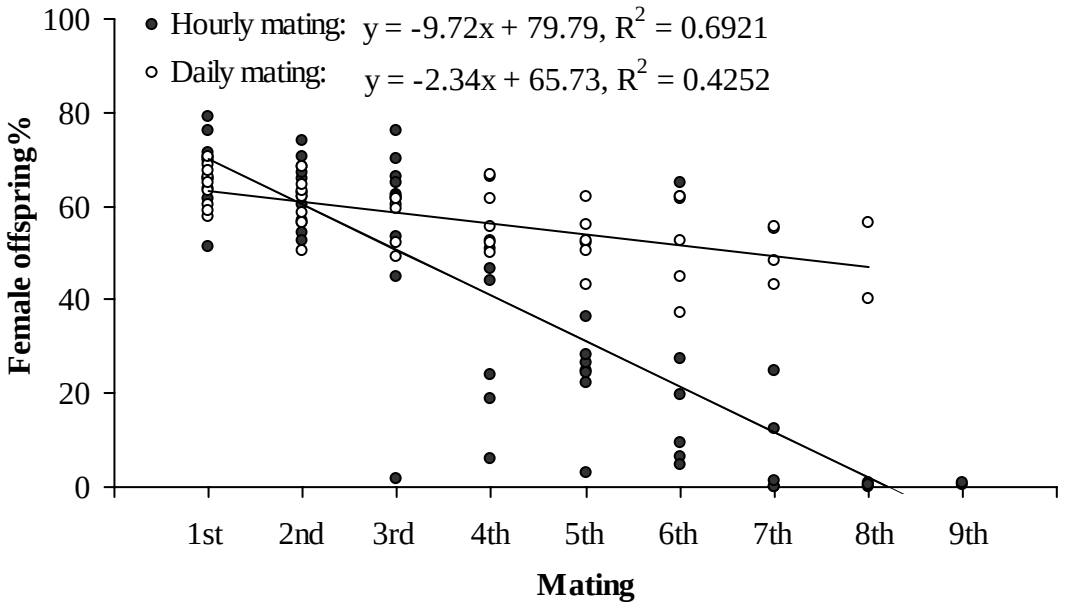

Figure 4.15 Relationship between proportion of female offspring and male mating history of *A. ervi*.

4.5.4 Discussion

In insects, males may transfer both sperm and nutrition or sperm only during copulation. In the aphidiid *Lysiphlebus delhiensis* (Subba Rao and Sharma), female age rather than male age at mating significantly reduces the fecundity (Srivastava and Singh 1995), suggesting that males of this species may transfer egg production stimulants and/or nutrients to females during mating, and if mating occurs when females are young, these materials could significantly increase egg production. However, like the aphelinid *A. asychis* Walker (Fauvergue et al. 1998), neither parental age at mating nor male mating history had any significant influence on the female fecundity in *A. ervi*. This implies that mating does not stimulate egg production and males do not transfer male-derived nutrients during mating in *A. ervi*. In our study of body size effect on fecundity (Section 4.4), results also suggest that there may be no nutrient transfer from males during mating.

The effect of mate age and male mating history on sperm production by male and sperm utilization by females is generally reflected in the proportion of female offspring produced. The proportion of female offspring declined with parental age at mating in this study. Similar to the aphidiid *L. delhiensis* (Srivastava and Singh 1995),

the lower production of daughters by older males may not be attributed to the sperm depletion in *A. ervi*. Rather, this could be due to diminishing sperm viability and mobility related to aging (Srivastava and Singh 1995). For the aging females, the reduction of daughter production could be explained by the constraints in fertilization process in *A. ervi*. Flanders (1946) found the depletion of spermathecal gland secretion resulting in a reduced availability of activated sperm for fertilization of ovulating eggs. According to Pandey et al. (1983), eggs may contain certain kind of sperm attracting chemicals, which deplete with the female age, leading to a reduced fertilization.

This study also indicated that female age at mating had more severe effect than male age on the production of daughters in *A. ervi*; between 20 and 40% of females when mating at the age of 5 d did not produce any daughters but none of the 5-d-old males failed to produce daughters. This suggests that fertilization process in females may be more sensitive to aging than sperm vigor in males. It is also likely that males adjust the amount of sperm transfer according to female age, delivering less sperm to older females (Srivastava and Singh 1995).

In the hourly mating experiment, *A. ervi* males started to show sperm depletion after three successive matings, while in the daily mating experiment, males still had sufficient sperm supply until having mated six times. In addition, the production of daughters decreased significantly faster in hourly mating than in daily mating treatments. These findings have two implications. First, males of this species appear to be at least moderately synspermatogenic because they can replenish sperm if allowed 24-h recovery period between matings up to six matings. As a quasi-gregarious species moderate synspermatogeny should be advantageous to some extent because it enables males to disperse and locate mates (Boivin et al. 2005) apart from partial sibmating and local mate competition (Mackauer and Völkl 2002). Moderate synspermatogeny also has been reported in a number of other parasitic hymenopteran families (Nadel and Luck 1985; review by Boivin et al. 2005). Second, because females are monandrous it could be difficult for *A. ervi* males to find a number of virgin females and mate several times within a day in the field. Therefore, *A. ervi* males may seldom suffer sperm depletion in the field. The reduction of daughter production in the daily mating experiment may be caused by the reduction in sperm

transfer and quality due to a decrease in males' vigour with the increased age (Srivastava and Singh 1995; King 2000; Jacob and Boivin 2004; Damiens and Boivin 2005) rather than sperm depletion.

In hymenopteran parasitoids, the number of daughters produced may be an estimate of the maximum effective quantity of sperm a male can carry or transfer (Henter 2004). The number of sperm carried by *A. ervi* males at emergence may be detected by the number of daughters produced in the hourly experiment. The mean number of daughters produced was 757.50 ± 72.05 and 920.50 ± 146.50 for males in hourly and daily mating experiments, respectively. The results imply that males can carry 82% mature sperm at emergence and produce only about 18% sperm during adult life. Higher amounts of sperm carried at emergence may enable males to maximize the reproductive fitness of both sexes by taking advantage of more females being inseminated early in their life, because males' vigour usually decreases over their age as discussed above and females carry mature eggs in ovaries and start oviposition soon after emergence.

In conclusion, females are pro-synovigenic and males are at least moderately synspermatogenic in *A. ervi*, supporting Boivin et al.'s (2005) prediction. The fact that *A. ervi* adults do not feed on hosts (unpublished observation) and both sexes produce gametes during the adult stage suggests that adult food sources containing amino acids may boost egg production and sperm replenishment in this parasitoid. As a result, supply of adult food such as pollen may increase reproductive potential of both sexes in this species. Aging affects sexes in different ways: for old males, diminishing sperm vigor may be the main cause of the reduced daughter production while for old females constraints of egg fertilization process in relation to aging may be the reason for the lower daughter proportions. Information provided in this study should be taken into consideration in manipulating optimal daughter production in both mass-reared and field populations of *A. ervi*.

CHAPTER 5
MATING BEHAVIOUR AND SEXUAL SELECTION OF *APHIDIUS ERVI*

5.1 Introduction

Mating is a central component of sexual reproduction in insects. Sexual reproduction involves separate reproductive strategies for males and females. Since Darwin (1859) highlighted the evolutionary importance of competition for fertilising the female ova, the study of mating behaviour and sex selection has received massive attention. In Hymenoptera, unmated females lay unfertilised haploid eggs that develop into males, and mated females can lay either unfertilised eggs that develop into males or fertilised diploid ones that develop into females (Quicke 1997). Thus, the mating behaviour contains fundamental information needed to understand parasitoids' reproductive biology, and the discovery of the traits selected for mating preference by males and females provides valuable information necessary for the development of biological control strategies.

5.2 General Methods

5.2.1 Data Recording

Experiments were conducted in Petri dishes (8.5 cm diameter × 1.3 cm height) that maintained experimental parasitoids. The mating behaviour was observed for 10 minutes and recorded using a Panasonic SVHS Camcorder (MS-4) (Panasonic, Japan) connected with a Samsung digital video cassette recorder (DVD-V530, Korea) and images were viewed on a Panasonic colour monitor (TC-21T1Z, Japan) (Figure 5.1). Experiments were carried out in the first four hours after light was on at 8:00am as *A. ervi* generally mate in the morning (Mackauer 1969).

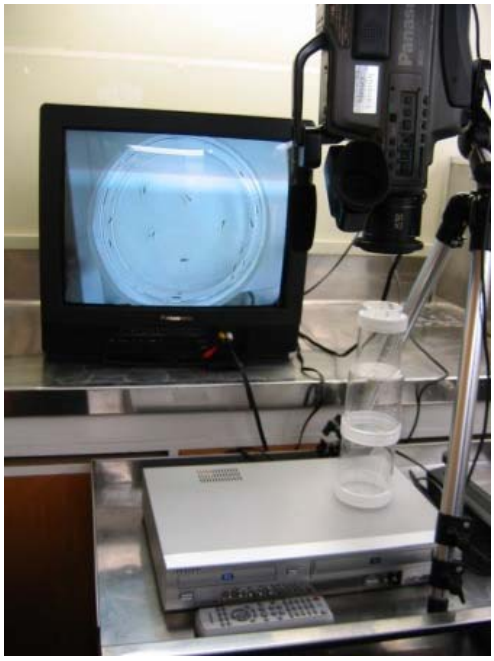

Figure 5.1 Equipment used for recording mating behaviour.

5.2.2 Behaviour Definition

Behavioural interactions between male and female parasitoids were defined as followings:

(a) Courtship - a male rapidly fanned his wings within 1 cm from a female;
(b) Approaching - a male approached a female with his wings fanning;
(c) Mounting - a male mounted a female;
(d) Homosexual behaviour - a male courted and mounted another male;
(e) Mating success - a male inserted his aedeagus into a female's genitalia and copulated;
(f) Mate competition - a male mounted another male when the latter had mounted and/or was mating with a female;
(g) Precourtship period - the period of a male needed to start wing fanning after he was released into a Petri dish where a female was preset;
(h) Homosexual period - the period of a male performing courtship display to and mounting another male;
(i) Courtship period - the period between initiation of wing fanning and mating;
(j) Competition period - the period of a male mounting another male that had mounted and/or was mating with a female;
(k) Mating period - the period from mating initiation to termination.

5.3 Mating Behaviour and Effect of Male Mating History on Mating Success in *A. ervi*

5.3.1 Introduction

Studies on mating behaviour are essential for understanding the reproductive behaviour of a parasitoid species. Mating behaviour can be studied for practical purposes, for example, the development of efficient techniques for the mass-rearing of large numbers for field or glasshouse release in a biological control program (Hardy et al. 2005). A major problem with mass-rearing of parasitoids is an overproduction of males (Waage et al. 1985; Waage 1986). A study of mating behaviour can help solve this problem and provide the key to the optimal production of males. Thus it can provide valuable information for the implementation and development of biological control programmes.

The primary mating behaviour of *A. ervi* has been described (Spencer 1926; Mackauer 1969; Battaglia et al. 2002; McClure et al. 2007) and three steps are identified: (1) females release sex pheromones, (2) males detect the sex pheromone and court the female, and (3) mating. The effect of male mating history on the reproductive fitness of both sexes has been studied (Section 4.5). However, no quantitative information on the behavioural sequences is available, making it difficult to understand the interactions between males and females and between males.

Aphidius ervi is a solitary endophagous parasitoid species with females being monandrous and males polygynous. Because its hosts are gregarious, it is a quasi-gregarious species (i.e. solitary species that develop in hosts that are gregarious) (Mackauer and Völkl 1993) where partial local mating may be common (Hardy et al. 2005). Under natural field conditions, virgin females may encounter mated and/or virgin males, and male-male competition may occur. Therefore, mated and virgin males and virgin females were used for this experiment. The aim of the section was to investigate the mating behaviour of *A. ervi* based on observations of male-female and male-male interactions in relation to male mating history.

5.3.2 Materials and Methods

Parasitoids emerged from aphid mummies that were parasitised at third instar (3 d old) and were all 1 day old when used.

To observe *A. ervi* mating behaviours defined in Section 5.2.2, a virgin male and a mated male were introduced into an above mentioned Petri dish containing a virgin female. Mating sequences were observed and recorded for 10 minutes as mentioned above. For each replicate, before introduced into the Petri dish, one of the two test males was randomly selected and marked on the front wings with Radiant Color (Magruder Color Company, NJ). The mated males were used 1 h later after individually copulating with a virgin female. Sixty-five replicates were performed.

5.3.3 Statistical Analysis

The number of mated and virgin males that undertook wing fanning, approaching, homosexual behaviour, mounting, competition and mating was analysed using a Chi-square test. A goodness-of-fit test was used to test the distribution of following data before analysis. Data on precourtship period were not normally distributed even after transformation and thus analysed using the nonparametric Kruskal-Wallis test. Data on homosexual behaviour, competition and mating period were normally distributed and analysed using ANOVA. Data on courtship period were square-root transformed prior to ANOVA.

5.3.4 Results

5.3.4.1 General Mating Behaviour

The observed behaviours of all individuals were pooled to illustrate the general mating behavioural sequences of *A. ervi* (Figure 5.2). The mating behaviours can be divided into two phases: courtship and mating. Courtship included male wing fanning, approaching and mounting.

After released into a Petri dish, males walked randomly in the dish and about 50 seconds later, 86% of them became excited and started fanning their wings upon encounter or detection of virgin females. When a male first approached a female with his fanning wings and attempted to mount the latter, the female often ran or flew away. After an average of 4.2 ± 0.4 attempts (mean ± SE), the male usually mounted the female successfully from rear or side, crawled forward, and grasped the female on the thorax and abdomen with his fore- and mid-legs. The male moved his antennae rapidly from side to side and touched those of the female. If the female rejected the male, she walked and tried to prevent the contact of their external genitalia by bending her abdomen downward. When the female accepted the male, she lowered her antennae and held them still, and raised her abdomen. When the female was in her receptive position, the male crawled backward, curved his abdomen, and inserted his aedeagus into her genitalia. The female usually terminated the mating by moving her antennae and walking away. After mating, both sexes usually cleaned their heads, mouthparts and antennae using their forelegs. There were 13.8 ± 4.6% males that did not perform courtship behaviour in 10 minutes. Among the courting males, 48.3 ± 14.4 and 42.9 ± 12.0% of them successfully mounted and mated with the females, respectively. Mating lasted an average of 59.8 ± 1.1 seconds.

When the courting males encountered other males, 30% of them showed homosexual behaviour by displaying courtship behaviours to each other. A male might compete for mating by mounting another male that had already mounted a female, displaying the same courtship behaviour and attempting to mate with the female. This behaviour might cause the couple to deleave from each other if mating had not occurred. However, in most cases (15/16), the male that first mounted the female would mate, and once the mating occurred, the subsequent male could not split the couple.

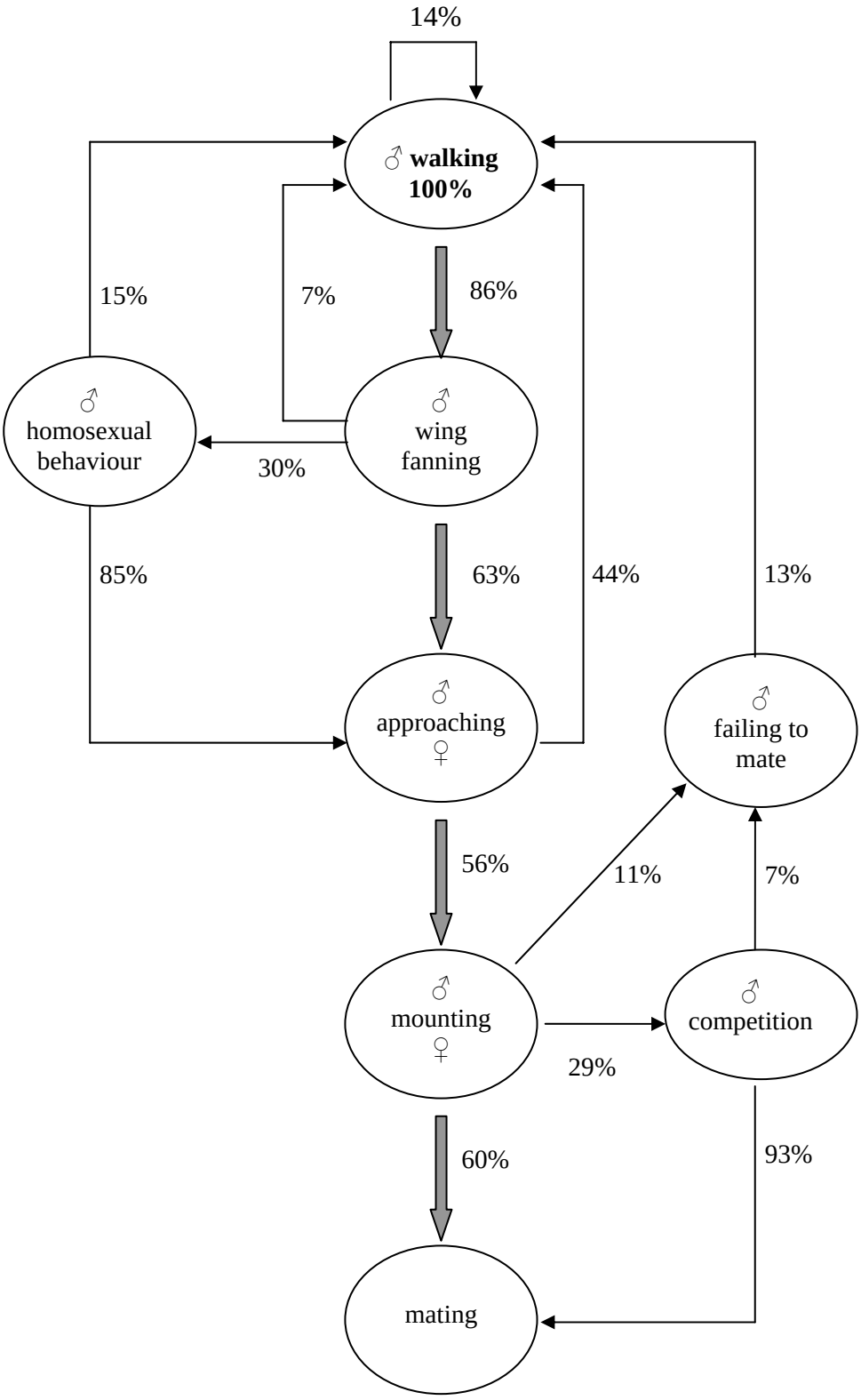

Figure 5.2 Mating behavioural sequences of *A. ervi*. Probabilities of a particular transition between stages are given.

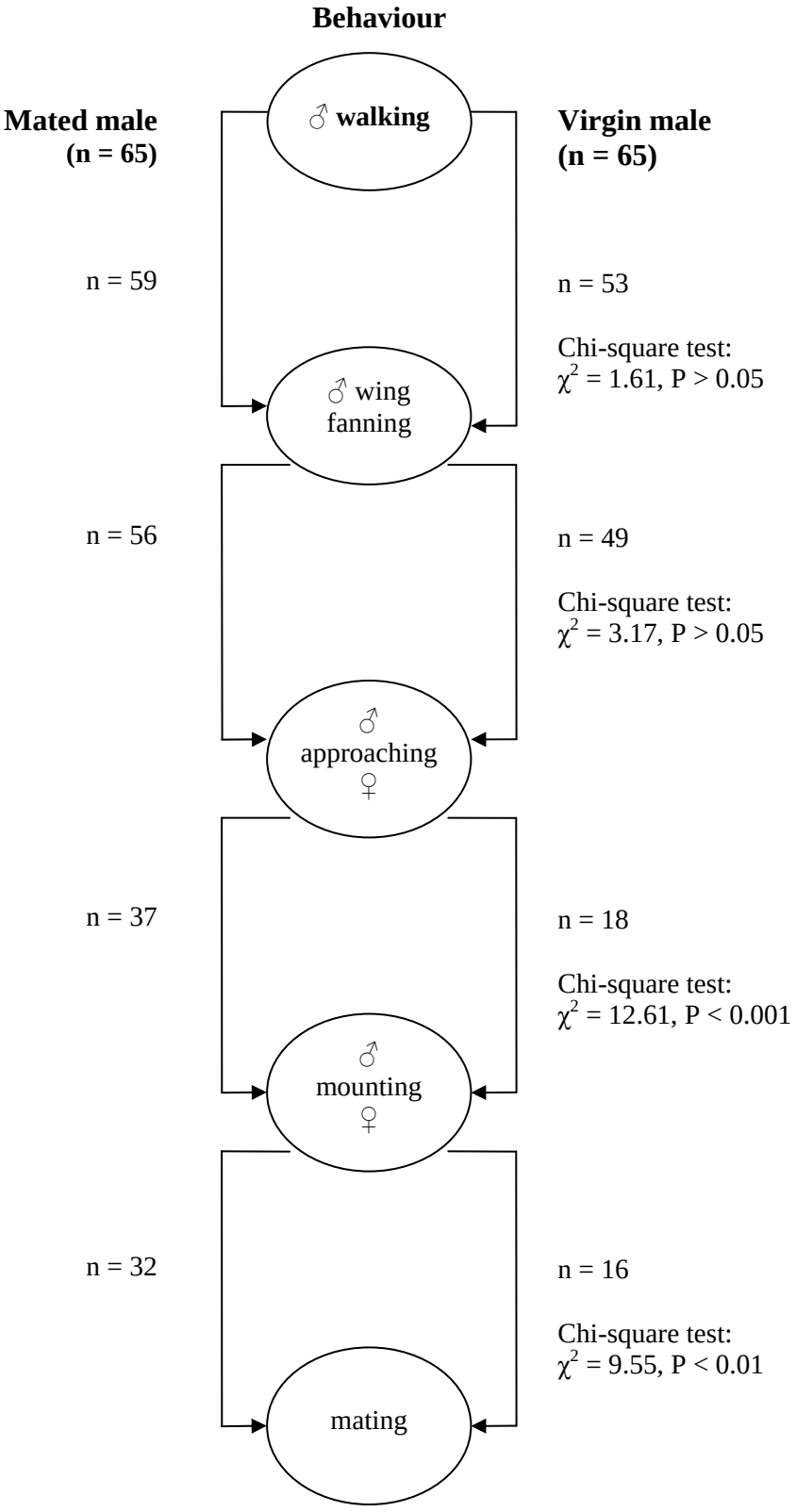

Figure 5.3 Comparison of the number of courtships and matings displayed by mated and virgin males when competing for a virgin female in 10 minutes. n = the number of males.

5.3.4.2 Mating Success in Mated and Virgin Males

The number of males that displayed courtship behaviours with wing fanning and approaching females was not significantly different between mated and virgin males (Figure 5.3). However, compared to the virgin males, mated males were significantly more likely to mount and mate with the females (Figure 5.3).

Mated males had significantly shorter precourtship (Kruskal-Wallis test: H = 4.35 > $\chi^2_{1,0.05}$ = 3.84; df = 1,110; P < 0.05) and courtship period (ANOVA: F = 5.43; df = 1,46; P < 0.05) than did virgin males (Table 5.1). However, there was no significant difference in mating period between mated and virgin males (ANOVA: F = 3.39; df = 1,46; P > 0.05) (Table 5.1).

Table 5.1 The mean (± SE) period (seconds) of *A. ervi* males performing mating behaviours.

	Precourtship	Courtship	Mating
Mated male	36.10 ± 4.35 b	134.00 ± 17.20 b	58.50 ± 3.35 a
Virgin male	64.77 ± 11.65 a	200.33 ± 33.71 a	63.13 ± 4.84 a

Means (± SE) followed by the same letters in columns are not significantly different (P > 0.05).

5.3.4.3 Male-Male Interaction

There was no significant difference in the number of males preforming homosexual (n = 13 and 16, for mated and virgin males, respectively) and mating competition behaviours (7 mated and 9 virgin males, respectively) between mated and virgin males (χ^2 = 1.433 and 0.001 for homosexual and mating competition behaviours, respectively; df = 1,110; P > 0.05). After mating occurred, no mated or virgin males could separate the mating couples.

Virgin males spent a slightly longer period in homosexual behaviour and mating competition but no significant difference was found when compared with

mated males (ANOVA: F = 0.097 and df = 1,17 for homosexual period; F = 1.519 and df = 1,14 for competition period; P > 0.05) (Table 5.2).

Table 5.2 The mean (± SE) period (seconds) of homosexual and competition behaviour performed by *A. ervi* males.

	Homosexual	Competition
Mated male	62.56 ± 15.56 a	52.25 ± 10.51 a
Virgin male	69.67 ± 16.65 a	69.13 ± 8.78 a

Means (± SE) followed by the same letters in columns are not significantly different (P > 0.05).

5.3.5 Discussion

Behavioural observations indicate that males appear to be more active than females in the mating behavioural sequence of *A. ervi*. This may be because a virgin female can reproduce without mating but the success of male reproduction absolutely relies on, and is mediated by females (Quicke 1997). Moreover, as detected in *A. nigripes* (McNeil and Brodeur 1995), *A. ervi* females release a sex pheromone that triggers the male behavioural sequence of wing fanning, approaching and mounting (Battaglia et al. 2002; McClure et al. 2007). My results show that the courtship behaviour by males (i.e. wing fanning and approaching) is non-random, indicating that in *A. ervi*, females produce signals and males do the searching.

Tactile stimulation may play an important role in mating success of *A. ervi*. The male moved his antennae rapidly from side to side and contacted those of the female. This behaviour seemed to have quietened the female. Previous reports show that males of many hymenopteran wasps have glands in their antennae (Isidoro and Bin 1995; Isidoro et al. 1996; Battaglia et al. 2002). Males of *A. ervi* emit secretion from their antennae when they are exposed to virgin females; the secretion functions specifically as a contact pheromone and the antennae play a pivotal role in sex recognition and partner acceptance (Battaglia et al. 2002).

In theory, female choice is clearly an important driving force in sexual selection in animals, and a few examples of adaptive choice are known in natural enemies, particularly fly predators such as the black-tipped hanging fly *Hylobittacus apicalis* (Hagen) (Thornhill 1988) and scorpion fly *Panorpa vulgaris* Imhoff and Labram (Thornhill and Sauer 1991). However, in parasitoids there is no evidence that females exhibit any active choice (Hardy et al. 2005). A virgin parasitoid female will usually mate with the first conspecific male encountered, for example, in a gregarious parasitoid *N. vitripennis* (van den Assem et al. 1980). In the present study, *A. ervi* females usually mated with the first mounting males regardless of their virginity. This may be because of the difficulty of finding another suitable partner (van den Assem et al. 1986). As found in other *Aphidius* species (Mackauer 1969), *A. ervi* females usually became receptive after several approachings by males. It is still not clear whether the females have means to assess their partners' quality during this period.

Obvious male aggression behaviour was not observed in *A. ervi*, but male-male interactive behaviour such as male courtship display and mating attempt to the rival was common when males encountered each other. Homosexual mounting between males is found in a number of insect species including aphidiids (Starý 1970; Thornhill and Alcock 1983; van den Assem 1986; Kaneshiro and Giddings 1987; Gillespie 1991; Wang et al. 1996; Serrano et al. 2000; Cheng et al. 2003; Switzer et al. 2004). It is expected that mated males have learnt from the previous mating in discerning the potential mates and rivals and avoid homosexual mounting; however, results indicate the frequency of homosexual mounting between mated and virgin males was similar. Homosexual interactions between males in *A. ervi* may be elicited by the female sex pheromone, as reported in another aphidiid, *Praon aguti* Smith (Sekhar 1957; Starý 1970). Visual cues may also result in male mistake in courtship in some insects (Thornhill and Alcock 1983; Wang et al. 1996), including some hymenopteran parasitoids (van den Assem 1986). According to Thornhill and Alcock (1983), althought males are at the risk of making some mistakes, mounting in *A. ervi* may be an important process in discriminating a mate.

Male mating experience is a factor that affects mating success in *A. ervi*. Results of hourly mating experiments (Section 4.5) indicate that females can produce similar number of female offspring when mated with virgin males or once-mated

males, thus females are not expected to have preference between virgin and once-mated males for mating. In the present study, virgin males invested more energy in courtship (i.e. wings fanning and approaching) for courting females than mated males. Mated males responded to females more quickly and performed fewer approaches to females before mounting, and achieved more matings. These suggest that mated males have learnt from the previous mating, so that they respond to females more quickly and perform better courtships than naïve males. It has been reported that in some hymenopteran parasitoids (Perez-Lachaud and Campan 1995; Fischer and King 2005) and other insects (Schwartz 1991; Cook 1995) male mating experience enhances mating success. However, McClure et al. (2007) indicated that under no-choice situation, *A. ervi* males that have previously mated have longer pre-courtship period but have similar mating success when compared to virgin males of similar age. Thus, it is suggested that under competitive situation, mated *A. ervi* males may sense the presence of rivals, court females quickly and then achieve better mating success.

5.4 Mate Choice of *A. ervi* in Relation to Body Size and Age

5.4.1 Introduction

Mate choice is an important factor in the mating system that influences the reproductive fitness of both sexes. According to Darwin (1859), females should be choosier than males, because eggs cost more to produce than sperm, females mate fewer times than males and females endure most of the parental care in order to achieve the maximum fitness return. This leads to the conclusion that females have more to lose from suboptimal matings than males do. However, in Hymenoptera, virgin females can still produce progeny without mating; success of male reproduction absolutely depends on whether they can inseminate the females.

In general, studies on mate choice have focused on female choice of males and paid less attention to male choice of females (Rosenqvist and Berglund 1992; Ahnesjö et al. 1993; Jennions and Petrie 1997, 2000; Bonduriansky 2001). Male choice of females has been reported for a range of taxa, although few have been studied in hymenopteran species (Bonduriansky 2001). Studies on mate choice in both sexes in a single insect species are uncommon (Bonduriansky 2001), especially in parasitoid species (Godfray 1994; King et al. 2005). In many parasitoids such as *A. ervi*, mate choice is poorly known. Body size and age of *A. ervi* males and females are two important factors that affect the reproductive fitness (see Sections 4.4 and 4.5). This section investigated the role of body size and age in the mate choice for both sexes of *A. ervi*.

5.4.2 Materials and Methods

5.4.2.1 Influence of Body Size on Mate Choice

The body size of “small” (S) and “large” (L) adults of both sexes was defined in Section 4.4.2.1. To investigate whether and to what extent mate body size influenced mate choice, four treatments were set up: (1) small female × (small male + large male), i.e. SF × (SM + LM); (2) large female × (small male + large male), i.e. LF × (SM + LM); (3) SM × (SF + LF); and (4) LM × (SF + LF). Parasitoids were all

virgin and one day old when used for testing. There were 80, 75, 70 and 75 replicates for treatments (1), (2), (3) and (4), respectively. To observe the mate choice behaviour, in each replicate, female(s) and male(s) were released into a closed Petri dish and mating sequences were observed and recorded for 10 minutes (Section 5.2.1).

The number of small and large males that undertook courtship (wing fanning), mounting and mating was analysed using a Chi-square test. A goodness-of-fit test was used to test the distribution of other data (i.e. precourtship, courtship and mating periods) before ANOVA. Data were base-10 logarithm transformed before ANOVA if they were not normally distributed.

5.4.2.2 Influence of Mate Age on Mate Choice

Experimental parasitoids emerged from aphid mummies that were parasitised at third instar (3 d old). To determine whether mate age affected mate choice, two treatments were set up: (1) one 1-d-old male + three females of 1, 3 and 5 days of age; (2) one 1-d-old female + three males of 1, 3 and 5 days of age. There were 78 and 61 replicates for treatments (1) and (2), respectively. To observe mate choice behaviour, female(s) and male(s) were released into a closed Petri dish and mating sequences were observed and recorded for 10 minutes (Section 5.2.1). Before introduced into the Petri dish, two of the three test mates of different ages were randomly selected and marked on their front wings with Radiant Color (Magruder Color Company, NJ). In this experiment, parasitoids were fed with 10% honey solution. They were all virgin and females did not lay eggs (no aphids provided) before testing.

The Marascuilo procedure of the nonparametric analysis (Daniel 1990) was used to assess the number of males performing mating behaviours (courtship, mounting and mating). The rejection level was when $U_0^{'} > \chi^2_{2,0.05} = 5.99$. A goodness-of-fit test was used to test the distribution of other data. Data of the mean number of a male approaching a female were not normally distributed even after transformation and thus analysed using the nonparametric Kruskal-Wallis test. All other data were analysed using ANOVA followed by a Tukey's studentized range (HSD) test. Data of

precourtship and courtship periods were base-10 logarithm transformed before ANOVA.

5.4.3 Results

5.4.3.1 Influence of Body Size on Mate Choice

When a small male and a large male were provided to a female, similar numbers of small and large males courted, mounted and mated regardless of female size ($P > 0.05$) (Table 5.3). No significant difference was detected in precourtship period between the larger and small males (Table 5.4). Compared to small males, large males had significantly shorter courtship and mating periods regardless of female size (Table 5.4).

When a small female and a large female were provided to a male, large males started courtship display (i.e. wings fanning) significantly earlier than did small ones (precourtship period = 45.95 ± 2.40 and 52.86 ± 7.90 seconds, n = 66 and 59 for large and small males, respectively) (ANOVA: $F = 5.58$; $df = 1,123$; $P < 0.05$). Small males did not have significant preference for mates of different size for mating (Chi-square test: $\chi^2 = 0.63$ and 1.67 for mounting and mating, respectively; $df = 1$; $P > 0.05$) but large males significantly preferred large females for mounting and mating (Chi-square test: $\chi^2 = 4.32$ and 7.35 for mounting and mating, respectively; $df = 1$; $P < 0.05$) (Tables 5.5). Regardless of male size, both courtship and mating periods were significantly longer when mating with large females than when mating with small females (Table 5.6).

Table 5.3 The number of small and large males (selectees) preformed mating behaviour in response to females (selectors) in *A. ervi*.

Selectees	Selectors					
	Small female (n = 80)			Large female (n = 75)		
	courtship	mounting	mating	courtship	mounting	mating
Small males	61 a	31 a	24 a	59 a	29 a	21 a
Large males	68 a	38 a	32 a	63 a	33 a	29 a

Chi-square test: χ^2 = 1.44, 0.92 and 1.35 for courtship, mounting and mating, respectively for small females; χ^2 = 1.10, 0.25 and 1.47 for courtship, mounting and mating, respectively for large females; df = 1; P > 0.05.

Table 5.4 The mean (± SE) period (seconds) of mating behaviours performed by males (selectees) to the females (selectors) in *A. ervi*.

Selectees	Selectors					
	Small female			Large female		
	Precourtship*	Courtship*	Mating	Precourtship*	Courtship	Mating
Small male	76.31 ± 8.54 a	202.63 ± 26.49 a	60.13 ± 1.25 a	73.56 ± 7.60 a	262.86 ± 29.15 a	65.52 ± 2.03 a
Large male	69.65 ± 9.34 a	117.16 ± 12.50 b	54.00 ± 1.17 b	61.27 ± 9.51 a	120.45 ± 11.67 b	58.83 ± 1.48 b
F	2.57	9.82	12.53	2.96	25.36	11.58
df	1, 127	1, 54	1, 54	1, 120	1, 48	1, 48
P	> 0.05	< 0.01	< 0.001	> 0.05	< 0.0001	< 0.001

Means (± SE) followed by the same letters in columns are not significantly different (ANOVA: P > 0.05).

* Data were base-10 logarithm transformed before ANOVA.

Table 5.5 The number of males (selectors) preformed mating behaviour in response to small and large females (selectees) in *A. ervi*.

Selectees	Selectors			
	Small male (n = 70)		Large male (n = 75)	
	mounting	mating	mounting	mating
Small female	24 a	19 a	23 b	17 b
Large female	27 a	24 a	33 a	29 a

Numbers followed by the same letters in columns are not significantly different (P > 0.05).

Table 5.6 The mean (± SE) period (seconds) of mating behaviours performed by males (selectors) to the females (selectees) in *A. ervi*.

Selectees	Selectors			
	Small male		Large male	
	Courtship*	Mating	Courtship*	Mating
Small female	92.95 ± 13.17 b	54.37 ± 1.92 b	86.24 ± 8.42 b	51.18 ± 1.81 b
Large female	167.71 ± 21.45 a	67.50 ± 2.25 a	123.76 ± 12.52 a	59.45 ± 1.46 a
F	10.14	18.48	4.41	12.30
df	1,41	1,41	1,44	1,44
P	< 0.01	< 0.001	< 0.05	< 0.001

Means (± SE) followed by the same letters in columns are not significantly different (ANOVA: P > 0.05).

* Data were base-10 logarithm transformed before ANOVA.

5.4.3.2 Influence of Mate Age on Mate Choice

When a 1-d-old male was provided with three females of different ages, 74.4% (58/78) of males courted. The number of males approaching 1- and 3-d-old females was significantly higher than that approaching 5-d-old females (Kruskal-Wallis Test: H = 9.32; df = 2,150; P < 0.01) (Table 5.7). Males tended to mount younger females more often but there was no significant difference in mounting between females of different ages ($U_0^{'}$ = 3.24 < $\chi^2_{0.05,2}$ = 5.99; df = 2; P > 0.05); however, upon mounting,

the probability of males dismounting their mates was significantly higher when they mounted 3- and 5-d-old females than when they mounted 1-d-old ones ($U_0^{'}$ = 11.47; df = 2; P < 0.001) (Table 5.7). Males significantly preferred young than old females for mating ($U_0^{'}$ = 10.49; df = 2; P < 0.001). Within 10 minues observation period, significantly more males recourted and remated (with other females) after they had mated with 3- or 5-d-old females than after they mated with 1-d-old females ($U_0^{'}$ = 37.45 and 29.51 for recourting and remating, respectively; df = 2; P < 0.0001) (Table 5.7).

Table 5.7 Mating behaviour of *A. ervi* males in the presence of females of different ages.

Female age	No. of approaching	No. of mounting	Dismounting % (n*)	No. of mating	Recourting % (n)	Remating % (n)
1 d old	2.25 ± 0.18 a	24 a	4.1 (1) b	23 a	16.7 (4) b	28.3 (2) b
3 d old	2.45 ± 0.21 a	20 a	30.0 (6) ab	14 ab	78.6 (11) a	71.4 (10) a
5 d old	1.66 ± 0.20 b	15 a	40.0 (6) a	9 b	88.9 (8) a	66.7 (6) a

Means (± SE) followed by the same letters in columns are not significantly different (P > 0.05). *n was the number of males performed dismounting, recourting or remating behaviour.

Males started to court an average of 49.47 ± 4.25 seconds (n = 58) after being released into the Petrel dishes and recourted 212.48 ± 14.69 seconds (mean, n = 23) after the termination of first mating. The mean courtship period was 128.78 ± 9.80 and 102.22 ± 22.17 seconds for the first and second mating, respectively. In the first mating, mating period was significantly longer when males mated with 1-d-old females than with 5-d-old ones (ANOVA: F = 6.31; df = 2,43; P < 0.01) (Table 5.8). However, no significant difference was found in mating duration between different female ages in the second mating (ANOVA: F = 0.72; df = 2,15; P > 0.05) (Table 5.8).

Table 5.8 Mean (± SE) mating period (seconds) of males when they mated with females of different ages.

	Female age		
	1 d old	3 d old	5 d old
1st mating	53.08 ± 1.73 a	49.64 ± 1.59 ab	42.67 ± 2.46 b
2nd mating	41.71 ± 2.68 a	43.25 ± 2.29 a	39.14 ± 1.71 a

Means (± SE) followed by the same letters in lows are not significantly different (P > 0.05).

When a 1-d-old virgin female was offered to three virgin males of different ages, significanly more 1-d-old males courted, mounted and mated with the females than did 5-d-old males ($U_0^{'}$ = 14.71, 10.91 and 7.77 > $\chi^2_{2,0.05}$ = 5.99 for mounting and mating, respectively; df = 2; P < 0.05) (Table 5.9). Before mounting, the 1-d-old male approached the female significantly more frequently than did 5-d-old male (mean ± SE = 3.34 ± 0.22, 2.97 ± 0.28 and 2.46 ± 0.24 for 1-, 3- and 5-d-old males, respectively; Kruskal-Wallis Test: χ^2 = 6.33; df = 2,106; P < 0.05). The 1-d-old males had significantly shorter precourtship period but mated for a significantly longer period than did 5-d-old males (Table 5.10). There was no significant difference in courtship period between the courting males of different ages (Table 5.10).

Table 5.9 Number of males of different ages that courted, mounted and mated with 1-d-old females.

Male age	Courtship	Mounting	Mating
1 d old	47 a	20 a	16 a
3 d old	35 ab	12 ab	10 ab
5 d old	28 b	7 b	6 b

Numbers followed by the same letters in columns are not significantly different (P > 0.05).

Table 5.10 The mean (± SE) period (seconds) of males of different ages performing mating behaviours to 1-d-old females in *A. ervi*.

Male age	Precourtship	Courtship	Mating
1 d old	37.17 ± 7.48 b	135.63 ± 19.64 a	58.50 ± 1.73 a
3 d old	45.57 ± 9.45 ab	177.20 ± 21.51 a	54.20 ± 1.25 ab
5 d old	60.29 ± 10.14 a	156.33 ± 24.35 a	51.00 ± 2.22 b
F	3.66	1.37	4.03
df	2,107	2,28	2,29
P	P < 0.05	P > 0.05	P < 0.05

Means (± SE) followed by the same letters in columns are not significantly different (ANOVA: P > 0.05).

5.4.4 Discussion

Previous studies have demonstrated that large *A. ervi* males have more sperm supply during mating (Section 4.4). Results of this section (Section 5.5) indicate that large males had shorter courtship and mating periods (Table 5.4) which may help them to gain access to mates and reduce the predation risk comparing to small males. However, when provided two males of different size, females did not significantly prefer large males for mating (Table 5.3). Generally, female mating preferences may be due to direct benefits which increase females' fecundity or survival as detected in some other insect species (Huignard 1983; Simmons and Gwynns 1993; Jiménez-Pérez and Wang 2004). In *A. ervi*, however, mating does not increase females' fecundity and longevity, and females do not obtain a nutritional contribution from males during copulation (Section 4.4). Moreover, *A. ervi* is arrhenotokously parthenogenetic, where inseminated females produce both sons and daughters and virgin females can still give birth to sons. Finally, *A. ervi* females do not suffer sperm insufficiency (i.e. proportion of female offspring larger than 50%) when they mate with small or large males (Section 4.4). Therefore, it is not surprising that *A. ervi* females do not show any observable active mate choice.

There have been many studies showing that larger males have a mating advantage over smaller males in mating competition and success, but most of them

have described aggressive male-male competition, defence of territory, or resource provision (Alcock 1979, 1984; McCauley 1979; Hughs and Hughs 1982; Johnson 1982; Karban 1983; Thornhill and Alcock 1983; Juliano 1985; Alcock andGwynne 1987; Vencl and Carlson 1998; Bateman 2000), and such behavioural characters are not observed in *A. ervi*. Thus it is suggested that similar to *Lariophagus distinguendus* (Förster) (van den Assem et al. 1989), body size is not important for *A. ervi* males to compete successfully.

When provided two females of different size, both small and large males preferred large females to small ones for mating, although they invested longer time to court the larger females (Table 5.6). This is probably because large females have higher reproductive potential and thus males mating with large females obtain maximum reproductive fitness return in terms of their female progeny (Section 4.4). Moreover, when compared to small males, large males were more likely to mate with large females, which may be attributed to males' superior behaviours (i.e. courted earlier with shorter courtship period). This may also explain the greater success of large males in mate searching when compared to small males, which has been demonstrated in other parasitoid species (van den Assem et al. 1989; Kazmer and Luck 1995).

Regardless of female size, larger males had shorter copulations than did small males. Regardless of male size, males copulated longer when they mated with larger females than with small ones. This may be a general rule in insects including parasitoids, for example, *L. distinguendus* (van den Assem et al. 1989). Usually insects will terminate mating when the maximum sperm has been transferred, or when the spermatheca is full (van den Assem et al. 1989). The relationship between body size of mates and copulation duration may be explained by three assumptions: (1) larger females have a larger spermatheca, (2) larger males have more sperm supply, and (3) larger males transfer more sperm per unit of time (van den Assem et al. 1989). The shorter copulation duration detected for larger males but longer copulation duration for larger females (this study) and greater number of daughters produced by larger males and females (Section 4.4) corroborates these assumptions. Thus, larger males may transfer more sperm at a faster rate than do small males, and males may need longer time to fill up a larger female's spermatheca. It is expected that the

shorter copulation plus shorter courtship period may enable larger males to have sufficient time in replenishing sperm and searching for a new mate. Thus, large body size seems to directly benefit a male's fertilization success because of more frequent matings, which has been found in many hymenopterans (van den Assem et al. 1989; Kazmer and Luck 1995; Sagarra et al. 2001) and also other insect species (Pitnick 1991; Warner et al. 1995; Danielsson 2001).

Results indicate that mate choice is influenced by mate age. *A. ervi* males were more likely to approach and mount young females. *A. ervi* females release a sex pheromone that triggers males' behavioural sequence such as wing fanning, approaching and mounting (Battaglia et al. 2002; McClure et al. 2007). However, as reported in hymenopterans (Simser and Coppel 980; McNeil and Brodeur 1995; Schworer et al. 1999) and other insect species (Teal et al. 1990; del Mazo-Cancino et al. 2004; Showler et al. 2005), sex pheromones produced and released by *A. ervi* females decrease with their age (McClure et al. 2007). Therefore, older females are less attractive to the males. On the other hand, because sperm is a limited resource in *A. ervi* males (Section 4.5), males preferring young females for mating have greater reward as younger females have greater potential fecundity due to their longer longevity and thus will produce more daughters. As a result, although males did not suffer sperm limitation, they dismounted the old females more frequently, shortened the mating period, and were more likely to perform the second mating when they mated with old females. It is suggested that males could discriminate the quality of their mates and optimize the use of their sperm based on the reproductive status of females (Pitnick and Markow 1994; Srivastava and Singh 1995).

Male age of *A. ervi* had a negative effect on courtship and mating success (Tables 5.8 and 5.9). As reported by McClure et al. (2007), under no-choice conditions, significantly fewer 8-d-old males performed courtship behaviour (wing fanning and approaching) and mate successfully than did 1-d-old males. These may be the results of decreased ability to recognize and respond to females' sex pheromone (Vetter and Visscher 1997; McClure et al. 2007) or decreased activity with aging (Cheng et al. 2003). The fact that young males achieve more matings than do old ones has also been reported in another parasitoid species, *Cephalonomia tarsalis* (Ashmead) (Cheng et al. 2003). It is expected that young males transfer more sperm than old ones

during copulation, which may explain why younger males were more likely to be accepted by females for mating.

5.5 Operational Sex Ratio and Density Influence Partial Local Mating Behaviour in *A. ervi*

5.5.1 Introduction

Hardy (1994) summarised three mating strategies in the hymenopteran parsitoids: (1) panmixis, which assumes that individuals mate randomly throughout the population (Fisher 1930); (2) fully local mating or local mate competition (LMC), which assumes that all matings are confined to the natal patch and only mated females disperse (Hamilton 1967); and (3) partial local mating, which is defined as an intermediate mating structure between panmixis and fully local mating (Hardy 1994). Theoretical and empirical studies have shown that LMC is common in parasitoid groups with female-biased sex ratio (King 1987; Godfray 1994; Hardy 1994). Martel and Boivin (2004) indicate that whether LMC occurs in a species depends on whether the species is truly solitary, quasi-gregarious or gregarious. The difference between solitary, quasi-gregarious or gregarious has been discussed in Section 4.5.

Partial local mating is influenced by the operational sex ratio (OSR) which is defined as the ratio of males searching for mates to females available for mating (Hardy et al. 2005). The OSR composes of two component variables, the male and female densities (Alonso-Pimentel and Papaj 1996). Local population density is defined as the total number of individuals that are ready to mate in a given space (Wang et al. 2008). OSR and local population density are temporally and spatially dynamic in the natural environment (Lawrence 1986; Krupa and Sih 1993; Wang and Chen 2005). Because parasitoid females are usually monandrous and males polygynous, the number of females available for insemination is usually far less than the number of males ready to mate (Hardy et al. 2005). Therefore, the OSR can be very male-biased even in those cases where the primary sex ratio is female-biased (van den Assem et al. 1980; Werren 1980). The influence of OSR and local population density on mating behaviour has been well studied in many insects (Arnqvist 1992; Parker and Simmons 1994; Jobloński and Vepsäläinen 1995; Alonso-Pimentel and Papaj 1996; Simmons 2001; Wang et al. 2008) but little is known in parasitoid species. Knowledge of how OSR and population density affect LMC and

mating success of parasitoids may provide valuable information in optimizing sex ratio in mass rearing programmes.

Aphidius ervi is an arrhenotokously reproducing species, and females are monandrous and males are polygynous. The field populations of *A. ervi* are female-biased (65.7%) (Sequeira and Mackauer 1993) and parasitised aphids often show a clumped distribution (Mackauer and Völkl 1993). Furthermore, males and females may emerge in temporal and spatial synchrony (Section 3.3). These conditions may favor local mating before dispersal. According to Hardy (1994) and van den Assem (1996), although the assumption of fully local mating may be incorrect for *A. ervi*, partial local mating may be common. This section investigated the effect of OSR and population density on the mating behaviour of *A. ervi*.

5.5.2 Materials and Methods

5.5.2.1 Experimental Insects

Parasitoids used in this study emerged from aphid mummies parasitised at third instar (3 d old). Parasitoids were all virgin and females did not lay eggs before being used for experiment.

5.5.2.2 Experimental Design

To determine how OSR and population density affected mating behaviour of *A. ervi*, four experiments were set up as follows:

Exp. A, fixed female density (n = 3) with increasing sex ratios (♂:♀): 1:3, 3:3, 6:3, 9:3, 12:3 and 15:3 with 20, 16, 15, 15, 16 and 16 replicates, respectively;

Exp. B, fixed male density (n = 3) with decreasing sex ratios (♂:♀): 3:1, 3:3, 3:6, 3:9, 3:12 and 3:15 with 16, 16, 15, 15, 16 and 14 replicates, respectively;

Exp. C, even sex ratio (1♂:1♀) with increasing density: 3:3, 6:6 and 9:9 with 16, 15 and 17 replicates, respectively;

Exp. D, fixed overall density with various sex ratios (♂:♀): D_1 (density = 12), 3:9, 6:6 and 9:3 with 15 replicates for each treatment; D_2 (density = 18), 3:15, 9:9 and 15:3 with 14, 17 and 16 replicates, respectively.

In each replicate, experimental parasitoids were released into an above mentioned Petri dish, and mating sequences were observed and recorded for 10 minutes as described above. Data presented in this section were the mean number of behavioural events (i.e. homosexual behaviour, mate competition and mating success) which occurred in a 10-minute period. The probability of female remating, and mating durations of first and second matings by females were also recorded.

5.5.2.3 Statistical Analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Data of mating duration were normally distributed and analysed using ANOVA followed by Tukey's studentized rang (HSD) test. Other data that were not normally distributed even after transformation were analysed using the nonparametric Kruskal-Wallis test. The interactions between male and female densities affecting homosexual behaviour, mate competition, mating success and mating duration were tested using the CCD model as described in Section 4.4. A log likelihood ratio test (McCullagh and Nelder 1989) was then applied to determine whether density of sexes had different effect on the mating behaviour of parasitoids. The Marascuilo procedure of the nonparametric analysis (Daniel 1990) was used to assess the frequencies of female remating with a rejection level of $U_0' > \chi^2_{2,0.05} = 5.99$.

5.5.3 Results

5.5.3.1 Homosexual Behaviour

The number of homosexual events depended on OSR, male density and population density (Table 5.11). It increased significantly with increasing male density when female density fixed to 3 (Kruskal-Wallis Test: H = 53.98 > $\chi^2_{4,0.05}$ = 9.49; df = 4,73; P < 0.0001) and when population density was 12 and 18 (Kruskal-Wallis Test: H = 36.36 and 38.49 98 > $\chi^2_{2,0.05}$ = 5.99; df = 2,42 and 2,44 for population density of 12 and 18, respectively; P < 0.0001). When the sex ratio was even (i.e. 1♂:1♀), the number of homosexual events significantly increased with the increasing population density (Kruskal-Wallis Test: H = 18.85 > $\chi^2_{2,0.05}$ = 5.99; df = 2,45; P < 0.0001). However, when male density was 3, increasing female density significantly reduced the number of homosexual events (Kruskal-Wallis Test: H = 16.23 > $\chi^2_{5,0.05}$ = 11.07; df = 5,86; P < 0.01).

The CCD model also indicates that both male and female densities significantly affected the homosexual behaviour of *A. ervi* (CCD: F = 163.98; df = 4,181; P < 0.0001; R^2 = 0.7837) (Figure 5.4A) with male density positively and female density negatively effecting the number of homosexual events (likelihood rate test: χ^2_2 = 1080.09; P < 0.0001) (Figure 5.4B).

Table 5.11 Effect of OSR and population density on mating behaviour of *A. ervi*.

OSR (♂:♀)	Number of homosexual events	Number of mate competition events	Number of mating successes	Mating period (seconds)
Exp. A: fixed female density (n = 3) with increasing sex ratios				
1:3			0.65 ± 0.11 c	52.77 ± 1.28 b
3:3	0.63 ± 0.24 c	0.55 ± 0.21 b	0.88 ± 0.18 bc	53.71 ± 1.47 b
6:3	5.13 ± 1.13 bc	2.30 ± 0.40 ab	0.93 ± 0.23 bc	56.36 ± 1.32 b
9:3	11.00 ± 1.00 ab	2.43 ± 0.84 ab	1.60 ± 0.24 a	61.94 ± 1.36 ab
12:3	15.44 ± 1.72 a	3.31 ± 0.41 a	1.31 ± 0.20 ab	63.43 ± 1.69 ab
15:3	16.63 ± 1.14 a	3.64 ± 0.75 a	1.31 ± 0.24 ab	67.10 ± 1.86 a
Exp. B: fixed male density (n = 3) with decreasing sex ratios				
3:1	1.81 ± 0.55 a	0.56 ± 0.18 a	0.56 ± 0.13 d	61.44 ± 3.02 a
3:3	0.63 ± 0.24 ab	0.55 ± 0.21 a	0.88 ± 0.18 cd	53.71 ± 1.47 a
3:6	0.33 ± 0.19 ab	0.54 ± 0.18 a	1.47 ± 0.22 bc	56.27 ± 2.63 a
3:9	0.27 ± 0.12 ab	0.85 ± 0.33 a	2.07 ± 0.25 ab	57.13 ± 1.82 a
3:12	0.25 ± 0.11 ab	0.38 ± 0.13 a	2.56 ± 0.13 a	56.32 ± 1.73 a
3:15	0.07 ± 0.07 b	0.29 ± 0.16 a	2.36 ± 0.17 a	56.91 ± 2.47 a
Exp. C: even sex ratio (1♂:1♀) with increasing population density				
3:3	0.63 ± 0.24 b	0.55 ± 0.21 b	0.88 ± 0.18 c	53.71 ± 1.47 b
6:6	2.33 ± 0.35 b	2.73 ± 0.41 a	2.07 ± 0.34 b	59.58 ± 2.17 ab
9:9	5.29 ± 1.07 a	3.24 ± 0.55 a	4.12 ± 0.43 a	63.30 ± 2.82 a
Exp. D: fixed population density with varying sex ratios				
D_1: population density = 12				
3:9	0.27 ± 0.12 c	0.85 ± 0.33 b	2.07 ± 0.25 a	57.13 ± 1.82 a
6:6	2.33 ± 0.35 b	2.73 ± 0.41 a	2.07 ± 0.34 a	59.58 ± 2.17 a
9:3	11.00 ± 1.00 a	2.43 ± 0.84 a	1.60 ± 0.24 a	61.94 ± 1.36 a
D_2: population density = 18				
3:15	0.07 ± 0.07 c	0.29 ± 0.16 b	2.36 ± 0.17 b	56.91 ± 2.47 b
9:9	5.29 ± 1.07 b	3.24 ± 0.55 a	4.12 ± 0.43 a	63.30 ± 2.82 ab
15:3	16.63 ± 1.14 a	3.64 ± 0.75 a	1.31 ± 0.24 c	67.10 ± 1.86 a

In each experiment, means (± SE) followed by the same letters in columns are not significantly different (P > 0.05).

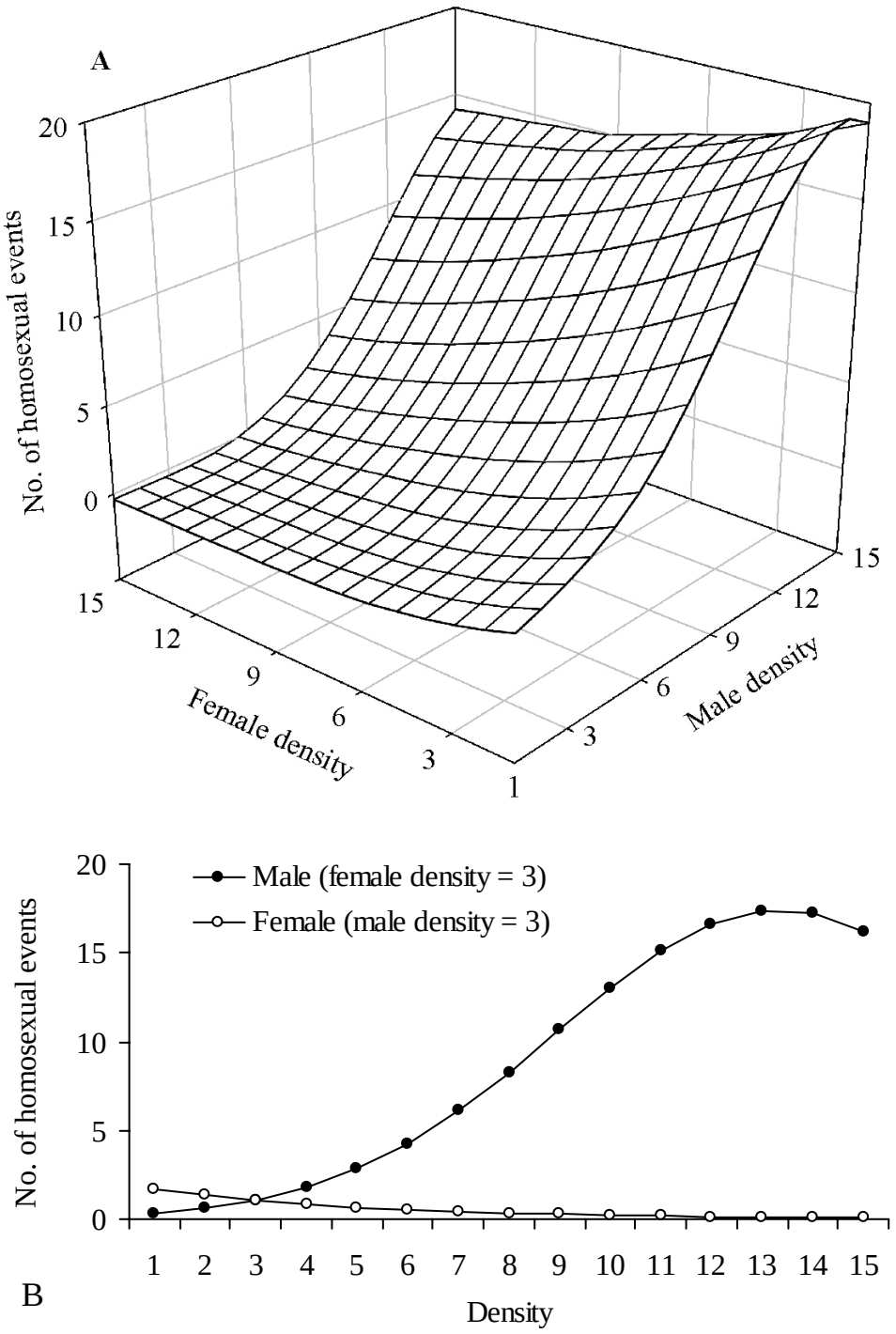

Figure 5.4 Interactions of male (χ_m) and female (χ_f) densities affecting homosexual behaviour in *A. ervi*: (A) number of homosexual events performed by males in combinations of different male and female densities ($y = \exp(- 0.9372 + 0.6421\chi_m - 0.0262\chi_m^2 - 0.2866\chi_f + 0.0188\chi_m\chi_f)$; (B) predicted number of homosexual events affected by male and female densities.

5.5.3.2 Competition for Mate

As shown in Table 5.11, the number of mate competition events increased significantly with increasing male density when female density was fixed to 3 (Kruskal-Wallis Test: H = 17.17; df = 4,60; P < 0.01), when population density was 12 (Kruskal-Wallis Test: H = 8.31; df = 2,39; P < 0.05) and 18 (Kruskal-Wallis Test: H = 13.06; df = 2,39; P < 0.01) or when the OSR was even (Kruskal-Wallis Test: H = 14.47; df = 2,40; P < 0.001). However, when male density was fixed, no significant difference was found in the number of competition events between various OSRs (Kruskal-Wallis Test: H = 4.34; df = 5,70; P > 0.05).

Both male and female densities significantly affected mate competition behaviour of *A. ervi* (CCD: F = 26.28; df = 4,157; P < 0.0001; R^2 = 0.4011) (Figure 5.5A) but male density had significantly more effect on competition behaviour than did female density (likelihood rate test: χ_2^2 = 34.28; P < 0.0001) (Figure 5.5B). The number of mate competition events was higher when female density was from 6 to 9 and male density from 12 to 15 (Figure 5.5A).

5.5.3.3 Mating Success

Mating success depended on the density of both sexes (Table 5.11, Figure 5.6). Mating success increased significantly with increasing density of males from 1 to 9 and females from 1 to 12, after which no future increase occurred (Kruskal-Wallis Test: H = 14.82 and 51.46 and df = 5,92 and 5,86 for male and females, respectively; P < 0.01). The likelihood rate test indicated that female density had significantly more effect on mating success than did male density (χ_2^2 = 9.84; P < 0.0001) (Figure 5.6B). At even OSRs, mating success increased significantly with increasing population density (Kruskal-Wallis Test: H = 25.65; df = 2,45; P < 0.0001) (Table 5.11). At the population density of 12, no significant difference was found between OSRs (Kruskal-Wallis Test: H = 2.28; df = 2,42; P > 0.05); however, at the population density of 18, significantly higher mating success was detected in the OSR of 9♂:9♀ (Kruskal-Wallis Test: H = 24.58; df = 2,47; P < 0.0001) (Table 5.11).

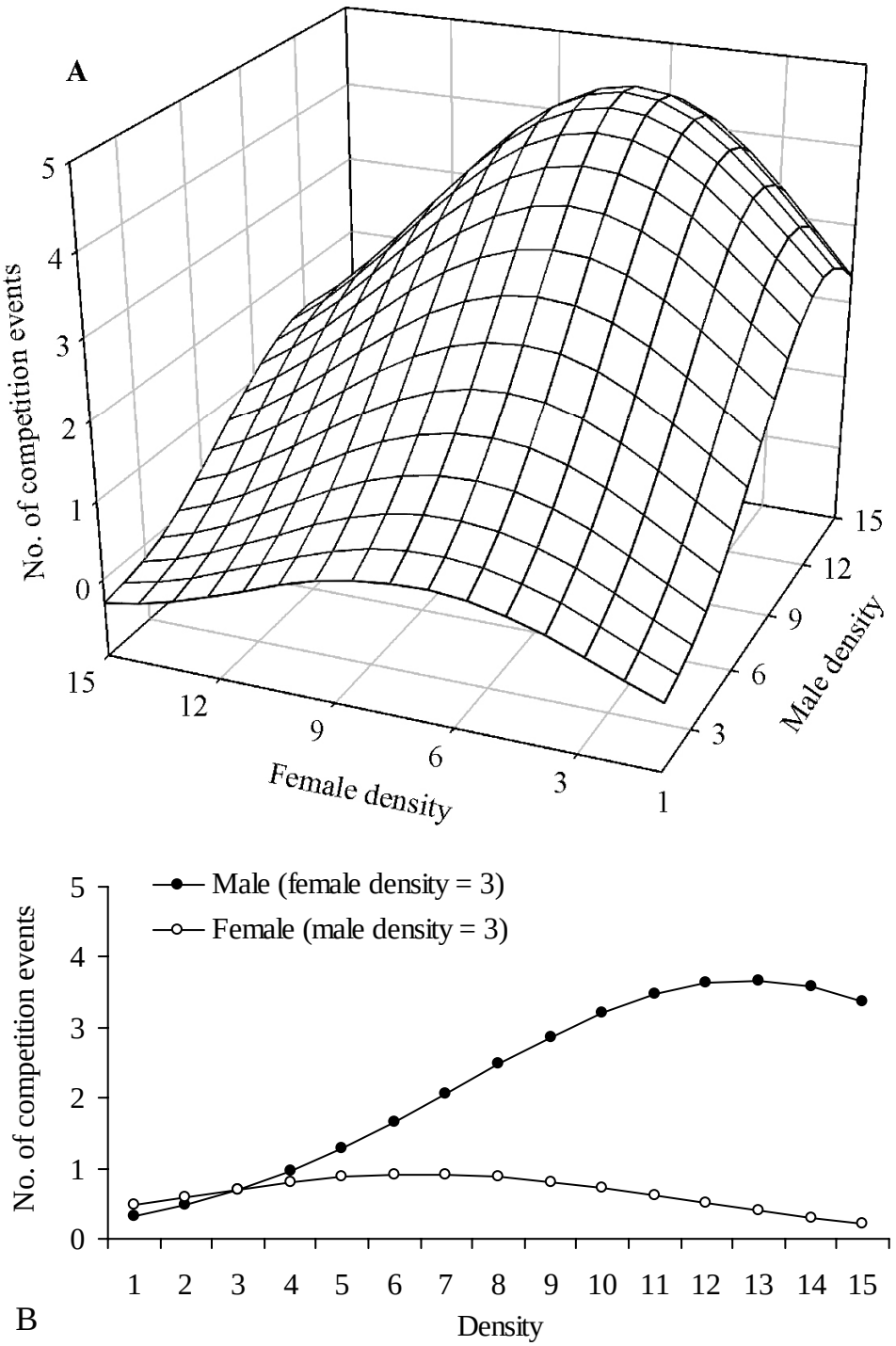

Figure 5.5 Interactions of male (χ_m) and female (χ_f) densities affecting mate competition in *A. ervi*: (A) number of mate competition events performed by males in combinations of different male and female densities ($y = \exp(-2.1592 + 0.4421\chi_m - 0.0173\chi_m^2 + 0.2742\chi_f - 0.0208\chi_f^2)$; (B) predicted number of mate competition events affected by male and female densities.

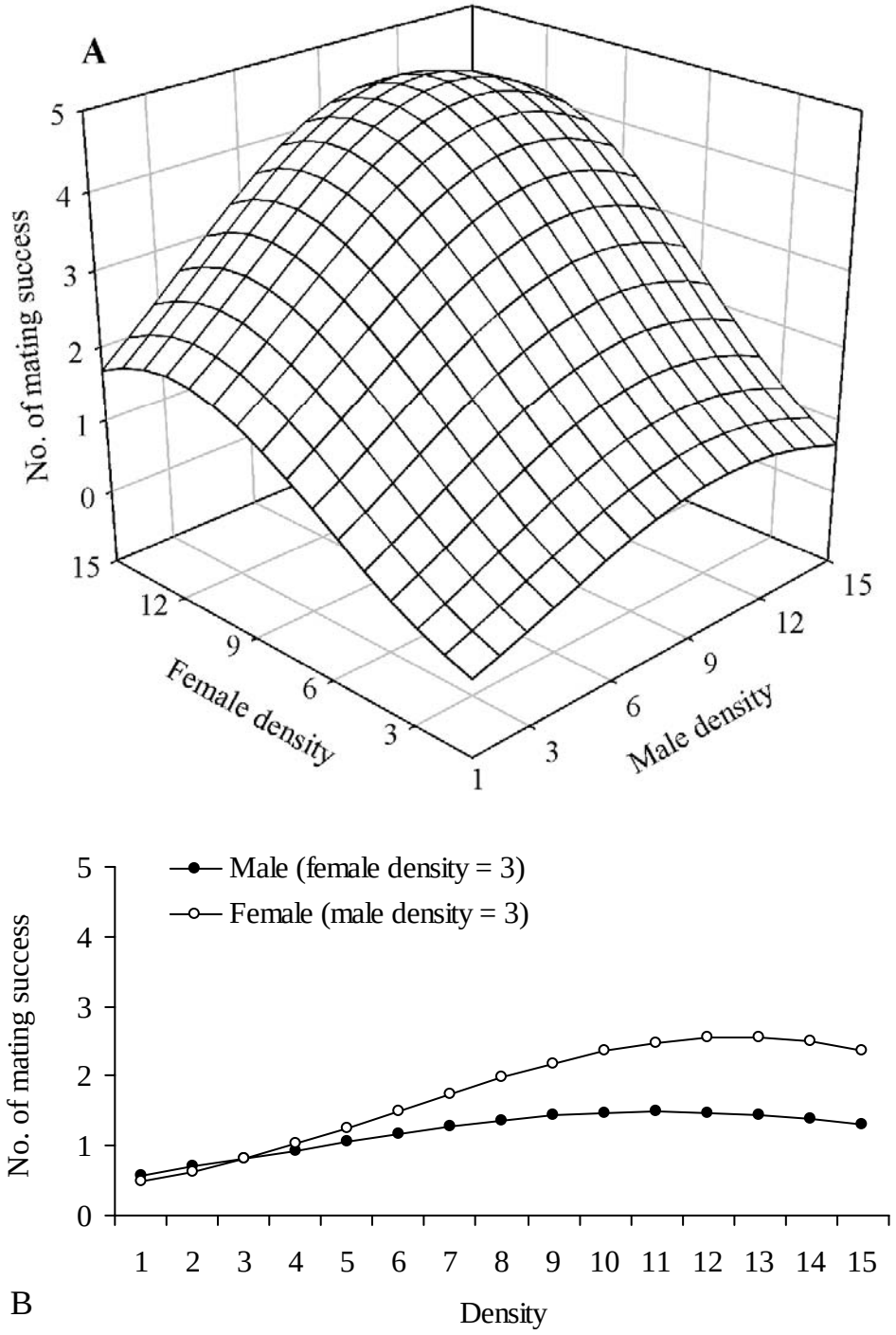

Figure 5.6 Interactions of male (χ_m) and female (χ_f) densities affecting mating success in *A. ervi*: (A) number of mating successes in combinations of different male and female densities (CCD: $y = \exp(-1.5704 + 0.2042\chi_m - 0.0092\chi_m^2 + 0.3159\chi_f - 0.0126\chi_f^2$; $F = 38.40$, $df = 4,201$, $P < 0.0001$, $R^2 = 0.4332$); (B) predicted number of mating successes affected by male and female densities.

5.5.3.4 Mating Period

At the fixed female density of 3, mating period significantly increased with male density (ANOVA: $F = 5.25$; $df = 5,103$; $P < 0.01$); but at the fixed male density of 3, increasing female density had no effect on mating period (ANOVA: $F = 0.52$; $df = 5,145$; $P > 0.05$) (Table 5.11). The CCD model and likelihood rate test also indicate that only male density had significant effect on mating period (CCD: $F = 25.73$, $df = 1,291$, $P < 0.0001$, $R^2 = 0.0805$; likelihood rate test: $\chi_2^2 = 605.74$, $P < 0.0001$) (Figure 5.7). At even OSR, mating period significantly increased with increasing population density (ANOVA: $F = 3.52$; $df = 2,57$; $P < 0.05$), and at the population density of 18, mating became significantly longer with the increasing male density (ANOVA: $F = 3.45$; $df = 2,78$; $P < 0.05$) (Table 5.11).

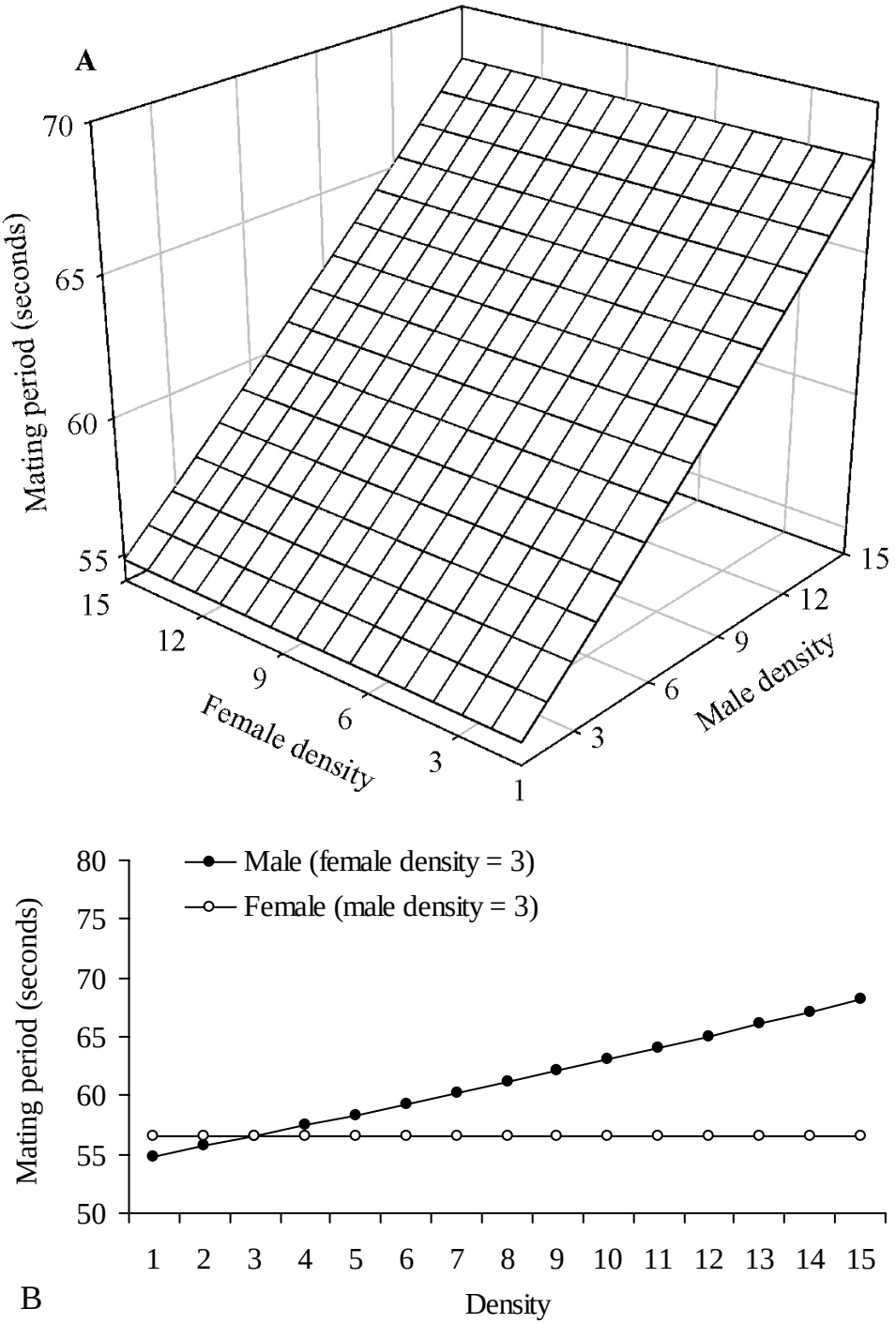

Figure 5.7 Interactions of male (χ_m) and female (χ_f) densities affecting mating period in *A. ervi*: (A) mating period in combinations of different male and female densities (y = exp(3.9891 + 0.0155χ_m); (B) predicted mating period affected by male and female densities.

5.5.3.5 Female Remating

Female remating was observed in most OSRs with the exceptions of 1:3, 3:1 and 3:15 (♂:♀) (Table 5.12). The proportion of mated females mounted by other males and females remated was significantly higher in male-biased OSRs than in female-biased ones (Table 5.12). Females remated 50.67 ± 9.39, 47.08 ± 3.66 and 47.50 ± 3.60 seconds after the first mating at female-biased, even and male-biased OSRs, respectively, with no significant difference (ANOVA: F = 0.09; df = 2,27; P > 0.05). For the remated females, mating period in the second mating was significantly shorter than in first mating (Table 5.13).

Table 5.12 The proportion of mated females mounted by other males and females remated in *A. ervi* in various OSRs.

OSR (♂:♀)	Total no. mated females	Mated females mounted by other males%	Females remated%
Female-biased (3:6, 3:9, 3:12)	94	3.19 b (n* = 3)	3.19 b (n = 3)
Even (3:3, 6:6, 9:9)	115	11.30 b (n = 13)	11.30 ab (n = 13)
Male-biased (6:3, 9:3, 12:3)	81	30.86 a (n = 25)	17.28 a (n = 14)
$U_0^{'}$		28.03	12.52
df		2	2
P		< 0.0001	< 0.01

Percentage values followed by the same letters in columns are not significantly different (P > 0.05). *n is the number of mated females mounted by other males or remated.

Table 5.13 The mean (± SE) mating period (seconds) of *A. ervi* females in the first and second matings.

	Female-biased OSR	Even OSR	Male-biased OSR
First mating	55.67 ± 7.31 a	60.54 ± 4.67 a	71.36 ± 4.40 a
Second mating	17.00 ± 7.00 b	31.15 ± 5.15 b	30.07 ± 5.74 b
F	14.59	17.86	32.62
df	1,4	1,24	1,26
P	P < 0.05	P < 0.001	P < 0.0001

Means (± SE) followed by the same letters in columns are not significantly different (ANOVA: P > 0.05).

5.5.4 Discussion

In *A. ervi*, the OSR of 1♂:3♀ may reflect the sex ratio in field condition (i.e. 65.7% females, Sequeira and Mackauer 1993). However, OSR varies from female-biased to male-biased because of the declining number of virgin females. Results of this study show that *A. ervi* males tended to have more homosexual and competition events with the increasing of OSR and male density, especially when the OSR skewed to be male-biased. The increasing male homosexual and male mate competition events may be due to the increasing encounter rate between searching males. Thornhill and Alcock (1983) proposed an evolutionary interpretation of insect homosexual mounting behaviour. They assume that if the male's main cost of reproduction is the time consumed in discerning the sex of potential mates, increasing the number of mountings per unit of time may be more advantageous rather than carefully choosing a partner of the right sex. Competition for mate increasing with OSR may be a general rule which has been demonstrated in many animals including insects (Hamilton 1979; Lawrence 1986; Enders 1993; Krupa and Sih 1993; Dick and Elwood 1996; Michner and Mclean 1996; Parker and Simmons 1996; Clutton-Brock et al. 1997; Jennions and Petrie 1997; Flanagn et al. 1998; Jirotkul 1999, Abe et al. 2003; Shuker et al. 2006). Mating success increased with the increasing density of males or females and population density in even OSR. This may be because of the increasing encounter rate between males and females leading to the easier mate location. Furthermore, CCD model and the likelihood rate test indicated that male

density had more effect on homosexual behaviour than did female density, but female density had more effect on mating success than did male density. It is further confirmed that production of highly female-biased offspring is advantageous to *A. ervi* in successful reproduction and improves mass-rearing programmes.

Generally, the switching-off of parasitoid females' receptivity following the end of a mating results from the presence of sperm in the spermatheca or the additional substances introduced by the male during copulation (Manning 1962; Merle 1968). It has been found in many parasitoid species, immediately following mating no further receptive signaling or response could be provoked, for example, in the *Spalangia cameroni* Perkins, *Asaphes vulgaris* Walker and *Anogmus strobilorum* Thompson (van den Assem 1986). Previous observations (personal observation in Section 4.5) showed that *A. ervi* females were unattractive to males one hour after mating. In this study, most mated females of *A. ervi* (> 82%) were unattractive to males soon after mating and no longer mated, indicating that they have switched off their receptivity. Furthermore, males are less likely to mount and mate with mated females, suggesting that *A. ervi* males may be able to discriminate females' mating status. Male discrimination against females' mating status has been demonstrated in some parasitoid species such as *L. distinguendus* (Ruther et al. 2000) and *Spalangia endius* Walker (King et al. 2005).

Female unreceptivity and male discrimination may benefit both sexes (van den Assem 1986; King et al. 2005). To mated females of *A. ervi*, because they have received sufficient sperm during the first mating (Sections 4.4 and 4.5), preventing males from mating may enable them to concentrate on host searching and oviposition. To males, female remating may provide low fitness to them because possible first-male sperm precedence may occur (El Agoze et al. 1995). Sperm precedence has not been examined in *A. ervi*, but first-male sperm precedence has been reported in many hymenopteran species (Wilkes 1966; Parker 1970; Holmes 1974; van den Assem et al. 1989; Allen et al. 1994; El Agoze et al. 1995). El Agoze et al. (1995) indicated that the difference in sperm precedence level between species may depend on variables such as the number of matings in nature and the time between successive matings. Results in this study have demonstrated that a mated *A. ervi* female is usually unattractive to males after 1 minute following a mating, and remating in females in

the field is thus rare. Therefore, females switching off receptivity soon after mating and male discrimination of females' mating status may reduce the probability of males mating with the already mated females and hence, allow them to avoid the sperm wasting and save time and energy for searching for other mates.

However, in some hymenopteran species, female unreceptivity after a mating is a gradual process which proceeds with time. For example, in *N. vitripennis*, responsiveness is switched off within 1 minute following the termination of mating (King 1960), and when more courtship is preformed by males, a second or third receptive signal could be provoked (van den Assem and Visser 1976). Results in this study showed that some females (< 18%) were still receptive to other males within 1 minute after the first mating. Moreover, in this study mating period of females in the second mating was significantly shorter than that in the first mating, and a short mating period of about 50 seconds (Section 5.4) is sufficient to fertilise a female's life (Section 4.4). Thus female remating may be due to the gradual process of switching off female receptivity rather than the insufficient sperm transferred during the first mating. A small proportion of females that remated (3.2~6.7%) is also reported in *Aphytis melinus* DeBach (Allen et al. 1994). However, McClure et al. (2007) reported that in *A. ervi*, 25% (4/16) females remated 24 hours after the first mating. They supposed that remating in their study is possibly the result of an unsuccessful first mating or due to a low quality mate. Results from Sections 4.4 and 4.5 indicate that one mating of females could fertilise more than 50% of their fecundity, and thus do not support McClure et al.'s (2007) supposition.

Obvious post-copulatory guarding is not common in the majority of parasitoid species (van den Assem 1986). In this and previous (Sections 5.3 and 5.4) studies, postcopulatory courtship and contact or non-contact mate guarding were not observed after the termination of mating. Therefore, mate guarding, if any, should occur during copulation in this species. Like some insects (Arnqvist 1992; Parker and Simmons 1994; Jobloński and Vepsäläinen 1995; Alonso-Pimentel and Papaj 1996; Simmons 2001; Wang et al. 2008), *A. ervi* prolonged copulation duration under male-biased OSR and high male density. The reason may be that when the OSR and male density increase, the probability that a male will find another mate decreases but possibility that his mate will remate increases.

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSION

6.1 Introduction

In this thesis, I report how *A. ervi* reduces aphids' reproductive potential, investigate the factors that affect the reproductive fitness of parasitoids and examine the mating behaviour of *A. ervi*. Such knowledge is important for a better understanding of biological control ecology of *A. ervi*.

In this chapter, I summarise and discuss my main findings and their relevance to the reproductive biology of *A. ervi* in order to provide valuable information necessary for the development of biological control strategies.

6.2 Reproductive Rhythms of *A. ervi*

In insects, adults' circadian activities are usually correlated with reproductive rhythms, such as mating and oviposition. *A. ervi* is an arrhenotokous and quasi-gregarious species (Section 4.5) with monandrous females and polygynous males (Mackauer 1969). Males' reproductive success mainly depends on the number of mates they inseminate. Males emerge earlier but become sexual mature later than females. On the basis of the above reproductive property of *A. ervi*, the emergence pattern is advantageous to males where they can coincide with the sexual receptivity of females and mate before the latter disperse and start oviposition.

Moreover, *A. ervi* adults usually emerge during the first few hours of the photophase and females mainly oviposit throughout the photophase. Virgin females may start oviposition within 30 min after emergence (unpublished data). Females attack more aphids and lay more eggs in aphids in the photophase than in the scotophase, suggesting that besides the chemical and physical cues (Vinson 1984; Schmidt 1991; Vet and Dicke 1992; Turlings et al. 1993; Godfray 1994; Quicke 1997), *A. ervi* females may also employ visual cues in host searching and oviposition (Battaglia et al. 1995). The oviposition pattern of *A. ervi* females ensures they reserve

energy and produce mature gametes during the scotophase. Therefore, the emergence of *A. ervi* synchronises the rhythmic function of mating and oviposition.

6.3 Reproduction of *A. ervi* in Relation to Host Age and Density

Aphidius ervi significantly reduces the reproductive potential of pea aphids in terms of longevity and progeny production (Section 3.4). Aphids parasitised in their early instars (1 to 3 d old) die before reproduction but those parasitised at later instar stages (4 to 6 d old) may reach the adult stage and produce a limited number of progeny before mummification; parasitised adults (7 to 10 d old) have significantly shorter reproductive period and produce significantly fewer progeny. These results suggest that attacking young hosts will be more effective in suppressing aphid populations.

However, *A. ervi* females prefer aphids that are 3 to 5 d old over the younger (1 and 2 d old) and older (6 and 7 d old) aphids for oviposition (Section 4.3). It is found that females that oviposit in 4- to 7-d-old aphids have more fitness gains in terms of progeny body size and egg load in female progeny (Section 4.3) and sperm load in male progeny at emergence (detected by proportion of female progeny in Section 4.4), but older aphids are more capable of physically defending themselves and escaping from parasitisation and thus cost more energy and time to the females in oviposition. Therefore, females' preference for 3- to 5-d-old aphids for oviposition may be based on the optimal balance between costs in terms of host defense and fitness gains in terms of progeny quality. Moreover, *A. ervi* more likely deposits fertilised eggs in large hosts and unfertilised eggs in small ones, supporing the host size-dependent sex allocation theory in parasitic Hymenoptera (Charnov et al. 1981; Charnov 1982).

The success of a parasitoid species in suppressing host populations greatly depends on its ability to find and parasitise hosts and to increase offspring numbers in response to increasing host density (Waage and Hassell 1982; Mackauer 1983). The number of aphids parasitised and eggs laid by *A. ervi* significantly increases with an

increase of host density. However, the number of eggs laid in each parasitised aphid significantly decreases (from 2.9 to 1.3 eggs) with the increase of host density from 15 to 75 aphids. These results suggest that the parasitoid adjusts oviposition strategy in response to increasing host density, and a host density equivalent to 50 aphids is the highest critical density where a parasitoid could maximise its control efficiency in 24 h period. Moreover, the highest proportion of female progeny is produced at host densities of 50 and 75 aphids, suggesting that the sperm limit occurs when host density reaches these density levels. However, at low host density of 15 and 25 aphids, the proportion of female progeny is also low, indicating that *A. ervi* females can adjust the sex allocation strategy in response to the host density rather than the limitation of sperm supply; this may be advantageous to parasitoids to persist under low host density conditions (Tripathi and Singh 1991).

On the basis of the above reproductive strategy, it is expected that parasitoids released in the early season when growing populations have a high proportion of young aphids (Schowalter 2000) should be more effective in inhibiting aphids' population build-up than later in the season. Moreover, in the laboratory mass rearing, aphids that are 3 to 5 d old appear to be the appropriate hosts because they give relatively higher quality of progeny, and 50 to 75 aphids/female/day may be provided to parasitoids as hosts to produce higher female progeny populations.

6.4 Factors Affecting Mate Choice and Reproductive Fitness of *A. ervi*

Factors affecting mate choice and reproductive fitness of *A. ervi* include mate body size and age, and male mating history.

Reproductive fitness of both sexes is positively correlated with their body size. For example, large individuals have greater longevity, large males father more progeny, and large females have higher fecundity and parasitism and greater ability in host searching and handling (Section 4.4). Therefore, it is not surprising that when given a choice of small and large females, both small and large males prefer large females for mating (Section 5.4), because these females had higher reproductive

potential and in turn males have maximum reproductive fitness return in term of their female progeny.

With increasing body size, females gain more than males in longevity and fecundity but males gain more than females in the number of daughters produced (Section 4.4). The asymmetrical size-fitness between sexes may be because the different reproductive strategies of different sexes, i.e. the reproductive success of *A. ervi* females directly relies on their potential fecundity and their ability to search and parasitise hosts whereas the success of male reproduction is absolutely mediated by females, and virgin females still reproduce with the absence of males. Thus, when given a choice of small and large males, both small and large females do not show any preference for small or large males for mating (Section 5.4).

The production of daughter declines with mate age at mating. For the aging males, the lower production of daughters may be due to the diminishing sperm viability and mobility related to aging rather than sperm depletion as reported in *L. delhiensis* (Srivastava and Singh 1995). Evidence for this is found in the daily experiment (Section 4.5), where a male could inseminate up to six females with similar number of daughters produced (> 50%) if allowed 24-h recovery period between matings. When three males of different ages compete for one mate, old males achieve significantly fewer matings (Section 5.4). These may be the results of decreased ability to recognize and respond to females' sex pheromone (Vetter and Visscher 1997; McClure et al. 2007) or decreased activity with aging (Cheng et al. 2003).

For the aging females, the reduction of daughter production could be explained by the constraints in fertilization process in *A. ervi* because in parasitoid females, the depletion of spermathecal gland secretion may result in a reduced availability of activated sperm for fertilization of ovulating eggs (Flanders 1946), and eggs may contain certain kind of sperm-attracting chemicals, which decrease with the female age, leading to a reduced fertilization (Pandey et al. 1983). *A. ervi* males may deliver fewer sperm to old females as found in *L. delhiensis* (Srivastava and Singh 1995). Mating behaviour observation (Section 5.4) shows that although 1-d-old males do not suffer from sperm limitation, they dismount the old females more frequently,

shorten the mating period, and are more likely to perform the second mating after they have mated with the old females within a 10 minute period. Results also indicate that between 20 and 40% of females when mating at the age of 5 d old do not produce any daughters (Section 4.5). *A. ervi* males are more likely to approach, mount and mate young females, because younger females have greater potential fecundity. Moreover, in *A. ervi*, females release a sex pheromone that triggers males' behavioural sequence (Battaglia et al. 2002; McClure et al. 2007), but sex pheromones produced and released by females decrease with their age (McClure et al. 2007). Therefore, older females are less attractive to the males.

Mating in *A. ervi* does not increase females' fecundity and longevity (Section 4.4 and 4.5), suggesting that *A. ervi* females do not obtain a nutritional contribution from males during copulation. These results support Godfray's (1994) conclusion that this may be a general rule in hymenopteran parasitoids, which is found in other parasitoid wasps (Madel et al. 1990; Srivastava and Singh 1995; Fauvergue 1998; Cheng et al. 2003; Jacob and Boivin 2004; Khanh et al. 2005).

6.5 Mating Behaviour of *A. ervi*

Aphidius ervi males appear to play a more active role in mating behaviour. This may be because a virgin female can reproduce without mating but the success of male reproduction absolutely relies on, and is mediated by females (Quicke 1997). The courtship behaviours of males (i.e. wing fanning, approaching and mounting) are associated with the release of female sex pheromone (McClure et al. 2007), indicating that in *A. ervi*, females produce signals and males do the searching.

It is found that both virgin and once-mated males father similar number of their progeny (hourly mating experiments in Section 4.5), thus females are not expected to have preference between virgin and once-mated males for mating. However, once-mated males achieve more matings. These results suggest that mated males have learnt from the previous mating so that they may respond to females more quickly and perform better courtships than naïve males (Perez-Lachaud and Campan 1995; Fischer and King 2005).

Homosexual behaviour and mate competition between *A. ervi* males are common and tend to be more intensive when the OSR, male density and population density increase, especially in the extremely male-biased conditions. These may be due to the increasing encounter rate between searching males. Thornhill and Alcock (1983) propose that in insects, male homosexual mounting may be an advantageous strategy of mate location, if the males' main cost of reproduction is the time consumed in discerning the sex of potential mates. Evidence also shows that external stimuli such as visual cues may result in male mistake in courtship in some insects (Thornhill and Alcock 1983; Wang et al. 1996) including hymenopteran parasitoids (van den Assem 1986).

Parasitoid females usually switch off their receptivity following mating due to the presence of sperm in the spermatheca or the additional substances transformed by the male during copulation (Manning 1962; Merle 1968). More than 82% mated females of *A. ervi* are unattractive to males soon after mating, suggesting they have switched off their responsiveness. Switching-off of receptivity by females may benefit both sexes (van den Assem 1986; King et al. 2005). To females of *A. ervi*, because they have received sufficient sperm during the first mating (Sections 4.4 and 4.5), preventing further mating may enable them to concentrate on host searching and oviposition. To males, the female unreceptivity may reduce the probability of males to mate with the already mated females and hence avoid sperm competition.

Under the male-biased OSR, a proportion of females (3~17%) accept the second males for mating within 1 min after the termination of the first mating, suggesting the female's switching-off of receptivity is a gradual process. The shorter mating period in the second mating suggests that female remating could result from the above gradual process rather than the insufficient sperm transformation during the first mating, because one mating is sufficient to fertilise a female for life.

Under the male-biased OSR, high male density or when other rivals compete for mating, *A. ervi* males prolong copulation duration. The reason may be that under these circumstances, the probability that a male will find another mate decreases but possibility that his mate will remate increases. In Aphidiidae, males will successfully

deliver sperm when copulation lasts for longer than 25 seconds (Wiackowski 1962; Straý 1970). Thus, sperm may be added by the second male if *A. ervi* females remate. Therefore, *A. ervi* males' prolonged copulation may serve as mate-guarding in order to reduce the probability of female remating and thus sperm competition.

6.6 Conclusion

In this thesis, I have reported and discussed the main findings of the reproductive behaviour, and factors affecting the reproductive fitness of *A. ervi*. This work has provided a much firmer basis of knowledge of this species, and a rounded perspective of its reproductive biology. Such knowledge is vital, as noted in this thesis, to appraising prospects for further investigation in improvement of biological control strategies.

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Reproductive strategies of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae)

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Abstract

Hymenopteran parasitoids are usually arrhenotokous parthenogenetic, where females arise from fertilized and males from unfertilized eggs. Therefore, the reproductive fitness of females is a function of egg production and furthermore affected by mating, whereas that of males is mainly determined by the number of daughters they father. *Aphidius ervi* Haliday is a quasi-gregarious parasitoid of a number of aphid pests on economically important crops such as legumes and cereals. Females are monandrous whereas males are polygynous. Here, we tested how parental age at mating and male mating history affected mating success, fecundity and daughter production in this species. Once-mated males perform significantly better than naïve males with regard to mating success, suggesting that males learn from previous matings. The fecundity of virgin females is not significantly different from that of mated females regardless of parental age at mating and male mating history, indicating that mating does not stimulate egg production or contribute to female nutrient supply. Males can replenish sperm supply after mating, implying that they are at least moderately synspermatogenic. Preference for young over old mates for mating by both sexes may be explained by the fact that aging of both sexes contributes to the reduction of daughter production. Rather than sperm depletion, the reduced daughter production may be attributed to diminishing sperm viability and mobility in aging males and increasing constraints in fertilization process in aging females. Our results also show that female age has a stronger impact on the production of daughters, suggesting that fertilization process in females is more sensitive to aging than sperm vigor in males.

Keywords: *Aphidius ervi*; Aging; Mating history; Fecundity; Sperm replenishment; Daughter production

1. Introduction

Hymenopteran parasitoids usually show arrhenotoky, where fertilized eggs produce diploid females and unfertilized eggs produce haploid males; Virgin females only produce sons while inseminated females produce both sons and daughters (Quicke, 1997). As a result, the reproductive success of females is primarily affected by egg production patterns and fertilization (Godfray, 1994; Boivin et al., 2005) while that of males is a function of the number of daughters they father (Quicke, 1997; Roitberg et al., 2001; Jacob and Boivin, 2004). For the parasitoid species with monandrous females and polygamous males, the net reproductive gain by females from mating may depend on the age of both sexes at mating as well as male mating history. Male reproductive gain, on the other hand, depends on the age of both sexes at mating, the number of females they inseminate, and oviposition history of their mates. The ways and extent of these effects may vary considerably between sexes as well as between species, depending on their life history strategies (Pandey et al., 1983; Srivastava and Singh, 1995; King, 2000; Jacob and Boivin, 2004; Damiens and Boivin, 2005).

The understanding of reproductive strategies in hymenopteran parasitoids underlies the success of biological control (Gordh and DeBach, 1976; Nadel and Luck, 1985; Godfray, 1994; King, 2000; Roitberg et al., 2001; Jacob and Boivin, 2004; Damiens and Boivin, 2005). In

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pro-ovigenic species, females emerge with their entire egg complement mature, whereas synovigenic females mature eggs during adult stage (Flanders, 1950; Quicke, 1997; Boivin et al., 2005). However, most species probably fall in between these two extremes, and are termed pro-synovigenic, i.e. females carry some mature eggs on emergence but continue to produce and mature additional eggs throughout adult lifespan (Quicke, 1997; Jervis et al., 2001, 2003). These life history parameters may be highly selected based on the expected rate of host encounter (Rosenheim, 1996; Sevenster et al., 1998). However, both pro-ovigenic and synovigenic traits are found in an encyrtid parasitoid, *Coccidoxenoides peregrinus* (Timberlake) (Ceballo and Walter, 2004), suggesting that female parasitoid life history strategies may be far more intricate. Spermatogeny has been categorized in analogy to ovigeny. Prospermatogenic males have fully developed their testes (which have fixed number of sperm) by the time they reach maturity, whereas synspermatogenic males produce sperm during adult stage (Gerling and Legner, 1968; Gordh and DeBach, 1976; Nadel and Luck, 1985; Jacob and Boivin, 2004; Boivin et al., 2005). Similar to females, many species appear to be moderately synspermatogenic, i.e. males emerge with some mature sperm, and can continue to produce and mature sperm throughout their lifespan (Boivin et al., 2005).

Hymenopteran parasitoids may develop gregariously, solitarily or quasi-gregariously. Gregarious parasitoid species favor sibmating on the natal patch and local mate competition (Mackauer and Völkl, 2002), and usually produce strongly female-biased broods, with only one or two males in each brood (Hardy et al., 1998). Solitary parasitoid species, in contrast, favor dispersal from natal patch and outbreeding (Nadel and Luck, 1992), and their offspring sex ratio is usually more balanced (Fisher, 1930). If the hosts of a solitary parasitoid species are gregarious such as aphids or coccids, quasi-gregarious broods of the parasitoid offspring can be produced (van den Assem et al., 1980), where partial sibmating and local mate competition may occur (Mackauer and Völkl, 2002). Furthermore, in quasi-gregarious parasitoid species, sex ratio is often moderately female-biased and unfertilized eggs are usually laid before fertilized eggs (Hardy, 1992).

Aphidius ervi Haliday is an endophagous, solitary and koinobiont parasitoid, and attacks a number of aphid species on economically important crops such as legumes and cereals (Starý, 1973, 1978). The field sex ratio is ca. 1 male to 1.9 females (Sequeira and Mackauer, 1993). Males are polygynous (Starý, 1962; our unpublished data), and females monandrous (our unpublished data). Under conditions as used in the present study, *A. ervi* adults can live for 11–14 d (He and Wang, 2006a); the female carries about 60 mature eggs on emergence (He and Wang, 2006b) but can parasitize more than 300 aphids in her lifespan (He et al., 2006), indicating that *A. ervi* females are pro-synovigenic with an ovigeny index of ca. 0.2 (calculated according to Boivin et al., 2005). On the basis of the above properties *A. ervi* should be a quasi-gregarious species, i.e. a solitary parasitoid species attacking gregarious hosts.

Prospermatogeny is expected to be advantageous for gregarious wasps where mating primarily takes place at emergence, while solitary species are expected to show synspermatogeny, as it enables males to disperse before locating mates (Boivin et al., 2005). Quasi-gregarious species, such as *A. ervi*, can be expected to take an in-between position. Boivin et al. (2005) argue that species that are prospermatogenic should also be pro-ovigenic and *vice versa* because the constraints linked to life history parameters are likely to act on gamete production in both sexes.

Here, we postulate that, in *A. ervi*, (1) both sexes prefer young to old mates to maximize their reproductive gain, (2) females prefer virgin to mated males for mating to obtain maximal number of sperm, (3) the age of males and females at mating has a differential effect on fecundity and daughter production because the number of matings varies between sexes, and (4) males are synspermatogenic or pro-synspermatogenic due to the quasi-gregarious and polygynous property of this species. We tested these hypotheses through investigating how the age at mating and male mating history affected mating success and reproductive outputs. Knowledge of these reproductive strategy parameters is essential to the understanding of life history evolution and manipulation of daughter production for mass-reared and field populations of *A. ervi*.

2. Materials and methods

2.1. Insects and experimental conditions

A breeding colony had been initiated in December 2002 from *A. ervi* emerged from *Acyrthosiphon kondoi* Shinji, collected on Lucerne in Palmerston North, New Zealand. The colony had been reared on *Ac. pisum* (Harris), feeding on potted broad bean, *Vicia faba* L. cv. Pride, for five generations before being used in our experiments. All experiments were carried out at 20 ± 1 °C and 60–70% RH with a photoperiod of 16 h light:8 h dark. All parasitoids used in this study emerged from aphids parasitized at third instar. To obtain virgin parasitoids, we individually maintained aphid mummies in glass vials (1.5 cm in diameter, 5.0 cm in height with a 0.5 cm mesh covered hole in lids) until emergence and recorded emergence time.

2.2. Effect of mate age and male mating experience on mating success

To determine whether mate age affected mating success, we set up two treatments: (1) one 1-d-old (24 h) male + three females of 1, 3 (72 h) and 5-d (120 h) of age, respectively; (2) one 1-d-old female + three males of 1, 3 and 5-d of age, respectively. There were 78 and 61 replicates for treatments (1) and (2), respectively. Parasitoids were all virgin and females did not lay eggs (no aphids provided) before being

used for experiments. For each replicate, we randomly selected two of the three test insects of different ages and marked them on their front wings with Radiant Color (Magruder Color Company, NJ), before releasing female(s) and male(s) into a closed Petri dish (5.5 cm in diameter × 1.3 cm in height). Upon release we observed their behavior for 10 min, and recorded mounting (a male mounted a female) and mating (a male inserted his aedeagus into a female's genitalia) events. Bioassays were recorded using a Panasonic SVHS camcorder (MS-4, Japan) connected with a Samsung digital video cassette recorder (DVD-V530, Korea).

To determine whether male mating experience affected mating success, we introduced one virgin male and one once-mated male (one of them was randomly selected and marked as above in each replicate) into an above mentioned Petri dish containing a virgin female. Experimental parasitoids were all 1-d-old when used. Mating behavior was observed and recorded as mentioned above. To obtain a once-mated male, we allowed a virgin male and a virgin female to copulate in the glass vial, and the male was used for experiment 1 h after copulation. Sixty-five replicates were performed.

2.3. Effect of parental age at mating on reproductive outputs

To examine whether parental age at mating affected *A. ervi* female reproductive output and sex allocation, we set up nine treatments, i.e. virgin males and females mated at nine combinations of three age classes: 1, 3 and 5-d-old. There were 10 replicates for each treatment. In each replicate a male and a female were mated in a glass vial as described above. Subsequently, the mated female was introduced into a transparent plastic cylinder (8.5 cm in diameter × 10.5 cm in height) with 50 healthy third instar aphids feeding on a bean plant cutting standing in a plastic container (6.5 cm in diameter × 8.5 cm in height) with tap water. The female was allowed to stay in the cylinder for 24 h, before being moved to another cylinder with 50 healthy third instar aphids. This was repeated until she died. Females mated when 3- and 5-d-old were also individually provided with 50 health aphids daily before mating, starting when females were 1-d-old. The number and sex of progeny produced by females following mating was used to calculate the sex ratio of her offspring. Ten 1-d-old virgin females, each provided with 50 healthy aphids daily throughout their life time, were used as control.

Honey solution (10%) was supplied daily as food for the parasitoids in a cotton wool wick (1 cm in length), inserted through a hole (0.6 cm in diameter) in the top of the cylinder. Broad bean cuttings were replaced when wilted.

Here, parasitism (total number of aphids parasitized by a female) was recorded as realized fecundity and the sex ratio as realized sex ratio. Our previous study (He et al., 2006) shows that females are not capable to parasitize 50 aphids a day so that superparasitism can be expected to be low at this host density/day. Furthermore, the mortality of the parasitoid and its hosts is very low (<5%) (unpublished data). Therefore, the realized fecundity and sex ratio recorded here are good estimates of primary fecundity and sex ratio.

2.4. Effect of male mating history on reproductive outputs

To determine how male mating history affected reproduction outputs, two experiments were conducted: (1) hourly mating and (2) daily mating. In the hourly mating experiment, a male was offered a 1-d-old virgin female hourly in the glass vial. Immediately after each mating, the mated females were removed, and individually kept in another glass vial. The number of females that a male could mate with during one day (16 h photophase) was recorded. In the daily mating treatment, a male was offered a 1-d-old virgin female once a day during his life. The number of females that a male could mate with was recorded. The males were supplied with 10% honey solution saturated in a cotton wool ball (0.5 cm in diameter) as food. All experiments were conduced during the photophase and ten males were used for each experiment.

Soon after mating, each female from the hourly or daily mating experiment was provided with 50 healthy third instar aphids daily in an above-mentioned cylinder until she died. Ten percent honey solution was also supplied as food for females. The fecundity and daughter production were recorded as above.

2.5. Statistical analysis

The incidences of mounting and mating were compared using the Marascuilo procedure of nonparametric analysis (Daniel, 1990). A goodness of fit test was used to test the distribution of other data. The data on the secondary fecundity were normally distributed and analyzed using ANOVA, followed by Tukey's studentized range (HSD) test. The data on proportion of daughters produced were not normally distributed even after transformation and thus analyzed using the nonparametric Kruskal–Wallis test followed by Dunn's procedure for multiple comparisons (Zar, 1999).

We used a central composite design (CCD) or response surface (Box and Draper, 1987) to analyze the effect of mate age at mating on the proportion of daughters produced. The relationship between mate age and proportion of daughters is given by the polynomial equation: $\text{daughter}\% = \exp(\beta_0 + \beta_1 x_f + \beta_2 x_m + \beta_{11} x_f^2 + \beta_{22} x_m^2 + \beta_{12} x_f x_m)$ where $\beta_0, \beta_1, \beta_2, \beta_{12}, \beta_{11}$, and β_{22} are model parameters, and x_f and x_m are female and male age, respectively. Only significant terms, after running the full regression models, were kept in the final models. A log likelihood ratio test (McCullagh and Nelder, 1989) was then applied to determine whether age of sexes had different effect on the proportion of daughters produced.

The relationship between the proportion of daughters produced and number of females a male mated with was analyzed using Analysis of Regression (AOR) in both hourly and daily mating experiments. ANCOVA was used to analyze the slopes.

All analyses were carried out using SAS (SAS Institute, 1996). Rejection level was set when $P > 0.05$.

3. Results

3.1. Effect of mate age and mating experience on mating success

Mate age affected mating behavior (Fig. 1). When a 1-day old male was placed together with three females of different ages, he tended to mate with younger females more often ($U'_0 = 10.47 > \chi^2_{0.05,2} = 5.99, P > 0.05$) (Fig. 1A), but the effect of female age on the number of mountings by males was not significant ($U'_0 = 3.52 < \chi^2_{0.05,2} = 5.99, P > 0.05$) (Fig. 1A).

When a 1-d-old virgin female was placed with three males of different ages, young males had significantly greater ability to mount and mate than old males ($U'_0 = 10.91$ and $7.77 > \chi^2_{0.05,2} = 5.99$ for mounting and mating, respectively; $P < 0.05$) (Fig. 1B).

When a virgin female was placed with a virgin male and a once-mated male, she was significantly more likely mounted and mated by the mated male ($U'_0 = 14.91$ and $12.00 > \chi^2_{0.05,2} = 5.99$ for mounting and mating, respectively; $P < 0.05$) (Fig. 2).

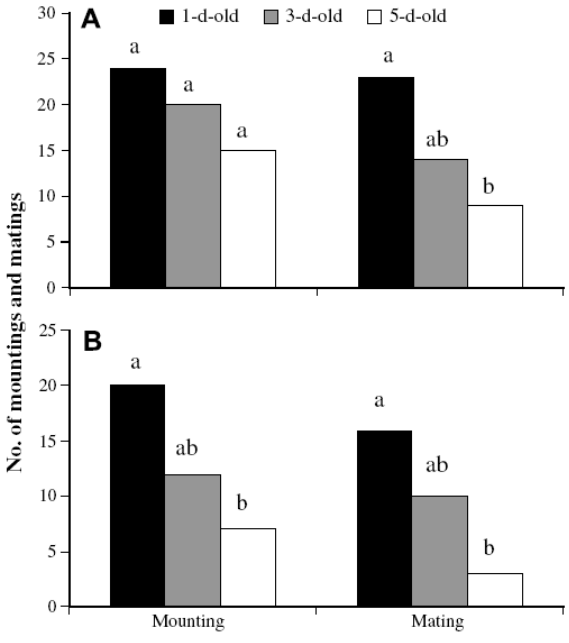

Fig. 1. Effect of mate age on mating success of *A. ervi*: (A) 1-d-old males × females of different ages: (B) males of different ages × 1-d-old females. Within the same category (mounting or mating) columns with the same letters are not significantly different ($U'_0 < \chi^2_{0.05,2}$).

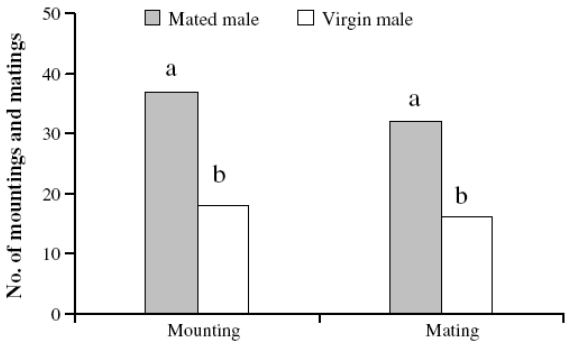

Fig. 2. Effect of male mating experience on mating success of *A. ervi*. Within the same category (mounting or mating) columns with the same letters are not significantly different ($U'_0 < \chi^2_{0.05,2}$).

3.2. Effect of parental age at mating on reproductive outputs

The realized fecundity of the nine mating treatments ranged from 326.8 ± 11.6 to 347.6 ± 9.4 offspring/female (means $\pm$ SE), and that of the control was 341.4 ± 5.1. Realized fecundity did not differ between treatments, nor between treatments and the control (ANOVA, $F_{9,90} = 0.40$, $P > 0.05$). This indicates that neither mating nor the parental age at mating had significant effect on a female's realized fecundity.

The proportion of daughters decreased significantly with the increasing age of both sexes at mating (CCD: $F_{2,87} = 7.14$, $P < 0.0001$, $R^2 = 0.1410$) (Fig. 3A). However, female age had a stronger effect on the proportion of daughters than did male age (likelihood rate test: $\chi^2_2 = 96.18, P < 0.0001$) (Fig. 3B). Furthermore, females of all age combinations produced some daughters with the exception of 5-d-old females, of whom 20–30% did not produce any daughters.

3.3. Effect of male mating history on reproductive outputs

Females from the hourly and daily mating treatments produced between 335.0 ± 24.9 and 352.6 ± 9.4 offspring/female. The realized fecundity did not differ between treatments nor between treatments and the control (virgin females) (ANOVA, $F_{17,102} = 0.09$, $P > 0.05$). This result shows that male mating history had no effect on the secondary fecundity of females.

The proportion of daughters produced generally declined with the number of matings males had had in both hourly and daily experiments. In the hourly experiment, this effect was significant after males had mated three times (Kruskal–Wallis test: $H = 59.75 > \chi^2_{0.05,8} = 15.51$; $P < 0.0001$) while in the daily trial it was significant after males had had six matings (Kruskal–Wallis test:

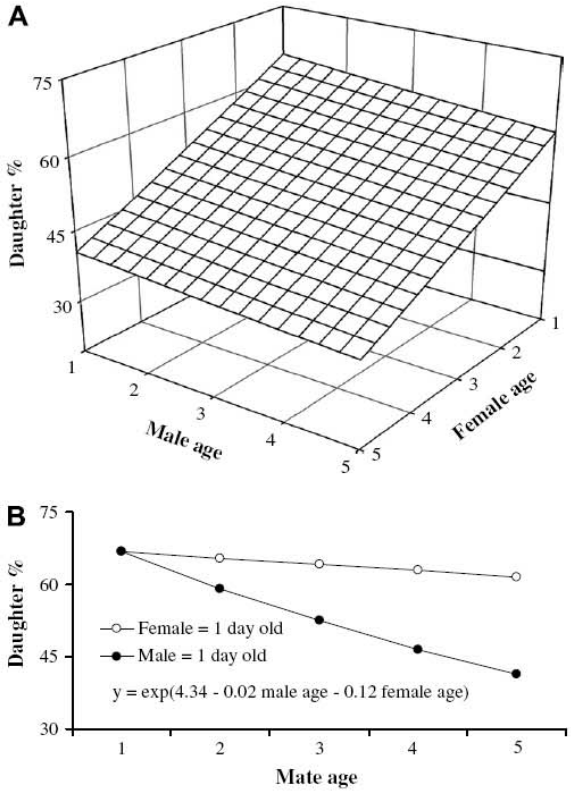

Fig. 3. Effect of mate age (d) at mating on daughters production of *A. ervi*: (A) proportion of daughters in pairs of different age combinations of sexes ($n = 90$); (B) predicted proportion of daughters produced by males and females of different ages.

$H = 21.78 < \chi^2_{0.05,7} = 14.07$; $P < 0.0001$) (Fig. 4). Furthermore, analysis of regression shows that in both hourly and daily experiments, the proportion of daughters significantly decreased with the increasing number of matings (AOR: $F_{1,59} = 132.62$ for hourly experiment and $F_{1,47} = 34.77$, for daily experiment; $P < 0.0001$) (Fig. 5) but decreased significantly faster in hourly than in daily experiments (ANCOVA: $F_{2,107} = 77.57$, $P < 0.0001$).

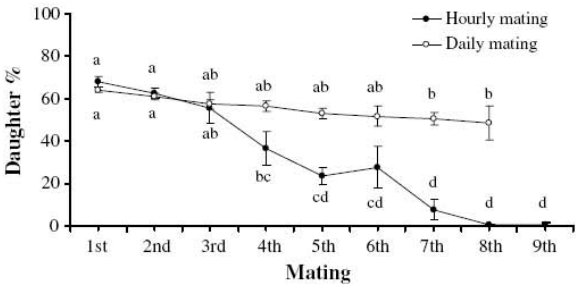

Fig. 4. Proportion of daughters of *A. ervi* males after successive matings.

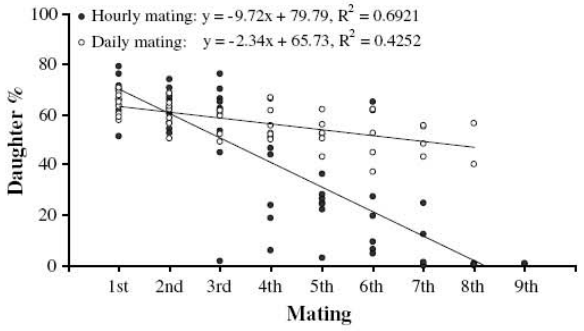

Fig. 5. Relationship between proportion of daughters and male mating history of *A. ervi*.

4. Discussion

In the present study, males produced similar numbers of daughters from their first few matings with different females. Therefore, to *A. ervi* females, once-mated and virgin males of the same age should have similar quality in terms of sperm numbers. However, our results show that once-mated males were more likely to mount and achieve successful mating when compared to virgin males. This suggests that mated males benefited from experience gained during previous mating. This operant learning allowed them to respond to females more quickly and successfully complete more courtships than naïve males. The fact that male mating experience enhances mating success had been previously reported in other hymenopteran parasitoids (Perez-Lachaud and Campan, 1995) as well as other insects (Cook, 1995).

For both sexes of *A. ervi*, young individuals have higher mating success than older ones. This may be partially explained by our findings that the proportion of daughters produced significantly decreased with parental age at mating. Furthermore, virgin females may start oviposition within 30 min after emergence (unpublished data), and in the natural environment older females are more likely to have laid eggs. Therefore, it is not to the males' benefit to mate with aged females due to the latter's lower reproductive potential. In addition, female sex pheromone production and release (Battaglia et al., 2002) may decrease with female age (Schworer et al., 1999; del Mazo-Cancino et al., 2004; Showler et al., 2005), which could make older females less attractive to males. Older males may have decreased ability to recognize and respond to female sex pheromones or decreased ability to court and mount females (Cheng et al., 2003; Damiens and Boivin, 2005), resulting in the reduced mating success.

Similar to the aphidiid *Lysiphlebus delhiensis* (Subba Rao and Sharma) (Srivastava and Singh, 1995), the lower production of daughters by older males may not necessarily be attributed to the sperm depletion in *A. ervi*. Instead, this could also be due to diminishing sperm viability and mobility related to aging (Srivastava and Singh, 1995).

For the aging females, the reduction of daughter production could be explained by the constraints in fertilization process in *A. ervi*. Flanders (1946) found the depletion of spermathecal gland secretion resulting in a reduced availability of activated sperm for fertilization of ovulating eggs. According to Pandey et al. (1983), eggs may contain certain kind of sperm attracting chemicals, which deplete with the female age, leading to a reduced fertilization.

Our study also indicates that in *A. ervi* female age at mating has a stronger impact on the production of daughters than male age; between 20% and 30% of females mated at the age of 5-d failed to produce any daughters, whereas all of the 5-d-old males yielded daughters. This suggests that fertilization process in females may be more sensitive to aging than sperm vigor in males. It is also possible that males adjust the amount of sperm transfer according to female age, delivering less sperm to older females (Srivastava and Singh, 1995).

In the aphidiid *L. delhiensis*, female age rather than male age at mating significantly reduces the fecundity (Srivastava and Singh, 1995). The authors suggested that males may transfer egg production stimulants and/or nutrients to females during mating, significantly increasing egg production in younger females. However, like the aphelinid *Aphelinus asychis* Walker (Fauvergue et al., 1998), mating in itself, nor parental age at mating or male mating history affected female realized fecundity in *A. ervi*. This implies that mating does not stimulate egg production and females do not obtain nutritional benefits from matings (He and Wang, 2006a).

In the hourly mating experiment, *A. ervi* males started to show sperm depletion after three successive matings, while in the daily mating experiment, males only started showing evidence of sperm depletion following six matings. Corresponding to this, the production of daughters decreased significantly faster in hourly mating than in daily mating treatments. These findings have two implications. First, males of this species appear to be at least moderately synspermatogenic because they can replenish sperm if allowed 24-h recovery period between matings up to six matings. As a quasi-gregarious species moderate synspermatogeny should be advantageous to some extent as it enables males to disperse and locate mates (Boivin et al., 2005) reducing partial sibmating and local mate competition (Mackauer and Völkl, 2002). Moderate synspermatogeny also has been reported in a number of other parasitic hymenopteran families (Nadel and Luck, 1985; review by Boivin et al., 2005). Second, because females are monandrous matings by *A. ervi* males may be limited by the number of virgin females in the field. Therefore, sperm depletion may be a rare event under field conditions. The reduction of daughter production in the daily mating experiment may be caused by the reduction in sperm transfer and quality due to a decrease in males' vigour with the increased age (Srivastava and Singh, 1995; King, 2000; Jacob and Boivin, 2004; Damiens and Boivin, 2005) rather than sperm depletion.

In conclusion, our results show that females are pro-synovigenic and males are at least moderately synspermatogenic in *A. ervi*, supporting Boivin et al.'s (2005) prediction. As *A. ervi* adults do not feed on hosts (unpublished observation) they may utilize other adult food sources containing amino acids like honeydew (Azzouz et al., 2004; Hogervorst et al., 2007) to boost egg and sperm production. The better performance by experienced males in mating success suggests they have learned from the previous mating. The fact that the monandrous females do not show any choice between naïve and experienced males of the same age for mating may be due to the synspermatogenic property in this species. Because mating has little effect on female secondary fecundity, female reproductive gain from mating should be mainly, if not solely, through the production of daughters. Aging affects sexes in different ways: for old males, diminishing sperm vigor may be the main cause of the reduced daughter production while for old females constraints of egg fertilization process in relation to aging may be the blame for the lower daughter proportions. Information provided in this study should be taken into consideration in manipulating optimal daughter production in both mass-reared and field populations of *A. ervi*.

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Asymmetric size effect of sexes on reproductive fitness in an aphid parasitoid *Aphidius ervi* (Hymenoptera: Aphidiidae)

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Abstract

Most studies on size–fitness relationships focus on females and neglect males. Here, we investigated how body size of both sexes of an aphid parasitoid, *Aphidius ervi* Haliday, affected the reproductive fitness. Reproductive fitness was generally positively correlated with body size for both sexes in this species. Large individuals of both sexes had greater longevity, large males fathered more progeny, and large females had higher fecundity, parasitism, and greater ability in host searching and handling. We demonstrated in this study that size effects of males and females were asymmetric on different reproductive fitness parameters. With increasing body size females gained more than males in longevity and fecundity while males gained more than females in the number of female progeny. Regardless of female size, large males sustained a female-biased population longer than small males. These results suggest that male body size should also be considered in the quality control of mass-rearing programs and the evaluation of parasitoid population growth.

Keywords: *Aphidius ervi*; Body size; Fecundity; Paternity; Longevity

1. Introduction

Body size of insects has usually been considered to be a key trait potentially affecting fitness in many ways, both directly and indirectly through correlated physiological parameters (Cloutier et al., 2000; Jiménez-Pérez and Wang, 2004). Many studies have provided strong support for the 'size-dependent fitness' model, i.e., large individuals often have physiological and behavioral advantages (Cloutier et al., 2000; Visser, 1994). For example, in hymenopteran parasitoids the reproductive fitness of females in terms of fecundity, parasitism, searching rate, and longevity is often positively correlated with their body size (Arakawa et al., 2004; Sagarra et al., 2001) and, in parasitoids that mature eggs throughout their lifetime, greater female longevity also allows them to produce more eggs (Cloutier et al., 2000). Large males usually have greater ability to inseminate and compete for mates (Kazmer and Luck, 1995) or have better genes and more sperm supply (van den Assem et al., 1989). Therefore, the fitness consequences of body size and its correlates, especially the supply of eggs or sperm and adult longevity, are important in population dynamics and essential for understanding and modeling the life history evolution and behavioral decisions.

On the basis of generally accepted 'size-dependent fitness,' Charnov's 'variation in fitness' model predicts that parasitoids lay fertilized eggs in larger hosts resulting in female progeny and unfertilized eggs in small hosts giving rise to male progeny, and female fitness increases more rapidly with body size than does male fitness (Charnov, 1982; Charnov et al., 1981). However, most studies on size–fitness relationships focus on females, neglecting males (King, 1987; Visser, 1994). Studies on the relationship between body size of male parasitoids and reproductive

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fitness usually concentrate on male mate searching behavior, competitive ability and copulation capacity (Kazmer and Luck, 1995; van den Assem et al., 1989) and rarely address the effect of male size on the other fitness parameters such as the number of resulting female progeny (Ode et al., 1996). To our knowledge, Heinz (1991) was the first author to consider the effect of male body size on the reproductive fitness in a wasp species, *Diglyphus begini* (Ashmead). He compared the male and female reproductive success in relation to their body size, and suggested that females gained more in terms of the number of progeny with increasing body size than did males. However, like many parasitic wasps, *D. begini* is a species of haplodiploid sex determination. His conclusion had thus overestimated the effect of female body size on reproductive fitness because the number of fathers' progeny (only female progeny) was always fewer than that of mothers' progeny (both female and male progeny).

Aphidius ervi Haliday is a cosmopolitan solitary endophagous parasitoid (Marsh, 1977) and a major biological control agent of several aphid species on economically important crops such as legumes and cereals (Powell, 1982; Starý, 1978). A positive relationship was found between *A. ervi* body weight and *Acyrthosiphon pisum* (Harris) body weight at parasitization (Sequeira and Mackauer, 1992). However, no study has addressed whether and how body size of both *A. ervi* males and females affects reproductive fitness. The fitness of parasitoids of different sizes needs to be known when calculating the value of particular hosts to foraging female parasitoids, which is important in modeling host selection strategies (Charnov and Stephens, 1988; Visser, 1994). Moreover, body size–fitness relationships are also relevant to mass-rearing programs as body size is commonly monitored as an indicator of parasitoids' quality (Jervis and Copland, 1996; Sagarra et al., 2001).

In the laboratory, *A. ervi* females mated only once while males could inseminate up to eight females if the mated male was offered a virgin female daily; the first six females inseminated by the same male produced similar number and proportion of female progeny (unpublished data). Similar results were also reported for another parasitoid species, *Trichogramma evanescens* Westwood (Jacob and Boivin, 2004). This information suggests that the greater longevity of adult wasps may be important not only for females to lay more eggs (Cloutier et al., 2000) but also for males to inseminate more females. However, a male in the field may not be able to inseminate as many females as in the laboratory because there are simply not enough virgin females available in the field, for example, the proportion of females in the field population is only slightly higher than that of males (Sequeira and Mackauer, 1993). Therefore, the first or first few matings should be the most important for the male's reproductive success. In the present study, we only recorded and analyzed the data from the first insemination.

Here, we report our work on the relationship between the body size of both sexes and reproductive fitness in *A. ervi*, with two objectives: (1) to determine how body size affected reproductive fitness in terms of fecundity, parasitism, number, and proportion of female progeny produced, searching efficiency, and longevity and (2) to evaluate whether body size of both sexes affected reproductive fitness in similar way.

2. Materials and methods

2.1. Insects and experimental conditions

A breeding colony started from *A. ervi* emerged from *Acyrthosiphon kondoi* Shinji, collected on lucerne in Palmerston North, New Zealand in December 2002. The colony was reared on *Ac. pisum*, feeding on potted broad bean, *Vicia faba* L. cv. Pride, for five generations before used for experiments. All experiments were carried out in transparent plastic cylinders (8.5 cm in diameter, 10.5 cm in height) with gauze-covered holes, one in the top (5 cm in diameter) and two (2 cm in diameter) in opposite sides for ventilation at 20 ± 1 °C and 60–70% RH with a photoperiod of 16 h light:8 h dark. A broad bean cutting standing in a plastic container (6.5 cm in diameter, 8.5 cm in height) with tap water was placed in the plastic cylinder and replaced when wilted. Honey solution (10%) was supplied daily in a cotton wool wick (1 cm in length), inserted through a hole (0.6 cm in diameter) in the top of the cylinder.

2.2. Measurement of body size

On the basis of previous work reported by various authors (e.g. Arakawa et al., 2004; Cloutier et al., 2000; Paine et al., 2004), we used head width of adults as the index of body size for *A. ervi*. We measured *A. ervi* under a stereomicroscope (Leica MZ12, Germany) equipped with an ocular micrometer.

The body size of emerging *A. ervi* wasps is host size-dependent. The mean head width (mean ± SE) of parasitoids emerged from aphids parasitized at the first instar (male, 0.547 ± 0.003 mm; female, 0.588 ± 0.004 mm) were significantly smaller than those from aphids parasitized at fourth instar (male, 0.612 ± 0.004 mm; female, 0.663 ± 0.003 mm) (ANOVA: $F = 184.14$ and 218.55 for male and female, respectively; $df = 1, 58$; $P < 0.0001$). The former was defined as "small" (S) and the latter as "large" (L) in this paper.

2.3. Experiments

To determine whether and to what extent body size affected reproductive fitness, we set up four treatments with 15 replicates per treatment and allowed them to mate once: small female (SF) × small male (SM), large female (LF) × large male (LM), SF × LM, and LF × SM.

For each replicate, one mated *A. ervi* female (<12 h old) was introduced into a plastic cylinder with 50 healthy third instar aphids feeding on a bean plant cutting. The female was allowed to stay in the cylinder for 24 h, and then moved to another cylinder with 50 healthy third instar aphids, etc. until she died. We recorded the data on the daily basis and all data collected during females' lifetime were included in the analysis. The total number of aphids used in the experiment was 8350, 8800, 10,550, and 10,600 for treatments SF × SM, SF × LM, LF × SM, and LF × LM, respectively. The once-mated males were numbered in correspondence with the females they mated with and reared individually in glass vials (1.5 cm in diameter, 5.0 cm in height) with a 0.5 cm mesh-covered hole in lids, and supplied with 10% honey solution in a cotton wool ball (0.5 cm in diameter) daily.

To estimate the number of eggs laid, we randomly selected 10 aphids from each cylinder 4 days after the removal of the female parasitoid and then dissected them in 70% alcohol under the stereomicroscope. In total, we dissected 1680, 1640, 2000, and 2030 aphids for above four treatments, respectively. The number of parasitoid larvae recorded from dissecting was assumed equal to the number of eggs laid (Bueno et al., 1993) or fecundity. The remaining aphids were reared until mummification. The number of parasitized aphids (parasitism) was the sum of those detected by both dissection and by counting mummies. The number and proportion of female progeny were counted and calculated from emerged progeny. Longevity of both sexes was also recorded for all treatments.

The host searching rate was estimated as $a = (1/T)\log_e(N_t/(N_t + N_a))$. The time spent on handling hosts T_h was estimated by solving the equation: $N_a = N_t(1 - \exp(-aT/(1 + aT_hN_t)))$ (Rogers, 1972). N_t is the number of hosts, N_a is the number of hosts parasitized within the duration T of the parasitoid in the cylinder.

2.4. Statistic analysis

A goodness of fit test was performed to test the normality of all data before analysis. The data on handling time and searching rate were not normally distributed and thus analyzed using the nonparametric Kruskal–Wallis t test followed by Dunn's procedure for multiple comparisons. The remaining data were normally distributed and analyzed using ANOVA followed by a Tukey's studentized range (HSD) test. The data on the proportion of female progeny were arcsine transformed prior to analysis.

We used a central composite design (CCD), i.e., response surface (Box and Draper, 1987), to analyze the differential size-dependent fitness gain of sexes in terms of the number and proportion female progeny produced. The relationship between parasitoid body size and reproductive fitness is given by the polynomial equation: $\text{fitness} = \exp(\beta_0 + \beta_1 x_f + \beta_2 x_m + \beta_{11} x_f^2 + \beta_{22} x_m^2 + \beta_{12} x_f x_m)$, where β_0, β_1, β_2, β_{12}, β_{11}, and β_{22} are model parameters, and x_f and x_m are female and male body size, respectively. Only significant terms, after running the full regression models, were kept in the final models. A log likelihood ratio test (McCullagh and Nelder, 1989) was then applied to determine whether body size of sexes had different effect on the number and proportion female progeny produced. We

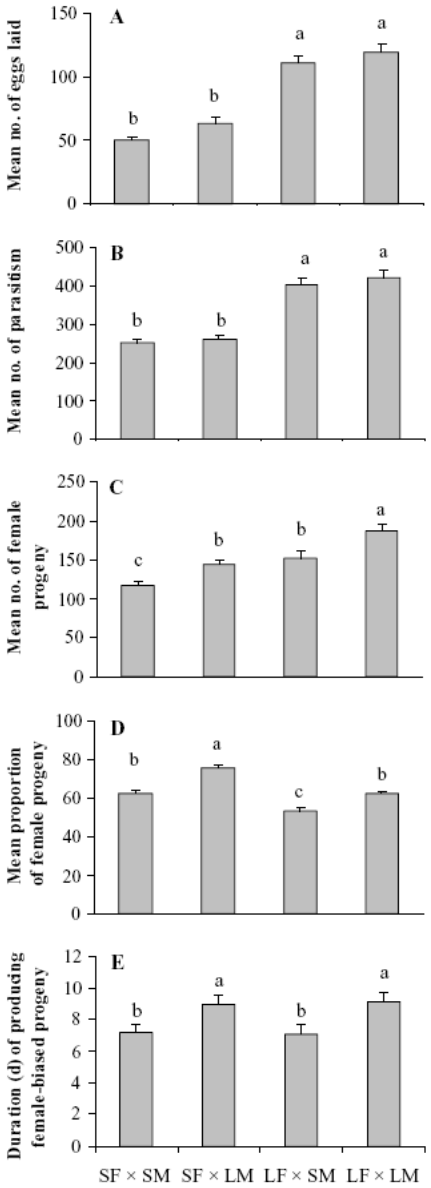

Fig. 1. Effect of body size of both sexes of *A. ervi* on the number of eggs laid or fecundity (A), number of parasitism (B), number of female progeny (C), proportion of female progeny (D), and duration (d) of producing female-biased progeny (E). Columns with the same letters were not significantly different ($P > 0.05$). (A–D): $F = 43.43$, 36.12, 13.69, and 29.68 for (A), (B), (C), and (D), respectively; $df = 3, 56$; $P < 0.0001$; (E): $F = 3.88$; $df = 3, 56$; $P < 0.05$.

also analyzed the slopes of the regression lines of size–longevity relationships of sexes using an analysis of covariance (ANCOVA).

All analyses were carried out using SAS (SAS Institute, 1996). Rejection level was set when $P > 0.05$.

3. Results

3.1. Male reproductive performance in relation to body size

Male body size had no significant effect on fecundity (Fig. 1A), parasitism (Fig. 1B), and searching rate and handling time (Table 1). However, females mated to large males produced significantly more female progeny (Fig. 1C) and had significantly higher proportion of female progeny (Fig. 1D) than those mated to small males. Moreover, females mated to large males produced female-biased progeny for significantly longer period than those mated to small males (Fig. 1E).

3.2. Female reproductive performance in relation to body size

Large females had significantly higher fecundity (Fig. 1A), parasitized significantly more aphids (Fig. 1B), and produced significantly greater number of female progeny (Fig. 1C) than small females. The proportion of female progeny was significantly higher for small females than that for large females (Fig. 1D). In addition, large females had significantly higher searching rate and shorter handling time than small females (Table 1). However, female body size did not affect how long females sustained the production of female-biased progeny (Fig. 1E).

3.3. Relative fitness gain of males and females in relation to body size

The number of female progeny significantly increased with the body size of both sexes (Fig. 2A) but log likelihood ratio test shows that males had significantly more gain than females in the number of female progeny with the increasing body size ($\chi^2 = 234.15$; $df = 2$; $P < 0.0001$). Unlike males, the female size had negative effect on the proportion of female progeny (Fig. 2B). Although large males and females lived significantly longer than small males and females (Table 1), females gained disproportionately greater longevity with the increase of body size than did males (ANCOVA: $F = 19.17$; $df = 2, 117$; $P < 0.0001$) (Fig. 3).

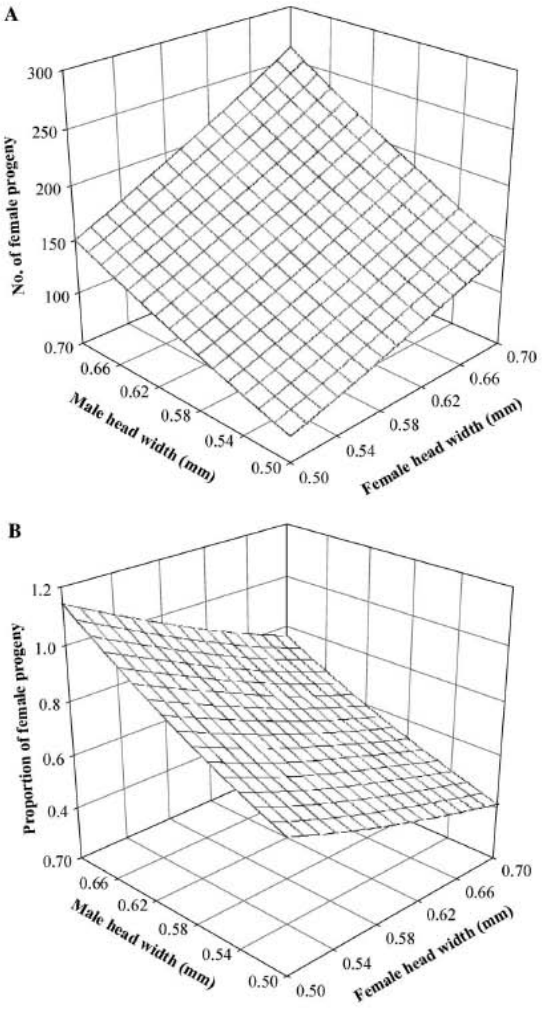

Fig. 2. Effect of female (x_f) and male (x_m) body size on the number of female progeny [(A): $y = \exp(1.26 + 2.97x_f + 3.23x_m)$ ($r^2 = 0.5222$; $F = 31.15$; $df = 2, 57$; $P < 0.0001$)] and proportion of female progeny [(B): $y = \exp(-0.67 - 2.25x_f + 2.77x_m)$ ($r^2 = 0.6328$; $F = 48.96$; $df = 2, 57$; $P < 0.0001$)] in *A. ervi*.

4. Discussion

The body size of *A. ervi* progeny is influenced by the host stage or age at parasitization (Sequeira and Mackauer,

Table 1
Effect of body size of both sexes on longevity and searching efficiency in *A. ervi*[a]

Fitness	SF × SM	SF × LM	LF × SM	LF × LM	F	H[b]	P
Female longevity (d)	11.13 ± 0.74 b	11.73 ± 0.63 b	14.07 ± 0.74 a	14.13 ± 0.86 a	4.35		<0.01
Male longevity (d)	12.13 ± 0.63 b	13.87 ± 0.52 ab	13.67 ± 0.45 ab	14.40 ± 0.45 a	3.51		<0.05
Searching rate (10^{-4} min)	1.05 ± 0.20 b	0.87 ± 0.08 b	1.35 ± 0.15 a	1.30 ± 0.10 a		13.34[b]	<0.01
Handling time (min)	8.34 ± 1.79 a	6.29 ± 0.50 a	3.83 ± 0.45 b	3.21 ± 0.12 b		25.31[b]	<0.0001

[a] Means (±SEs) followed by the same letters in rows were not significantly different ($P > 0.05$); $df = 3, 56$.
[b] Kruskal–Wallis t test: means (±SEs) were significantly different when $H > \chi^2_{0.05,3} = 7.82$.

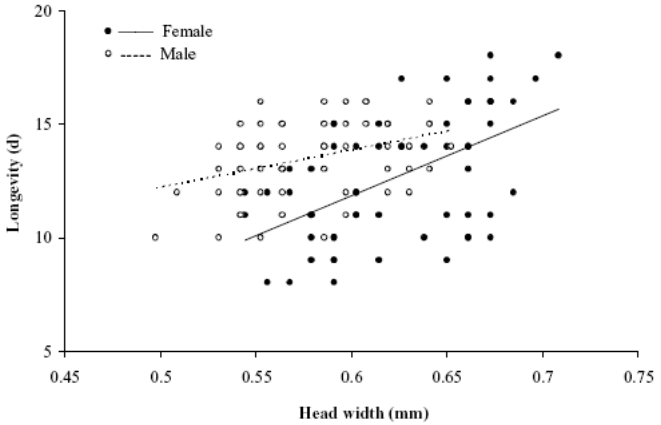

Fig. 3. Relationship between body size and longevity in *A. ervi*. For female: $y = 35.05x - 9.16$ ($r^2 = 0.2895$; $F = 23.63$; $df = 1, 58$; $P < 0.0001$); for male: $y = 16.54x + 3.93$ ($r^2 = 0.1452$; $F = 9.85$; $df = 1, 58$; $P < 0.01$).

1992). Like many hymenopteran species (Arakawa et al., 2004; Godfray, 1994; Honék, 1993; Sagarra et al., 2001; Visser, 1994), the reproductive fitness of *A. ervi* females and males depends on their body size.

The increase in the number of eggs laid with the increasing female body size in *A. ervi* may result from increasing longevity and greater ability to generate eggs when needed, as detected in *Aphidius nigripes* Ashmead (Cloutier et al., 2000). A higher number of eggs laid by large females may also translate into a higher parasitism due to their greater longevity and searching rate and shorter handling time. However, unlike many other insects where nutrients contributed by males through copulation are used by females for egg production (Huignard, 1983; Jiménez-Pérez and Wang, 2004; Simmons and Gwynns, 1993), large *A. ervi* males do not increase the number of eggs laid by females. This suggests that *A. ervi* females do not obtain a nutritional contribution from males during copulation as suggested by Godfray (1994) and Fauvergue et al. (1998).

The higher number of female progeny produced by larger females may be due to the greater storage capacity of the spermatheca and more production of eggs in those females (Lauzière et al., 2000). As found in other hymenopteran species, for example, the encyrtid parasitoid, *Anagyrus kamali* Moursi (Sagarra et al., 2001), the proportion of female progeny of *A. ervi* is higher for small females than that for large females. This may be because small females live shorter and deposit fewer eggs than large females and never become sperm limited. Furthermore, females mated with large males laid female-biased eggs for a longer period of time, suggesting that large males supply more sperm and thus sustain a female-biased population longer than small males.

The fitness data of *A. ervi* are generally in agreement with the main premise of Charnov's 'variation in fitness' model, which concerns the differential size–fitness relationship in males vs. females (Charnov, 1982; Charnov et al., 1981). However, our study demonstrates that body size of *A. ervi* females and males affects reproduction in different ways, i.e., with increasing body size females gained more than males in longevity and fecundity while males gained more than females in the number of female progeny. It is indicated that females concentrate their resource gain (body size) on egg production and oviposition period while males allocate their resource gain more to sperm production and insemination than on longevity. This suggests that for males the benefit from longevity is less certain than inseminating virgin females they can find at their earliest stage possible. The asymmetrical size–fitness between sexes may be because reproductive success of parasitoids directly relies on females' potential fecundity and their ability to search and parasitize hosts, whereas the success of male reproduction absolutely relies on, and is mediated, by females. In addition, virgin females still reproduce with the absence of males. Therefore, the different reproductive strategies of different sexes may result in the asymmetrical size-dependent fitness.

In conclusion, the body size of both sexes contributes to population growth and sex ratio distribution in *A. ervi*. Our results of the asymmetric size-dependent fitness in different sexes provide us useful information when we manage to predict parasitoid population dynamics and practice effective mass-rearing and release programs.

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HOST AGE PREFERENCE IN *APHIDIUS ERVI* (HYMENOPTERA: APHIDIIDAE)

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ABSTRACT

Host age preference by *Aphidius ervi* Haliday on pea aphid, *Acyrthosiphon pisum* (Harris), and the effects of host age on *A. ervi* reproductive fitness and sex allocation were studied in the laboratory. *Aphidius ervi* preferred aphids that were 3-5 days old over the younger (1 and 2 days old) and older (6 and 7 days old) aphids for oviposition. The body size and egg load at emergence of *A. ervi* progenies significantly increased with the increase of host age at the time of parasitisation from 1 to 4 days old, after which no further increase occurred. The results support the size-dependent sex ratio theory that *A. ervi* deposits fertilised eggs in large hosts and unfertilised eggs in small ones. The potential impact of the host age preference on biological control is discussed.

Keywords: *Aphidius ervi*, age, preference, body size, sex allocation, *Acyrthosiphon pisum*.

INTRODUCTION

Many studies have demonstrated that parasitism can influence development, fecundity, and population growth of the host aphids (Tsai & Wang 2002; Lin & Ives 2003; He et al. 2005). Generally, aphids parasitised in early instars are mummified before reaching reproductive maturity, whereas those parasitised in late instars are able to reach adult stage and produce progeny. Therefore, the parasitoid impact on the aphid population growth largely depends on the pattern of the host age selected for parasitisation (Hågvar & Hofsvang 1991; Tsai & Wang 2002) and host age preference is critical to the success of biological control of aphids (Tsai & Wang 2002).

Foraging parasitoids usually encounter hosts of different ages or sizes and have opportunities to select the most suitable hosts to maximise their reproductive fitness. For some solitary species of parasitoids, hosts selected for oviposition are determined by host size (Kouamé & Mackauer 1991) because large hosts contain more resources for parasitoid progeny development than small hosts (Charnov et al. 1981; Liu 1985). Many studies suggest that host size preference by parasitoids affect their progeny fitness, such as the body size (Liu 1985; Lampson et al. 1996) and egg load at emergence (Liu 1985; Mills & Kuhlmann 2000). Host age may also affect sex allocation of parasitoids (Godfray 1994). According to Charnov et al. (1981) and Charnov (1982), parasitoids can make efficient use of the size variation in the hosts encountered by allocating fertilised diploid eggs to large hosts and unfertilized eggs to small ones.

Aphidius ervi Haliday is a cosmopolitan endophagous parasitoid (Marsh 1977) of several aphid species on economically important crops such as legumes and cereals (Starý 1978; Powell 1982). Previous studies have demonstrated that the impact of *A. ervi* on population growth of the pea aphid, *Acyrthosiphon pisum* (Harris), largely depends on the host age at parasitism (He et al. 2005), and body size of pea aphid is positively correlated with its age (Sequeira & Mackauer 1992). However, so far little is known about the host age preference by *A. ervi*. Knowledge of host preference would lead to a better understanding of the population dynamics of the host and parasitoid (Nechols &

Kikuchi 1985). Therefore, to provide useful information for the development of biological control strategies, this study investigated the host age preference by *A. ervi* and its effect on the reproductive fitness and sexual allocation.

MATERIALS AND METHODS

Insects and experimental conditions

A breeding colony was started from *A. ervi* that emerged from blue-green lucerne aphid, *Acyrthosiphon kondoi* Shinji, collected on lucerne in Palmerston North, New Zealand, in December 2002. The colony was reared on pea aphid, feeding on potted broad bean, *Vicia faba* L. cv. Pride, for five generations before being used for experiments. All experiments were carried out in transparent plastic cylinders (8.5 cm in diameter, 10.5 cm in height) with gauze-covered holes, one in the top (5 cm in diameter) and two (2 cm in diameter) in opposite sides for ventilation at 20±1°C and 60-70% RH with 16:8 h light:dark. A broad bean cutting standing in a plastic container (6.5 cm in diameter, 8.5 cm in height) with tap water was placed in the plastic cylinder and replaced when wilted. Honey solution (10%) was supplied daily in a cotton wool wick (1 cm in length), inserted through a hole (0.6 cm in diameter) in the top of the cylinder. Parasitoid adults used for the experiments emerged from pea aphids parasitised at the third instar.

Experiment

To determine host age preference by *A. ervi* in relation to host age and its effect on *A. ervi* reproduction, mated females were supplied with host aphids of different ages, from 1 to 7 days old. Ten females (replicates) were tested in this experiment. For each replicate, one mated female parasitoid (< 12 h old) was introduced into an above-mentioned plastic cylinder with 105 healthy aphids (15 aphids of each age class) feeding on a bean plant cutting. The female was allowed to stay in the cylinder for 24 h, and then moved to another cylinder with 105 healthy aphids, etc. until she died. After the removal of the female parasitoid, aphids of different age classes were separated and transferred to an uninfested bean plant in a cylinder.

To estimate the number of eggs laid, five aphids of each age class were randomly selected from each cylinder 4 days after the removal of the female parasitoid, and dissected in 70% alcohol under a stereomicroscope (Leica MZ12, Germany). The number of parasitoid larvae recorded from dissecting was assumed equal to the number of eggs laid (Bueno et al. 1993). The remaining aphids were reared until mummification. The emerged progeny were counted and sexed.

To determine the effect of host age preference on reproductive fitness of parasitoids, the body size was measured and the egg load of newly emerged parasitoids from each host age class was determined. The head width (a measure of body size) of 30 newly emerged progeny of each sex randomly selected from each host age class was measured using the above stereomicroscope. The egg load of parasitoid females at emergence was determined by dissecting 30 newly emerged females randomly selected from each host age class. They were dissected in 70% alcohol on a slide under the above stereomicroscope. One drop of acid fuchsin was added to the alcohol and allowed to stand for 3–5 min for staining. The number of eggs in the ovaries was counted under a compound microscope (Olympus, Japan).

Statistical analysis

A goodness of fit test was used to test the distribution of data before analysis. Data of the number of egg load were not normally distributed even after transformation and thus analysed using the nonparametric Kruskal-Wallis test followed by Dunn's procedure for multiple comparisons (Zar 1999). Other data were normally distributed and analysed using ANOVA followed by a Tukey's studentized range test. Data of proportion of female offspring were arcsine transformed prior to analysis. The relationship between body size and egg load of newly emerged females was analysed using an analysis of regression.

RESULTS

Aphidius ervi females significantly preferred aphids that were 3-5 days old for oviposition with significantly higher number of aphids parasitised (Fig. 1) and eggs laid (Fig. 2) (P<0.0001).

Host age also had significant effect on proportion of female progeny produced (P<0.0001), which was highest in 6-day-old aphids and lowest in 1-day-old aphids (Fig. 3).

The head width of newly emerged adults of both sexes significantly increased with the increasing host age (from 1 to 4 days old) at the time of parasitisation (P<0.0001) (Table 1). Regardless of the host age when parasitised, females were significantly larger than males (P<0.01) (Table 1). The egg load of newly emerged females was also significantly higher when parasitoids attacked aphids that were 3-7 days old (P<0.0001) (Fig. 4). There was a positive linear relationship between the body size and egg load of newly emerged females (P<0.0001) (Fig. 5).

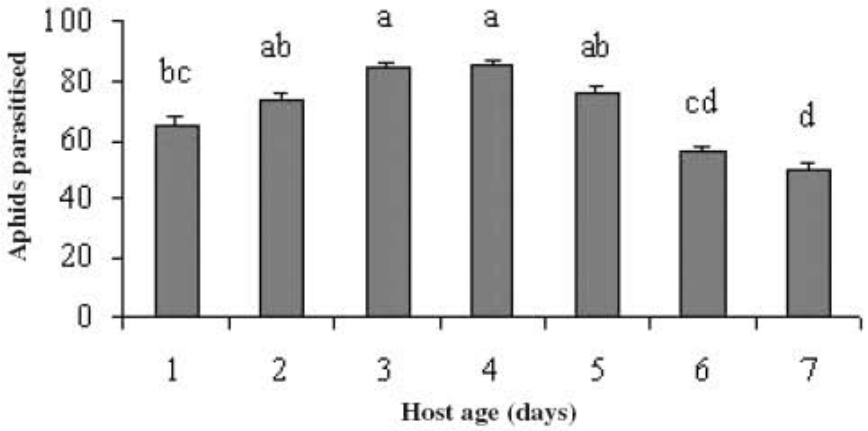

FIGURE 1: Mean number of aphids parasitised per *A. ervi* female in relation to host age. Columns with the same letters are not significantly different (P>0.05).

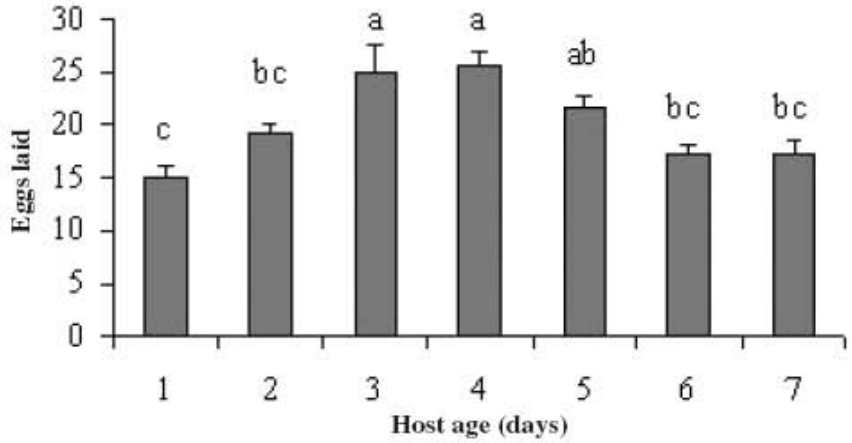

FIGURE 2: Mean number of eggs laid per *A. ervi* female (estimated from dissection) in relation to host age. Columns with the same letters are not significantly different (P>0.05).

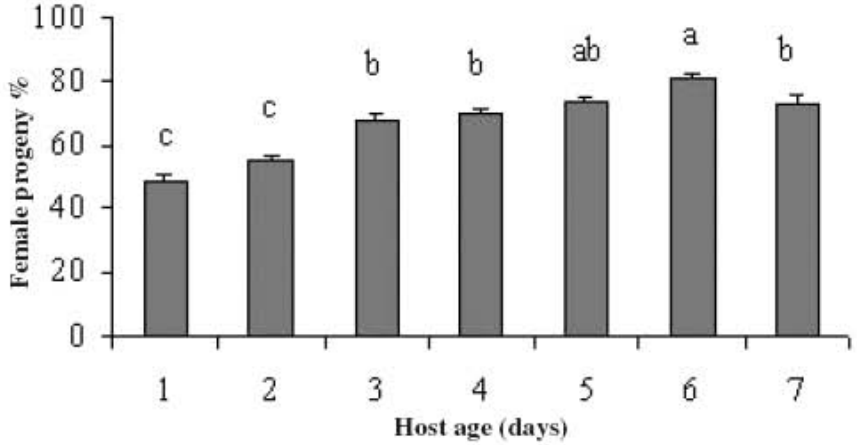

FIGURE 3: Mean proportion of female progeny emerged from hosts of different age classes. Columns with the same letters are not significantly different (P>0.05).

TABLE 1: Mean (± SE) head width (mm) of newly emerged *A. ervi*. Means followed by the same letters within rows were not significantly different (P>0.05).

	Host age (days) at parasitisation						
Sex	1	2	3	4	5	6	7
Male	0.535 d ± 0.0034	0.556 c ± 0.0032	0.580 b ± 0.0031	0.601 a ± 0.0037	0.599 a ± 0.0045	0.596 ab ± 0.0049	0.598 a ± 0.0051
Female	0.585 d ± 0.0027	0.613 c ± 0.0032	0.642 b ± 0.0031	0.660 a ± 0.0037	0.656 ab ± 0.0029	0.646 ab ± 0.0054	0.649 ab ± 0.0059

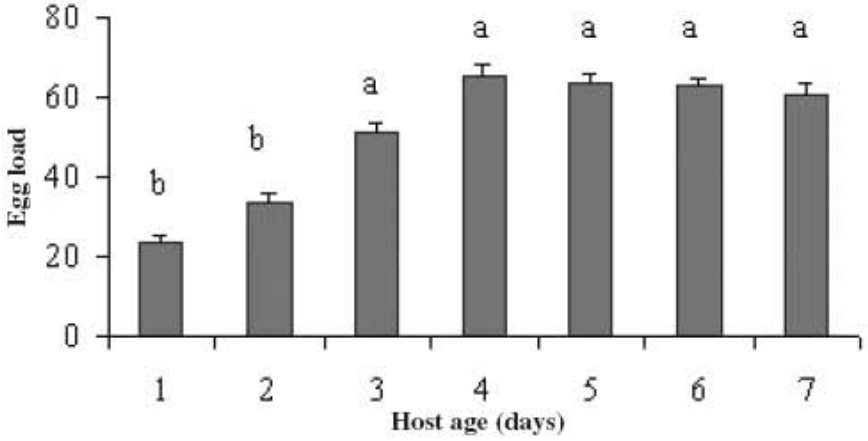

FIGURE 4: Mean egg load per female progeny emerged from hosts of different age classes. Columns with the same letters are not significantly different (P>0.05).

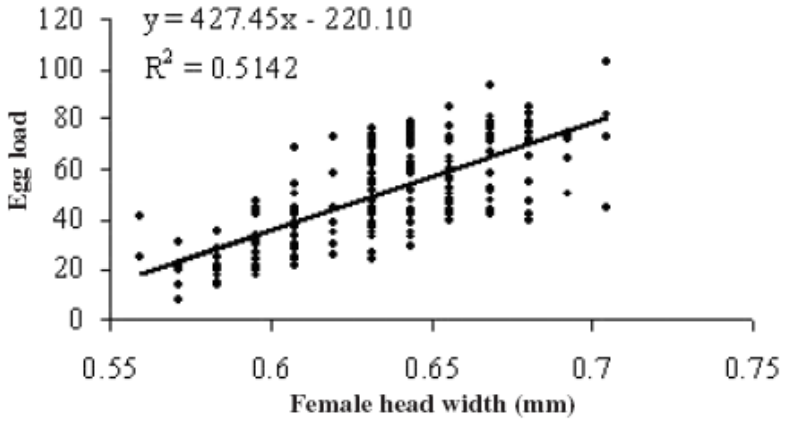

FIGURE 5: Relationship between body size and egg load per newly emerged female of *A. ervi*.

DISCUSSION

Female *A. ervi* attacked pea aphids of different ages. The immature pea aphids grow in size as they develop to adult stage and larger aphids have more food resource for *A. ervi* larvae (Li et al. 2002; He et al. 2005). According to the theory of optimal host acceptance in parasitoids, which is based on the model of optimal diet in predators (Stephens & Krebs 1986), *A. ervi* should prefer older aphids for oviposition. However, older aphids may be more capable of physically defending themselves from parasitisation and cost more to the female parasitoids in oviposition (Chau & Mackauer 2001). The present results indicate that *A. ervi* preferred aphids that were 3-5 days old over the younger (1-3 days old) and older ones (6-7 days old) for oviposition. Therefore, the food resource at the point of oviposition is not the only factor that determines the oviposition decision by parasitoids, and the parasitoids may select hosts according to the optimal trade-off between food supply and oviposition costs.

The results in this study also indicate that host age preference by *A. ervi* is related to its progeny's reproductive fitness. The body size of progeny and egg load of newly emerged females were similar when aphids were attacked at 4-7 days old, suggesting that the quantity and quality of food resource for the parasitoid progeny are similar in aphids of this age range.

The increase in proportion of female progeny of *A. ervi* with host size supports the host size-dependent sex allocation theory in parasitic Hymenoptera (Charnov et al. 1981; Charnov 1982). There is also field evidence that host instar plays a role in sex ratios, as reported for *A. ervi* parasitising pea aphid, where a male-biased population emerged from small aphids in the early season (Sequeira & Mackauer 1993).

Although the body size of parasitoids emerged from aphids parasitised at 4 days old was similar to that emerged from aphids parasitised at 6 days old, the proportion of female offspring was significantly lower when parasitoids selected 4-day-old aphids for oviposition than that when parasitoids selected 6-day-old aphids. Sex ratio data suggest that the body size of hosts at the point of oviposition is the major factor affecting the sex allocation by *A. ervi*.

The findings of this study have implications for laboratory mass rearing and field release of *A. ervi*. For example, aphids that are 3-5 days old appear to be the most appropriate hosts in the mass-rearing program because they gave the best fitness return for the parasitoids. The timing for field release of the parasitoids should be set to when the 3- to 5-day-old aphids are most abundant because parasitism in these age-classes produces no or few aphid progenies (He et al. 2005) and gives a greater parasitoid population of better reproductive fitness in the next generation.

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OVIPOSITION STRATEGY OF *APHIDIUS ERVI* (HYMENOPTERA: APHIDIIDAE) IN RESPONSE TO HOST DENSITY

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ABSTRACT

The reproductive response of *Aphidius ervi* Haliday to the density of pea aphid, *Acyrthosiphon pisum* (Harris), was investigated in plastic cylinders (10.5 cm high × 8.5 cm diameter). Mean number of aphids parasitised and eggs laid by a single *A. ervi* significantly increased with an increase of host density. Numbers of eggs laid per parasitoid reached a plateau at host densities of 75 aphids/cylinder and above. However, the number of eggs laid in each parasitised aphid significantly decreased (from 2.9 to 1.3 eggs) with the increase of host density from 15 to 75 aphids/cylinder, after which no further decrease occurred. These results suggest that the parasitoid adjusts oviposition strategy in response to increasing host density through increasing parasitism and decreasing superparasitism. The proportion of female progeny developed from fertilised eggs increased (up to 70%) with the increase of host density from 15 to 50 or 75 aphids/cylinder, after which it gradually declined, suggesting that the sperm limit occurs when host density reaches 50 to 75 aphids/cylinder.

Keywords: *Aphidius ervi*, host density, reproduction, sex allocation, *Acyrthosiphon pisum*.

INTRODUCTION

Hymenopterous parasitoids of aphids have provided spectacular success in biological control (Starý et al. 1988). The impact of a parasitoid on its host population greatly depends upon its ability to find and parasitise hosts and to increase offspring numbers in response to increasing host density (Waage & Hassell 1982; Mackauer 1983). It is important to identify the form of the parasitoid response to prey density in population modelling, from which biological control programmes can be developed (Mills & Lacan 2004). In analytical host-parasitoid models, changes in the density-dependent sex ratio of parasitoids influence the level of host population equilibrium and the stability of the host-parasitoid relationships thus affecting the success of biological control (Waage & Hassell 1982; Hassell & Waage 1984).

Aphidius ervi Haliday is a cosmopolitan, solitary, endophagous parasitoid (Marsh 1977) and a major biological control agent of several aphid species on economically important crops such as legumes and cereals (Powell 1982; Starý et al. 1988). Ives et al. (1999) studied some aspects of the functional response of *A. ervi* on pea aphid, *Acyrthosiphon pisum* (Harris), with emphasis on the parasitoid's behavioural decisions and their influence on the parasitism rate. In this paper, the relationship between host density and reproductive fitness of *A. ervi* was investigated by determining how host density affected parasitoid reproductive output and sex allocation. The aim was to provide further information for the assessment and improvement of *A. ervi*'s effectiveness in biological control.

MATERIALS AND METHODS

Insects and experimental conditions

A breeding colony of *A. ervi* was established from parasitised blue-green lucerne aphid, *Acyrthosiphon kondoi* Shinji, collected on lucerne in Palmerston North, New Zealand, in December 2002. The colony was subsequently reared on pea aphid, feeding on potted broad bean, *Vicia faba* L. cv. Pride, for five generations before being used for experiments. All experiments were carried out in transparent plastic cylinders (8.5 cm in diameter, 10.5 cm in height) with gauze-covered holes in the top and sides for ventilation. A broad bean cutting standing in a plastic container (6.5 cm in diameter, 8.5 cm in height) with tap water was placed in the plastic cylinder and replaced when wilted. Honey solution (10%) was supplied daily in a cotton wool wick (1 cm in length), inserted through a hole (0.6 cm in diameter) in the top of the cylinder. Parasitoid adults used for the experiments emerged from pea aphids parasitised at third instar. The experiment was carried at 20±1°C and 60-70% RH with 16:8 h light:dark.

Experiment

To determine whether and to what extent host density affected reproduction of *A. ervi*, six densities of aphids (15, 25, 50, 75, 100 and 125 third instars/cylinder/parasitoid female/day) were tested, with 10 replicates for each density. One mated *A. ervi* female (<12 h old) was introduced into an experimental cylinder with aphids feeding on a bean plant cutting. The female was allowed to stay in the cylinder for 24 h, and then moved to another cylinder with the same number of healthy third instar aphids, etc. until she died.

Because superparasitism was common under laboratory conditions, the oviposition potential of *A. ervi* was estimated by counting both the number of eggs laid (fecundity) and aphids parasitised (parasitism). To estimate the daily number of eggs laid in a parasitised aphid, five aphids from each cylinder at densities of 15 and 25 aphids, 10 aphids at density of 50 aphids, and 20 aphids at densities of 75, 100 and 125 aphids were randomly selected from each cylinder 4 days after the removal of the female parasitoid. These selected aphids were dissected in 70% alcohol under the stereomicroscope (Leica MZ12, Germany). The numbers of parasitoid larvae recorded from dissecting were assumed equal to the number of eggs laid (Bueno et al. 1993). The remaining aphids were reared until mummification. The number of eggs laid was assumed equal to the total number of aphids parasitised (= the sum of mummies and aphids parasitised detected by dissecting)×the average number of eggs laid in a parasitised aphid detected by dissecting. The emerged offspring were counted and sexed.

Statistical analysis

Goodness of fit tests were used to test whether the data were normally-distributed. Effect of aphid density on the number of parasitoid eggs laid was assessed by one-way ANOVA and means separated by Tukey's studentised range (HSD) test. Data for percent female progeny, parasitism rate and eggs per parasitised aphid were not normally distributed after transformation and were thus analysed using the nonparametric Kruskal-Wallis test. Means were subsequently separated by Dunn's procedure for multiple comparisons (Zar 1999). A significance level of $P<0.05$ was used for all tests.

In this study, more than 80% of eggs were laid within the first 8 days, thus all data presented were from the first 8 days.

RESULTS

Mean number of aphids parasitised and eggs laid (actual fecundity) during *A. ervi* females' life time increased with the increase of host density ($P<0.0001$) (Fig. 1). Fecundity reached a plateau at host densities of 75/cylinder and above (Fig. 1). The number of eggs laid in each parasitised aphid decreased with the increase of host density from 15 to 75/cylinder, after which no further decrease occurred ($P<0.0001$) (Fig. 2). The daily parasitism rate decreased when the host density increased to 50/cylinder ($P<0.0001$) (Fig. 3). The proportion of female progeny was greatest at host densities of 25 to 100/cylinder, and least at extremes of low and high host densities ($P<0.0001$) (Fig. 4).

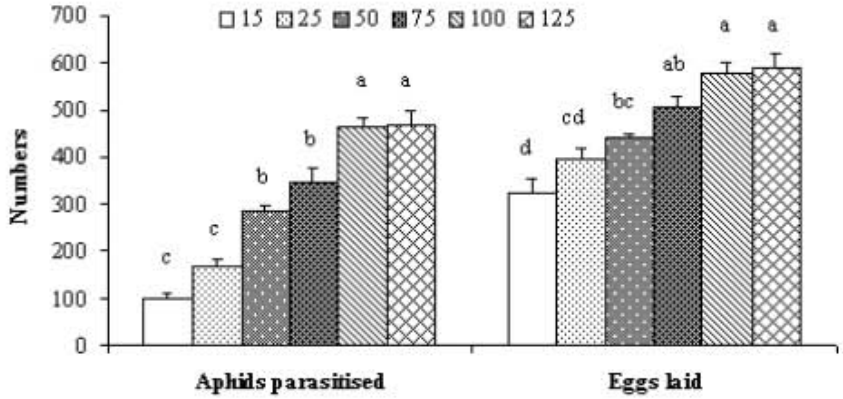

FIGURE 1: Mean number of aphids parasitised and eggs laid by *A. ervi* at different host densities (15, 25, 50, 75, 100 and 125 aphids/cylinder). Within the same category (Aphids parasitised or Eggs laid) columns with the same letters are not significantly different (Kruskal-Wallis test for aphids parasitised and ANOVA for eggs laid at P>0.05). Error bars are SEM.

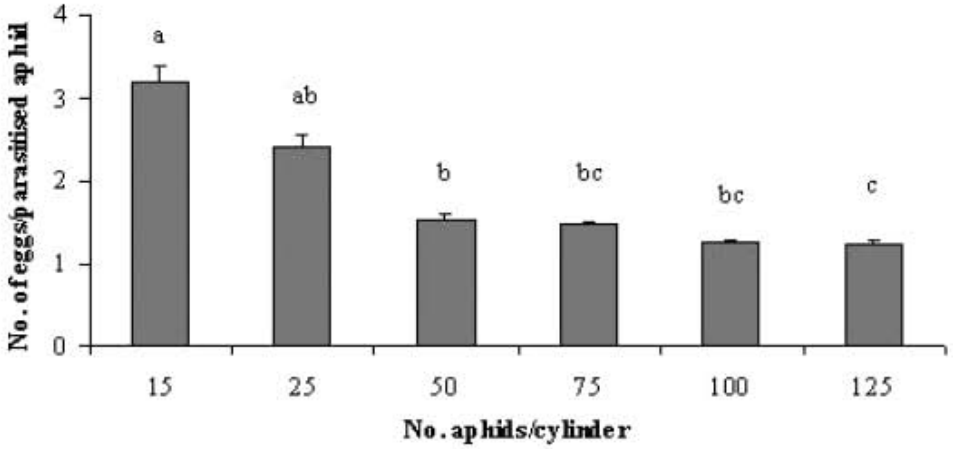

FIGURE 2: Mean number of eggs per parasitised aphid laid by *A. ervi* at different host densities. Columns with the same letters are not significantly different (Kruskal-Wallis test at P>0.05). Error bars are SEM.

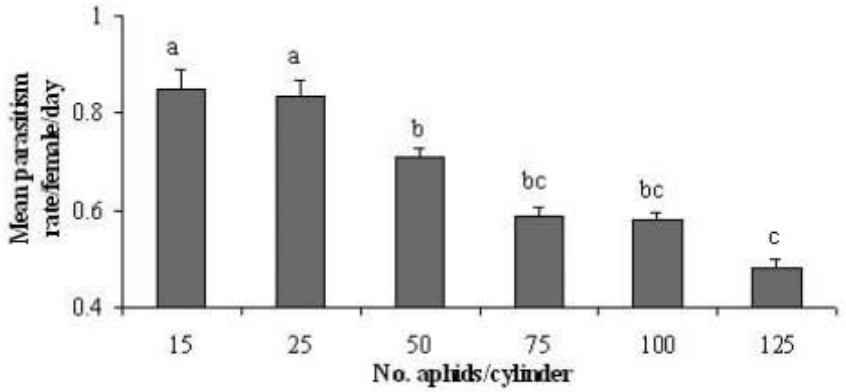

FIGURE 3: Mean daily parasitism rate of *A. ervi* at different host densities. Columns with the same letters are not significantly different (Kruskal-Wallis test at P>0.05). Error bars are SEM.

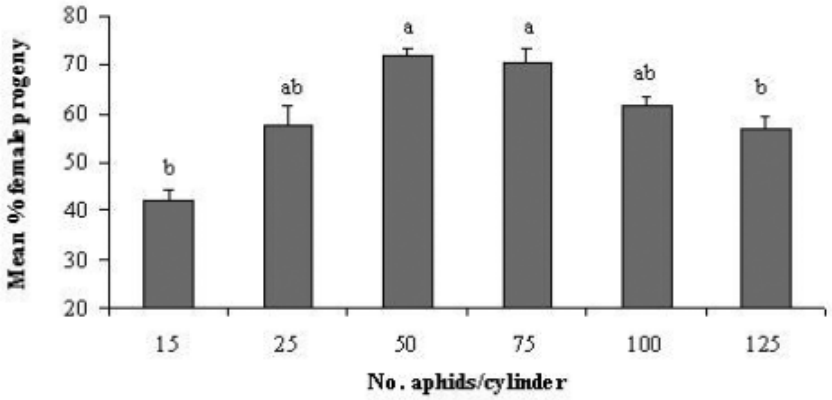

FIGURE 4: Mean proportion of female progeny of *A. ervi* at different host densities. Columns with the same letters are not significantly different (Kruskal-Wallis test at P>0.05). Error bars are SEM.

DISCUSSION

High fecundity and parasitism rates are considered necessary for parasitoids to be able to respond rapidly to the increases in pest density (Waage & Hassell 1982; Waage 1990). The results of the present study indicate that *A. ervi* can adjust oviposition strategy in response to increasing host density through increasing parasitism and decreasing superparasitism (Figs 1 & 2). These data alone suggest that this parasitoid has high potential to suppress the aphid population when the latter increases. However, when the host population was 50/cylinder, mean daily parasitism rate significantly decreased (Fig. 3). The behaviour of *A. ervi* in this experiment is consistent with the classic Holling type II functional response (Holling 1959), i.e. the number of aphids parasitised increased with an increase in aphid density but at a progressively decreasing rate. A type II functional response was also found in some other *Aphidius* species, such as the *A. smithi* Sharma & Subba Rao (Mackauer 1983) and *A. sonchi* Marshall (Liu 1985). Results of this study suggest that a host density equivalent to 50 aphids/cylinder is the highest critical density where the parasitoid could maximise its control efficiency. This information could be important when considering host-parasitoid density ratio in biological control programmes (Hassell & Waage 1984). For example, to maximise biological control efficiency of pea aphids, parasitoids could be released at a rate of about one female parasitoid/50 aphids.

The proportion of female progeny developed from fertilised eggs quickly and significantly increased with the increase of host density from 15 to 50 aphids/cylinder, after which it gradually declined. This suggests that the highest potential proportion of female progeny that *A. ervi* can produce is about 70% and sperm limit occurs when host density reaches 50 to 75 aphids/cylinder. Offspring sex ratio of other *Aphidius* species, such as *A. smithi* (Mackauer 1983) and *A. sonchi* (Liu 1985), has also been found to vary with host density. In hymenopteran parasitoids, males can mate several times while females mate only once. It is thus to the female's benefit that she produces the minimum number of males possible. However, when hosts occur at low density, a high parasitoid female sex ratio should reduce the ability of parasitoid persistence (Tripathi & Singh 1991), leading to extinction of the host population and eventually the parasitoid population. This suggests that the female parasitoids can adjust the sex allocation strategy in response to the host density. A similar case was found in *Aphelinus mali* Hal, which attacks the woolly apple aphid (Mueller et al. 1992).

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THE EFFECT OF PARASITISM BY *APHIDIUS ERVI* ON DEVELOPMENT AND REPRODUCTION OF THE PEA APHID, *ACYRTHOSIPHON PISUM*

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ABSTRACT

The effect of parasitism by *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) on development, survival and reproduction of pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), of different ages was studied in the laboratory. Aphids parasitised when 1 and 2 days old (1st and 2nd instar) died at the 4th instar. However, those parasitised when 3 to 6 days old (3rd and 4th instar) could reach the adult stage following parasitism and those that were parasitised after 4 days old (late 3rd instar) were still able to produce progeny. In comparison with the unparasitised aphids, the parasitised aphids had a significantly shorter reproductive period and produced significantly fewer progeny, and thus had significantly lower intrinsic rates of increase, net reproductive rates, shorter generation time and longer doubling time. The potential impact of the parasitoid on host population growth is discussed.
Keywords: pea aphid, *Acyrthosiphon pisum*, *Aphidius ervi*, reproduction, population growth.

INTRODUCTION

Many studies have reported that hymenopteran endoparasitoids can reduce the reproductive potential of aphids (Lui & Hughes 1984; Mackauer & Kambhampati 1984; Sequeira & Mackauer 1988; Tang & Yokomi 1996; He et al. 2003; Lin & Ives 2003). The majority of these studies indicate that aphids parasitised in their early instars die before reproduction but those parasitised at later stages of development may reach the adult stage and produce a limited number of progeny before mummification. Most aphid populations consist of multiple stages of development. Therefore, knowledge of the effect of parasitism on development, reproduction and population growth of aphids of different ages is critical to the success of biological control (Tsai & Wang 2002; Zhang & Hassan 2003).

The pea aphid (*Acrythosiphon pisum* (Harris)) is a pest of lucerne in New Zealand (Cameron & Walker 1989), originally probably from the Palaearctic (Blackman & Eastop 2000). It was first found in Auckland in October 1976 (Archibald 1979). *Aphidius ervi* Haliday is a solitary endophagous parasitoid of several pest aphid species on economically important crops, such as legumes and cereals (Starý 1978; Powell 1982). It was imported to New Zealand from California to control the pea aphid and blue green lucerne aphid, *Acrythosiphon kondoi* Shinji (Cameron & Walker 1989).

Several authors (Pennacchio et al. 1995; Digilio et al. 1998; Rahbé 2002) have studied biochemical changes in pea aphid caused by *A. ervi*. However, little is known about how parasitism by *A. ervi* affects the population of the pea aphid, making it difficult to evaluate the biological control efficiency of this parasitoid. This paper is the first to investigate the effect of parasitism by *A. ervi* on the development, reproduction and population growth of *A. pisum* at different stages of development.

MATERIALS AND METHODS

Breeding colony and experimental conditions

A breeding colony of *A. ervi* was established from individuals that emerged from blue-green lucerne aphids which were collected from lucerne on the AgResearch farm at Aorangi near Palmerston North in December 2002. Prior to the experiments, the colony was reared on pea aphid feeding on potted broad bean, *Vicia faba* L. cv. Pride, for five generations. The colony was maintained and all experiments were carried out at 20 ± 1°C and 60-70% RH with 16:8 h light: dark.

Development and reproduction

The effect of parasitism by *A. ervi* on the growth and reproduction of pea aphids at different stages of development (1–10 days old = 1st instar to adult) was investigated. To obtain parasitised aphids of the same age class, a single mated *A. ervi* female was released into a Petri dish (8.5 cm diameter × 1.3 cm height) containing 10 aphids of the same age. Aphids that received a single oviposition strike from the female parasitoid were removed and replaced with unparasitised individuals of the same age until 15 parasitised aphids were collected. Parasitised aphids were placed individually onto a bean plant, which was held in a transparent plastic cylinder (He et al. 2003). There were 10 treatments (age classes) and 15 replicates (number of parasitised aphids) for each treatment in this study. Survival, development and reproduction of parasitised aphids were monitored at 24 h intervals. Any offspring from these aphids were counted and removed. In addition, the survival and reproductive periods of aphids were also recorded. Fifteen unparasitised aphids (start at 1 day old) reared individually in cylinders served as controls and their survival and reproduction were monitored as above.

Population growth

The daily survival rate and reproduction of aphids were compiled into a life table to assess the population growth according to the method of Jervis & Kidd (1996). The intrinsic rate of increase (female/female/day; r_m) was estimated by solving the Lotka-Euler equation ($\sum e^{-r_m \mathbf{x}} l_x m_x = 1$). Other calculations included the net reproductive rate (females/females/generation; $R_0 = \sum l_x m_x$), mean generation time (days; $T = \log_e(R_0)/r_m$) and doubling time (days; $DT = \log_e(2)/r_m$). In these calculations $\mathbf{x}$ is the pivotal age, l_x is the proportion of the females surviving to age $\mathbf{x}$, and m_x is the number of offspring produced per female at age $\mathbf{x}$. For each treatment and the control, a jackknife method (Caswell 2001) was used to estimate the means and standard errors of the above life table parameters by partitioning the 15 parasitised aphids into 5 groups of 3 individuals.

Statistical analysis

A linear regression (PROC REG) was used to identify the relationships between aphid age at the point of parasitism, reproductive period and the number of progeny produced. A logistic regression (PROC NILN) was applied to analyse the relationships between aphid age ($\mathbf{x}$) at the point of parasitism and r_m (i.e. $r_m = c/(1+\exp(a+b*\ln(\mathbf{x})))$, where the constant c represents the maximum possible value of r_m; and a and b are constants to fit the curve). All other data were analysed using an ANOVA (PROC GLM). When significant differences in variables occurred, means were separated using a Tukey's studentised range (HSD) test ($P>0.05$). All analyses were performed using SAS.

RESULTS

Development and survival

Aphids parasitised at 1 and 2 days old (1st and 2nd instar) became mummified in the 4th instar. However, aphids parasitised at 3 to 6 days old (3rd and 4th instar) continued development to the adult stage. For all ages, parasitised aphids died about 7 days after being parasitised and their longevity was significantly shorter than unparasitised aphids ($P<0.0001$) (Table 1).

TABLE 1: Effect of parasitism of pea aphid by *A. ervi* on survival (days), reproductive period (days) and number of progeny. Means (± SE) followed by the same letters in columns are not significantly different (P>0.05).

Aphid age (days)	Survival (days)	Reproductive period (days)	No. of progeny
1 (1st instar)	8.13 ± 0.09 j	---	---
2 (2nd instar)	9.13 ± 0.09 ij	---	---
3 (3rd instar)	10.20 ± 0.11 hi	---	---
4 (3rd instar)	11.13 ± 0.09 gh	2.07 ± 0.15 g	4.93 ± 0.43 g
5 (4th instar)	12.07 ± 0.07 fg	2.33 ± 0.16 fg	10.07 ± 0.90 g
6 (4th instar)	13.13 ± 0.09 ef	3.13 ± 0.13 f	19.40 ± 1.13 f
7 (adult)	14.07 ± 0.07 de	4.20 ± 1.75 e	30.47 ± 1.48 e
8 (adult)	15.13 ± 0.09 cd	5.20 ± 0.11 d	39.07 ± 1.49 d
9 (adult)	16.20 ± 0.11 c	6.33 ± 0.19 c	52.33 ± 0.88 c
10 (adult)	17.47 ± 0.17 b	7.60 ± 0.19 b	62.60 ± 1.92 b
Control	27.87 ± 0.82 a	15.60 ± 0.38 a	111.20 ± 3.17 a

Reproduction

Only aphids parasitised when ≥ 4 days old (late 3rd instar) produced any progeny. Most of these aphids started reproduction from 8 days old. Parasitised aphids had a significantly shorter reproductive period and produced significantly fewer progeny than healthy aphids (P<0.0001) (Table 1). For the parasitised aphids, the reproductive period and the number of progeny produced increased significantly with the age when the aphid was parasitised (P<0.0001) (Figs 1 & 2, respectively).

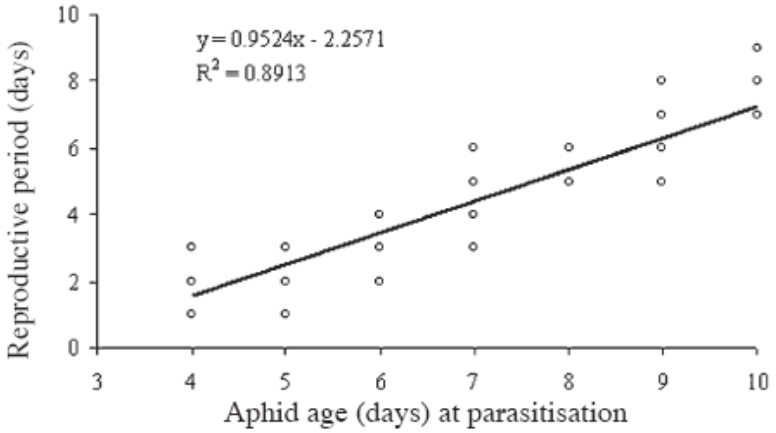

FIGURE 1: Relationship between host age at parasitisation and reproductive period.

Population growth

Parasitism by *A. ervi* significantly affected the population growth of pea aphids (P<0.05), reflected in changes in the intrinsic rate of increase (r_m), net reproductive rate (R_0), generation time (T), and doubling time (DT) (Table 2). Aphids parasitised as immature stages (≤ 6 days old) had a significantly lower r_m and R_0, shorter T, and longer DT than those parasitised as adults and healthy aphids (P<0.0001). The r_m increased rapidly with the increase of aphids' age when they were parasitised and reached a plateau at 9 and 10 days old (P<0.0001) (Fig. 3).

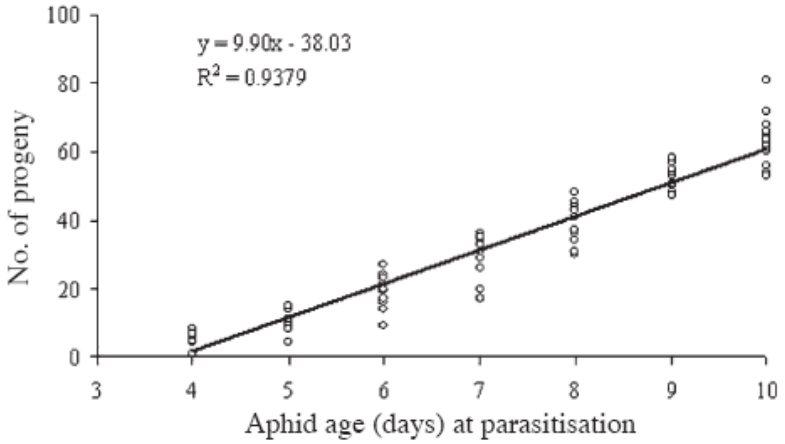

FIGURE 2: Relationship between host age at parasitisation and reproduction of progeny.

TABLE 2: Effect of parasitism by *A. ervi* on life table parameters of pea aphid. Means (± SE) followed by the same letters in columns are not significantly different (P>0.05).

Aphid age (days)	r_m	R_0	T	DT
4 (3rd instar)	0.1799 ± 0.0083 e	4.93 ± 0.39 g	8.71 ± 0.09 d	3.95 ± 0.21 a
5 (4th instar)	0.2573 ± 0.0050 d	9.13 ± 1.17 g	8.86 ± 0.07 d	2.94 ± 0.35 b
6 (4th instar)	0.3398 ± 0.0075 c	19.40 ± 1.18 f	8.69 ± 0.02 d	2.05 ± 0.05 c
7 (adult)	0.3705 ± 0.0056 b	30.47 ± 0.85 e	9.21 ± 0.07 c	1.87 ± 0.03 d
8 (adult)	0.3924 ± 0.0072 ab	39.07 ± 1.53 d	9.33 ± 0.07 c	1.77 ± 0.03 d
9 (adult)	0.3972 ± 0.0031 a	52.07 ± 0.67 c	9.95 ± 0.05 b	1.74 ± 0.01 d
10 (adult)	0.3979 ± 0.0033 a	62.60 ± 2.52 b	10.29 ± 0.06 b	1.73 ± 0.01 d
Control	0.4018 ± 0.0023 a	113.00 ± 2.51 a	11.89 ± 0.13 a	1.74 ± 0.02 d

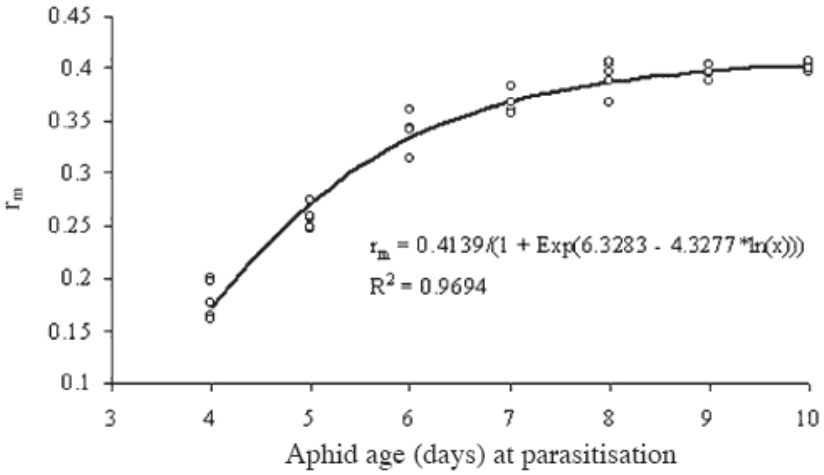

FIGURE 3: Relationship between host age at parasitisation and r_m.

DISCUSSION

Results of this study show that, in comparison with the unparasitised pea aphid controls, parasitism by *A. ervi* significantly reduced pea aphids' survival period, reproductive potential and population growth. However, when compared to *Praon pequodorum* Viereck (Sequeira & Mackauer 1988), another parasitoid of pea aphid, *A. ervi* has greater potential to suppress aphid populations because those parasitised by *P. pequodorum* produce 20 to 30 more progeny than those parasitised by *A. ervi*. The effect of parasitism by *A. ervi* on pea aphid reproduction is similar to that by *Aphidius eadyi* Starý, González & Hall (He et al. 2003).

The efficiency of parasitoids in the control of aphids depends on their ability to suppress aphids' population growth. Aphids parasitised by *A. ervi* continued to feed and grow until they were killed by the developing parasitoid larva. Because the time from being parasitised to mummification is about 7 days for all parasitised aphids, their survival and reproduction depend on the time when they are parasitised. Aphids parasitised ≤3 days old died at the fourth instar or early adult stage. In contrast, those parasitised when ≥4 days old were able to reach the adult stage and successfully reproduce. This may be because the aphid embryos in the later instar and adult stages have developed a resistant cuticle by the time of oviposition by the parasitoid (Polaszek 1986) and have thus escaped consumption by the developing parasitoid larvae.

The present study indicates that parasitised aphids' development, survival, reproduction and population growth vary with their age at the time of parasitism. It appears that attacking young hosts will be more effective in suppressing aphid populations than attacking the adult aphids. According to Schowalter (2000), early in the season, growing populations have a high proportion of aphids in young age classes. Thus, the application of *A. ervi* early in the season could significantly suppress populations of pea aphid in the field, inhibiting its dispersal and population build-up later in the season.

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Host stage preference and reproductive fitness of *Aphidius eadyi* (Hymenoptera: Aphidiidae) on *Acyrthosiphon pisum* (Hemiptera: Aphididae)

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Abstract Host stage preference by *Aphidius eadyi* Starý, González & Hall on pea aphid, *Acyrthosiphon pisum* (Harris), and its effects on the reproductive fitness of *A. eadyi*, were studied in the laboratory at 20 ± 1°C and RH 60–70% with a photoperiod of 16 h light:8 h dark. *Aphidius eadyi* females accepted aphids of all stages but preferred fourth instar nymphs and adults for oviposition. Females developed increasingly faster with the increase of host stages, and males developed faster than females in all host stages. The sex ratio of resulting parasitoids was close to 1:1 from all host stages. The egg load and body size of *A. eadyi* progeny at emergence increased with increasing host stage at the time of parasitisation. The host stage had nonlinear relationships with the number of eggs laid and aphids parasitised by *A. eadyi*, and the body size of its resulting progeny. Both host stage and body size of resulting parasitoid progeny affected the egg load of newly emerged parasitoids but the latter had more effect. The fourth instar aphids appeared to be the most appropriate hosts for the mass-rearing programme because they gave the best fitness return for the parasitoids. The field release of parasitoids may be better timed when fourth instar aphids are the most abundant because aphids parasitised at this stage produce few progeny.

Keywords Hymenoptera; *Aphidius eadyi*; host stage; preference; fitness; pea aphid; *Acyrthosiphon pisum*

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INTRODUCTION

Many studies suggest that host stage at the time of parasitism affects the reproductive fitness of resulting parasitoids, altering for example the developmental time (Sequeira & Mackauer 1993), progeny sex ratio (Godfray 1994; Napoleon & King 1999), body size (Liu 1985; Lampson et al. 1996), and egg load (Liu 1985; Visser 1994; Mills & Kuhlmann 2000). Foraging parasitoids usually encounter hosts of different life stages and have opportunities to select the most suitable hosts to maximise their reproductive success. Host preference by parasitoids exists when the frequency of some host types parasitised is higher than that of others (Hopper & King 1984). Oviposition decision by parasitoids usually depends on the host stage or size, which is often considered as an index of host quality; for example, large hosts contain more resources than small hosts (Charnov et al. 1981; Liu 1985).

Pea aphid, *Acyrthosiphon pisum* (Harris), is one of the important pests of lucerne in New Zealand (Kain et al. 1979). *Aphidius eadyi* Starý, González & Hall is a specific solitary parasitoid of *Ac. pisum*, and potentially important biological control agent of this pest. It was imported from California (Cameron et al. 1979), and now occurs throughout New Zealand (Cameron & Walker 1989). Recent research demonstrated that *A. eadyi* could significantly reduce the reproductive potential of *Ac. pisum* (He et al. 2003). However, little is known about whether and to what extent the parasitoid females choose their hosts according to the latter's stage and how such choice affects the reproductive fitness of the parasitoid's resulting progeny. This information can help us design

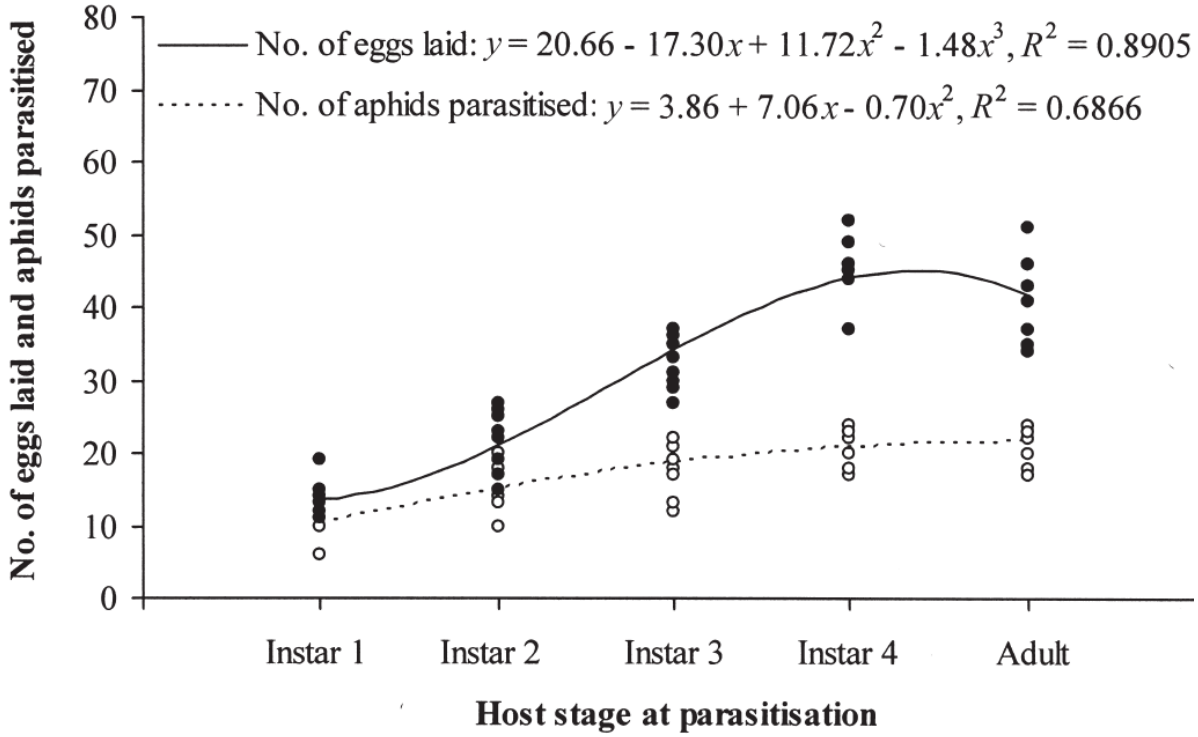

Fig. 1 Relationships between the number of eggs laid or aphids parasitised by *Aphidius eadyi* and the host stage at parasitisation.

the optimal mass-rearing programme for *A. eadyi*, better understand its effect on aphid populations in the field, and improve the timing for mass-releasing programme.

The aim of this study was to examine the host stage selection by *A. eadyi* on *Ac. pisum* with two objectives: (1) to evaluate the host stage preference of *A. eadyi*, and (2) to determine the effect of host stage on the reproductive fitness of *A. eadyi* progeny.

MATERIALS AND METHODS

Breeding colony and experimental conditions

A breeding colony of *A. eadyi* was established from individuals emerged from bluegreen lucerne aphids, *Ac. kondoi* Shinji, which were collected from lucerne on an AgResearch farm at Aorangi, near Palmerston North in late December 2002. Prior to the experiments, the colony was reared on *Ac. pisum* feeding on potted broad bean, *Vicia faba* L. cv. 'Pride', for five generations. The colony was maintained and all experiments were conducted at 20 ± 1°C and RH 60–70% with a photoperiod of 16 h light:8 h dark. Lighting was provided by broad-spectrum, high frequency light tubes (Osram L36W/72-965) with a light intensity of 430 lux. The experimental containers were transparent plastic cylinders (8.5 cm in diameter, 12 cm in height) with three gauze-covered holes, one (5 cm in diameter) in the top and two (2 cm in diameter) in opposing sides of the container for ventilation. Ten percent honey solution was supplied as food for parasitoids by applying it to a cotton wool wick (0.6 cm in diameter and 1 cm in length), inserted through a small hole (0.6 cm in diameter) in the top of the container.

Host stage preference

To determine whether *A. eadyi* females had any preference for different host stages of *Ac. pisum* for oviposition, we used five host stages including four nymphal instars and adult (1-, 2-, 3-, 5-, and 7-day-old stages for instars 1, 2, 3, 4, and the adult, respectively). Parasitoid adults used for the experiment emerged from aphids that had been parasitised at instar 3. For each replicate, we released one mated female (<12 h old) into a plastic cylinder with a bean plant infested with 50 aphids (10 of each host stage). The female parasitoid was allowed to stay in the cylinder for 24 h, and then moved to another

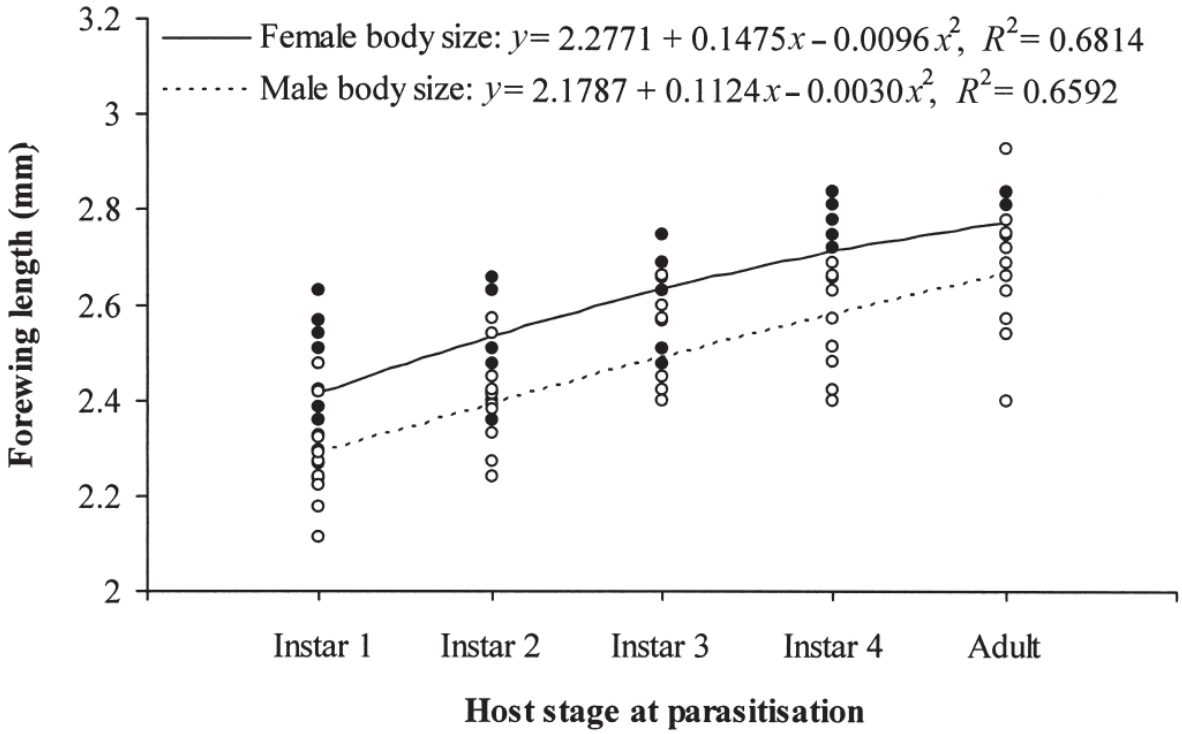

Fig. 2 Relationships between *Aphidius eadyi* adult forewing length and the host stage at parasitisation.

cylinder with 50 healthy aphids of different stages, etc., until she died. Aphids of different life stages from each cylinder were separated, and each stage was transferred to a clean bean plant in a container. We repeated the above experiment 10 times.

Our records show that *A. eadyi* females laid more than 80% of their total eggs in the first 5 days of their oviposition period (unpubl. data). Therefore, for this experiment, we dissected aphids and analysed the data from the first 5 oviposition days of each parasitoid. We randomly selected and dissected five of the 10 aphids of each host stage in each cylinder. As a result, we dissected a total of 250 aphids for each host stage (five aphids of a life stage × five cylinders (5 days) × 10 parasitoid females). These aphids were killed 4 days after being parasitised and dissected in 70% alcohol under a stereomicroscope. The number of larvae recorded from dissected aphids was assumed to equal the number of eggs laid by female parasitoids (Bueno et al. 1993). The remaining aphids were reared until mummification.

Progeny fitness

Mummies were checked every 12 h for emergence. The progeny emerged from each parasitised host stage were counted and sexed. Developmental time of parasitoids from egg to adult in hosts of each stage was recorded. For each host stage, 30 females and 30 males of the resulting parasitoids were killed by freezing at –20°C and their forewing length was measured to the nearest 0.01 mm using a stereomicroscope equipped with an ocular micrometer. To determine the egg load of newly emerged females (<12 h old), we randomly selected 15 females from each host stage and dissected them in 70% alcohol on a slide. One drop of acid fuchsin was added to the alcohol and allowed to stand for 3–5 min for staining. The number of mature eggs in the ovaries was counted and recorded.

Statistical analysis

Regression analyses were used to determine the relationships between host stage and several parasitoid reproductive fitness parameters (Fig. 1, 2) (PROC REG, SAS Institute 1996). Because the egg load of newly emerged females increased with both host stage and female parasitoid body size, a central composite design (CCD) (Box & Draper 1987) was used to estimate the interaction between the two variables (PROC GENMOD, SAS Institute 1996). The estimated value of the response variable logit $\hat{g}(z)$ is given by the polynomial equation: $\hat{g}(z) = \beta_0$

$+ \beta_1 x + \beta_2 y + \beta_{11} x^2 + \beta_{22} y^2 + \beta_{12} xy$, where $\hat{g}(z)$ is the estimated value of log (egg load), β_0 is the intercept on y of $\hat{g}(z)$, and x and y are host stage at parasitisation and resulting parasitoid size, respectively. The rate of change (slopes) associated with host stage and parasitoid size and their interactions is represented by β_1, β_2, and β_{12}; slopes of the quadratic effect on host stage and parasitoid size are presented by β_{11} and β_{22}, respectively. Only significant terms, after running the full regression model, were kept in the final models. We then used a log likelihood ratio test (McCullagh & Nelder 1989) to determine whether host stage and parasitoid size had a different effect on egg load.

An analysis of variance (ANOVA) followed by a Tukey's studentised range (HSD) test ($P < 0.05$) was used to analyse other data. Before ANOVA, data on the proportion of female offspring were transformed to arcsine values. All analyses were performed on SAS STAT 8.1 (SAS Institute 1996).

Table 1 Mean (± SE) number of eggs laid and aphids parasitised by *Aphidius eadyi* in hosts of different stages. Means followed by the same letters in columns are not significantly different ($P > 0.05$).

Host stage at parasitisation	Total no. of aphids dissected (*n*)	No. of aphids parasitised/female	No. of eggs laid/female
Instar 1	250	10.10 ± 0.78 c	13.40 ± 0.78 d
Instar 2	250	15.70 ± 1.17 b	21.90 ± 1.33 c
Instar 3	250	17.90 ± 1.05 ab	33.00 ± 1.13 b
Instar 4	250	21.50 ± 0.75 a	44.90 ± 1.53 a
Adult	250	21.40 ± 0.83 a	41.60 ± 1.80 a

Table 2 Mean (± SE) developmental time (days) of *Aphidius eadyi* from egg to adult in different host stages and proportion of resulting female progeny. Means followed by the same letters in columns are not significantly different ($P > 0.05$).

Host stage at parasitisation	(*n*) Female	(*n*) Male	Total no. of parasitoids emerged (*n*)	Female progeny (%)
Instar 1	30 15.6 ± 0.1 a	39 14.9 ± 0.1 a	100	45.96 ± 5.28 a
Instar 2	57 15.2 ± 0.1 b	55 14.8 ± 0.1 a	160	50.43 ± 5.28 a
Instar 3	58 15.1 ± 0.1 bc	59 14.7 ± 0.1 a	182	53.40 ± 4.18 a
Instar 4	55 15.1 ± 0.1 bc	68 14.7 ± 0.1 a	197	59.46 ± 3.36 a
Adult	67 15.0 ± 0.1 c	58 14.7 ± 0.1 a	181	52.20 ± 4.22 a

Table 3 Mean (± SE) forewing length (mm) and number of egg load of newly emerged *Aphidius eadyi* from different host stages. Means followed by the same letters in columns are not significantly different ($P > 0.05$).

Host stage at parasitisation	Male forewing length ($n = 30$)	Female forewing length ($n = 30$)	No. of egg load ($n = 15$)
Instar 1	2.28 ± 0.02 d	2.43 ± 0.03 c	18.13 ± 0.03 c
Instar 2	2.40 ± 0.02 c	2.51 ± 0.03 c	27.53 ± 0.03 c
Instar 3	2.49 ± 0.02 b	2.64 ± 0.02 b	45.80 ± 0.02 b
Instar 4	2.57 ± 0.03 ab	2.73 ± 0.02 a	53.00 ± 0.02 ab
Adult	2.67 ± 0.03 a	2.76 ± 0.01 a	60.73 ± 0.01 a

RESULTS

Host stage preference

Our results show that *A. eadyi* females accepted aphids of all stages but significantly preferred fourth instar nymphs and adults to younger aphids for oviposition ($F_{4,45}$ = 95.1 and 25.91 for the number of eggs laid and aphids parasitised, respectively, $P < 0.0001$) (Table 1). The number of eggs laid and aphids parasitised increased with the increasing host stages but the difference was not significant between fourth instar nymphs and adults ($P > 0.05$). Regression analyses show that the number of eggs laid had a cubic relationship with the host stage ($F_{3,46} = 0.8905$, $P < 0.0001$) while the number of aphids parasitised possessed a quadratic correlation with the host stage ($F_{2,47} = 0.6866$, $P < 0.0001$) (Fig. 1).

Progeny fitness

Host stage at the time of parasitisation had a significant effect on the developmental time of female *A. eadyi* progeny ($F_{4,363} = 8.77$, $P < 0.0001$), but no effect on that of male progeny ($F_{4,264} = 1.46$, $P > 0.05$) (Table 2). Female progeny developed faster in older stages. Regardless of the host stage, the developmental time of males was significantly shorter than that of females ($F_{1,67} = 20.15$, $F_{1,110} = 17.62$, $F_{1,116} = 8.89$, $F_{1,121} = 16.93$, and $F_{1,113} = 14.13$, $P < 0.01$, for instars 1, 2, 3, 4, and adult hosts, respectively) (Table 2). Except for instar 1, the sex ratio of the resulting parasitoids was slightly female-biased, however, there was no significant difference in sex ratio between host stages ($F_{4,45} = 1.99$, $P > 0.05$) (Table 2).

The forewing length of newly emerged adults of both sexes increased significantly with the host stage at the time of parasitisation ($F_{4,70} = 34.03$ and 39.35, $P < 0.0001$, for male and female, respectively), but no significant difference was found between fourth instar nymphs and adults (Table 3). Regardless of the host stage when parasitised, females were significantly larger than males ($F_{1,28} = 11.55$, 10.50, 23.63, 25.16, and 7.91, $P < 0.01$, for instars 1, 2, 3, 4, and adult, respectively) (Table 3). Regression analyses indicate that body size of both sexes of resulting progeny had quadratic correlations with the stage of hosts their mother parasitised ($F_{2,72} = 78.06$ and $F_{2,72} = 67.31$, for females and males, respectively; $P < 0.0001$) (Fig. 2).

The mean egg load of female progeny at emergence also increased significantly with host stage at the time of parasitisation ($F_{4,70} = 27.83$, $P < 0.0001$), but no significant difference was detected between instars one and two as well as between instar four and adults (Table 3). The equation for CCD model used to fit the relationship between egg load (z) and both host stage at parasitisation (x) and resulting female parasitoid body size (y) can be expressed as: $z = \text{Exp}(-30.03 + 0.35x + 23.84y - 0.03x^2 - 4.27y^2)$ with $R^2 = 0.7026$. This indicates that both the host stage at parasitisation and body size of resulting female progeny had significant effects on egg load at emergence ($F_{4,70} = 41.35$, $P < 0.0001$). However, the likelihood ratio test shows that female size had significantly more effect on egg load than host stage at the time of parasitisation ($\chi_2^2 = 67.93$, $P < 0.0001$).

DISCUSSION

Aphidius eadyi laid eggs and developed successfully in all life stages of *Ac. pisum*, but preferred later instar nymphs and adults for oviposition. Such a preference may be attributed to maximal fitness return (Charnov & Stephens 1988). Regression analyses show differential preference patterns in the number of eggs laid and the number of aphids parasitised. A cubic relationship was demonstrated between the number of eggs laid and the host stage, i.e., *A. eadyi* laid increasingly more eggs with the increase of host stages until instar four, after which stage the number of eggs laid decreased slightly. The higher protein content in late stages of *Ac. pisum* (Li et al. 2002) may explain the preference for late stage aphids by *A. eadyi*. Other studies also suggest that large hosts may generate superior parasitoid fitness (Charnov et al. 1981; Liu 1985; Harvey et al. 1994). A quadratic relationship was detected between the number of aphids parasitised and the host stage, i.e., *A. eadyi* attacked increasingly more aphids with the increase of the host age only among nymphs, and no further increase in attacks occurred after aphids reached instar four. These results may be interpreted by the fact that *Ac. pisum* adults are more capable of defending themselves from parasitisation and require more effort for female *A. eadyi* to parasitise (Gerling et al. 1990, unpubl. data). Similar results were also reported in other aphidiid species (Shaw & Huddleston 1991). Therefore, the reproductive success of a female parasitoid reaches a maximum when her host choice is based on the optimal balance between the cost for finding and capturing a host and the host quality for the growth and development of her progeny (Godfray 1994).

As reported in other koinobiont parasitoids (Sato 1980; Sequeira & Mackaure 1992), *A. eadyi*

developed faster in older than in younger life stages of *Ac. pisum*. Although the proportion of resulting *A. eadyi* female progeny slightly increased with the increasing host stages, no significant difference was detected between host stages. It is suggested that our results do not support the general theory of host size-dependent sex allocation in parasitic Hymenoptera (Charnov 1982) that parasitoids deposit fertilised eggs in large hosts and unfertilised eggs in small hosts.

The body size of *A. eadyi* progeny increased with the host stage parasitised, generally supporting the studies by various authors (e.g., Liu 1985; Sequeira & Mackauer 1992). However, the quadratic relationship between host stages attacked and the size of the resulting progeny suggested that *A. eadyi* gain similar body size when attacking the fourth instar nymphs and adults.

Results of the present study demonstrate that egg load of female parasitoids increased with increasing host stage at parasitisation and body size of resulting female progeny, but the female body size had more effect on egg load than host stage. This may be because aphids parasitised at nymphal stages continue to feed and grow, and thus the host stage when attacks occur is not a final determinant factor affecting egg load.

The findings of this study have implications for laboratory mass rearing and field release of *A. eadyi*. For example, the fourth instar aphids appear to be the most appropriate hosts for a mass-rearing programme because the parasitoid gains greater fitness return by attacking the host of this stage. Moreover, the timing for any field release of parasitoids may be optimally set when the fourth instar aphids are the most abundant, because aphids parasitised in this stage produce few aphid progeny (He et al. 2003).

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EMERGENCE, SEXUAL MATURATION AND OVIPOSITION OF *APHIDIUS ERVI* (HYMENOPTERA: APHIDIIDAE)

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ABSTRACT

Aphidius ervi Haliday is an important parasitoid of several aphid species, and information is needed for the development of mass-rearing techniques and better understanding of biological control ecology. The emergence, sexual maturation and oviposition of *A. ervi* on pea aphid, *Acyrthosiphon pisum* (Harris), was studied in the laboratory at 20±1°C and 60-70% RH with 16:8 h light:dark. About 95% of parasitoids emerged during the photophase. Females needed a significantly longer time than males to complete their life cycle. Newly emerged males were able to perform their courtship display but failed to mate until they were 4 h old; newly emerged females were able to respond to males' courtship display and mate. Females attacked aphids in both light and dark conditions. The number of eggs laid and parasitism (number of aphids parasitised) per oviposition bout (2 h oviposition period) were significantly greater in the photophase than in the scotophase.
Keywords: Hymenoptera, *Aphidius ervi*, emergence, mating, oviposition.

INTRODUCTION

Insect emergence events are usually rhythmic (Saunders 1982). In the parasitic hymenopterans, such rhythmicity is often synchronised with mating (Gordh & DeBach 1976; Nadel & Luck 1985) and oviposition (Armstrong et al. 1996; Couch et al. 1997) activities for an optimal reproductive fitness.

Daily activity patterns have been studied in detail in some parasitic hymenopterans (Vogt & Nechols 1991; Armstrong et al. 1996; Couch et al. 1997). Quicke (1997) suggested that for many parasitoid species, most oviposition occurs in the morning, for example, the squash bug egg parasitoid, *Gryon pennsylvanicum* (Vogt & Nechols 1991). However, in some other species, such as the *Sitona* weevil parasitoid, *Microctonus aethiopoides* Loan, oviposition may occur during light and dark with the circadian oviposition activity corresponding with its host activity (i.e. feeding and oviposition) (Armstrong et al. 1996; Couch et al. 1997). Knowledge of parasitoids' emergence, mating and oviposition patterns is vital to an understanding of the ecology and evolution of their reproductive strategies, which in turn contributes to the development and implementation of biological control programs.

Aphidius ervi Haliday is a solitary endophagous parasitoid of several pest aphid species on economically important crops, such as legumes and cereals (Star 1978; Powell 1982). It was imported into New Zealand from California to control *Acyrthosiphon pisum* (Harris) and *Ac. kondoi* Shinji and has successfully established (Cameron & Walker 1989). Michaud & Mackauer (1994) reported that *A. ervi* could successfully oviposit in *Ac. pisum* in both photophase and scotophase, but the circadian emergence and oviposition patterns were still unknown prior to this study. To provide information for the development of mass-rearing and field releasing techniques and better understanding of biological control ecology of *A. ervi*, the circadian patterns of emergence and oviposition, and sexual maturation in *A. ervi* were investigated.

MATERIALS AND METHODS

Breeding colony and experimental conditions

A breeding colony of *Aphidius ervi* was established from individuals emerged from blue-green lucerne aphids, *Acyrthosiphon kondoi* Shinji, which were collected from lucerne on an AgResearch farm at Aorangi near Palmerston North in late December 2002. Prior to the experiments, the colony was reared on pea aphid, *Acyrthosiphon pisum* (Harris), feeding on potted broad bean, *Vicia faba* L. cv. Pride, for five generations. The colony was held and all experiments were carried out at 20±1°C and 60-70% RH with 16:8 h light:dark.

Experimental parasitoids and hosts

All parasitoids used for experiments emerged from mummies that were parasitised in third instar (3 days old), and third instar pea aphids were used as hosts in all experiments.

Emergence

To observe the 24-h emergence patterns of *A. ervi*, two bioassay rooms were set up. The photophase in one room was set from 0800-2400 h (normal-light regime) and in the other room the photophase was between 1800-1000 h (reverse-light regime). To obtain parasitised aphids, a mated female parasitoid (<12 h old) was introduced into a Petri dish (5.5 cm in diameter, 1.3 cm in height) containing 25 third instar aphids for a period of 5 h. Fifty-eight females in individual Petri dishes were used.

Fifty parasitised aphids were reared on the bean plant in a transparent plastic cylinder (8.5 cm in diameter, 12 cm in height) with 3 gauze-covered holes, one (5 cm in diameter) in the top and two (2 cm in diameter) in the opposite sides of the container for ventilation. Fifteen and nine cylinders were maintained in the normal- and reverse-light regimes, respectively. Parasitoid emergence was observed from 675 mummies in the photophase in the normal-light regime and 378 in the scotophase in the reverse-light regime. The emergence incidence was recorded hourly and emerged adults were sexed. Developmental time from eggs to adults of both sexes was also recorded.

Sexual maturation

Because most matings occurred during the photophase (X.Z. He, unpubl. data), all experiments on sexual maturation were carried out during this period. To detect the sexual maturation period of adult parasitoids, two experiments were set up, each with seven treatments. In the first experiment 12-h-old virgin females were paired with 0 (newly emerged), 2, 4, 6, 8, 10 and 12-h-old virgin males, and in the second experiment 12-h-old virgin males were paired with 0 (newly emerged), 2, 4, 6, 8, 10 and 12-h-old virgin females.

A virgin male was paired with a virgin female in a clear glass vial (1.5 cm in diameter, 5.0 cm in height) with a 0.5 cm mesh covered hole in lids. Twenty pairs were established for each treatment. The sexual behaviour of both sexes in a 30 min period was observed and the number of courtship displays (male wing fanning) and matings (insemination) was recorded.

Oviposition

To determine the oviposition patterns of *A. ervi* on a 24 h basis, an experiment was carried out with 20 *A. ervi* females in the normal-light regime and another 20 *A. ervi* females in the reverse-light regime. Each mated female (<12 h old) was introduced into a Petri dish containing 20 healthy aphids and allowed to stay for 2 h (first oviposition bout). She was then transferred to another Petri dish with 20 healthy aphids and allowed to stay for 2 h (second oviposition bout). This was repeated until 8 and 4 oviposition bouts were completed in the photophase and scotophase, respectively.

The parasitised aphids from each oviposition bout (in a single Petri dish) were transferred to and reared in an above-mentioned plastic cylinder. To determine the number of eggs laid by *A. ervi* in oviposition bouts, 10 aphids were dissected in each oviposition bout 4 days after parasitisation and the number of larvae in each aphid was counted under the stereomicroscope (Leica MZ12, German). The remaining parasitised aphids were reared until mummification. The number of parasitisms in each oviposition bout was recorded as the number of aphids parasitised.

Statistical analysis

A chi-square test was used to determine the difference in emergence incidence between the photophase and scotophase. The rejection level was when $\chi^2 < \chi^2_{1,0.05} = 3.8415$. The Marascuilo procedure of the nonparametric analysis (Daniel 1990) was used to assess the sexual maturation. The rejection level was when $U_0' < \chi^2_{6,005} = 12.59$. All other data were analysed using an ANOVA. When significant differences in variables occurred, means were separated using a Tukey's studentised range (HSD) test ($P<0.05$). The proportion of female offspring data was subject to arcsine transformation before ANOVA, but untransformed means are presented in the paper. All analyses were conducted using SAS.

RESULTS

Emergence

The developmental time from oviposition to emergence was significantly shorter for males (mean±SE, 13.94±0.03 days) than that for females (14.31±0.03 days) ($P<0.0001$).

The hourly emergence rate (mean±SE) was significantly higher in the photophase (5.99±0.94%) than in the scotophase (0.74 ± 0.32%) ($P<0.001$). About 95% of parasitoids emerged in the photophase in both light regimes. On a 24 h basis, the male emergence peaked 2 h after light-on and the female emergence peaked between 3-6 h after light-on (Fig. 1).

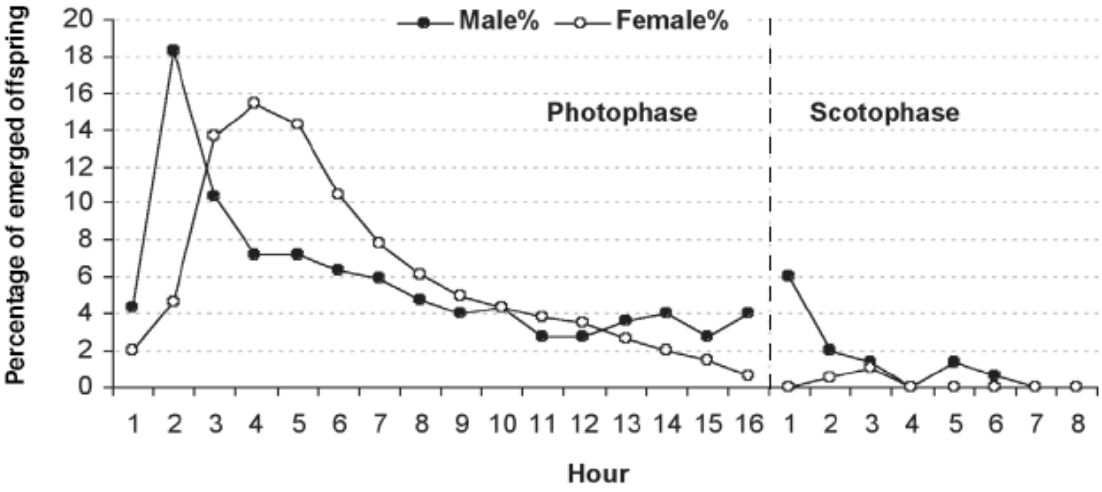

FIGURE 1: The percentage of female or male *A. ervi* offspring emerging throughout photophase or scotophase.

Sexual maturation

Newly emerged males were able to perform their courtship display to 12-h-old females but failed to mate until they were 4 h old (Fig. 2). However, 10- and 12-h-old males were significantly more likely to court females than ≤4-h-old males ($U_0'=105.22$, $P<0.0001$); mating success of 12-h-old males was also significantly higher than that of ≤4-h-old males ($U_0' =80.01$, $P<0.0001$) (Fig. 2).

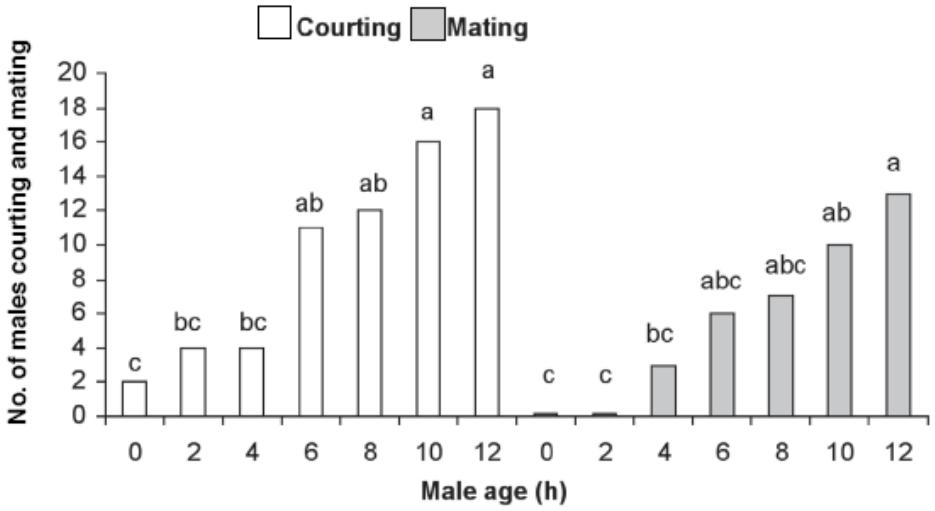

FIGURE 2: The number of *A. ervi* males courting or mating at 0, 2, 4, 6, 8, 10 or 12 h after emergence. Bars with the same letters are not significantly different (P>0.05).

Newly emerged females were able to entice the courtship display by 12-h-old males and successfully mate (Fig. 3). Twelve-h-old females were significantly more likely to respond to males' courtship display than of ≤4-h-old females ($U_0^{|} = 32.35$, P<0.0001); mating success of 12-h-old females was significantly higher than that of ≤2-h-old females $U_0^{|}$=14.97, P<0.0001).

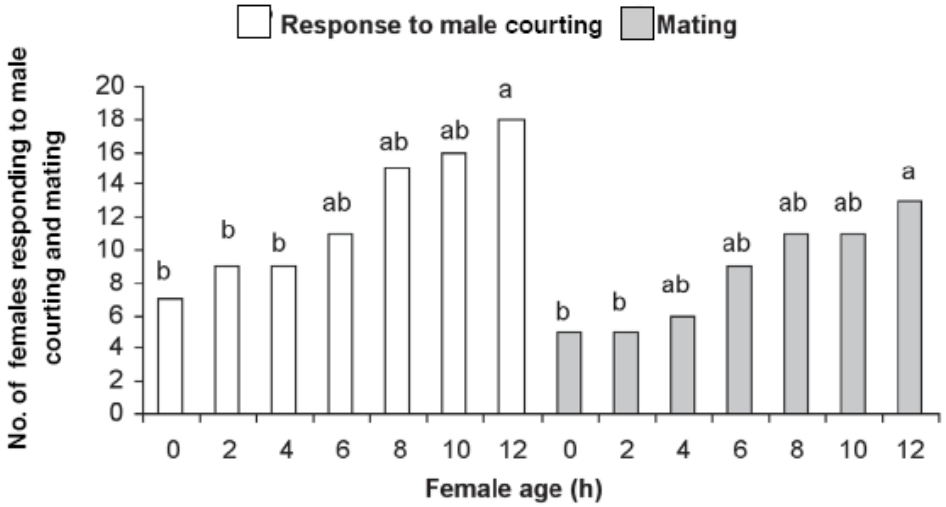

FIGURE 3: The number of *A. ervi* females responding to male courting or mating at 0, 2, 4, 6, 8, 10 or 12 h after emergence. Bars with the same letters are not significantly different (P>0.05).

Oviposition

Females oviposted in both photophase and scotophase. In the photophase, the mean (±SE) number of eggs laid (11.73±2.73) and parasitism (8.6±1.03) per oviposition bout was significantly greater than that in the scotophase (4.18±0.88 eggs and 3.43±0.69 parasitism, respectively) (P<0.01). In the photophase, the numbers of eggs laid and parasitism were significantly higher in the first oviposition bout (P<0.0001) (Fig. 4). However, no significant difference in number of eggs laid or parasitism between oviposition bouts was detected in the scotophase (P>0.05) (Fig. 4).

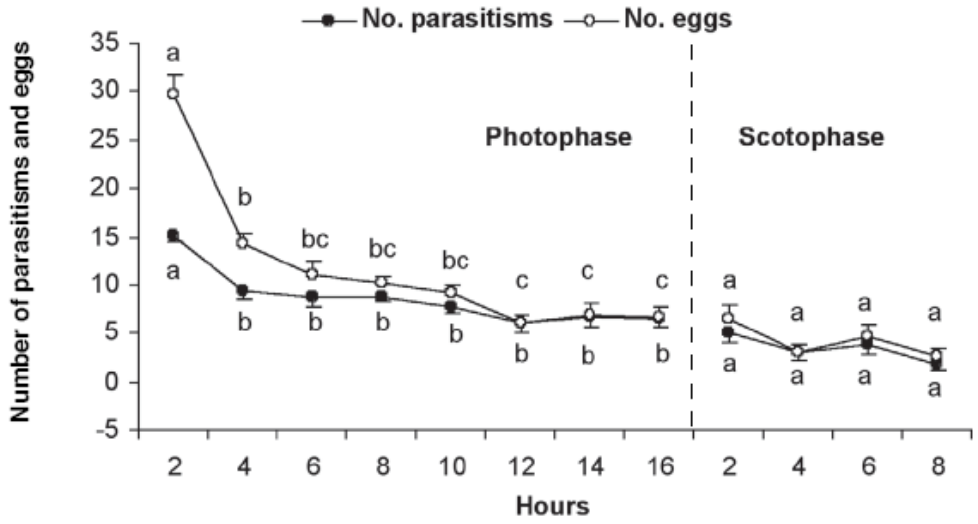

FIGURE 4: The number of parasitisms and eggs laid by *A. ervi* females throughout photophase or scotophase. Means (± SE) followed by the same letters within each line are not significantly different (P>0.05). Data from the photophase and scotophase were analysed separately.

DISCUSSION

Among parasitoid insects, adult emergence restricted to certain times of the day is known in several species, including *Trichogramma minutum* Riley (Corrigan et al. 1995), *Telenomus busseolae* Gahan (Fantinou et al. 1998) and *Encarsia formosa* Gahan (Lenteren et al. 1992). The results of the present study demonstrate that *A. ervi* males and females have an emergence peak during the first few hours of the photophase (Fig. 1). This suggests that the onset of light may act as a signal that synchronises the rhythmic function of *A. ervi* adult emergence.

This study has shown that *A. ervi* is a protandrous species with males emerging ca 9 h earlier than females. Singer (1982) argued that selection for protandry can only occur when generations are discrete. *Aphidius ervi* does not appear to match to that hypothesis because generations usually overlap in the field and females are readily available. However, *A. ervi* females mate only once during their lifespan, limiting the chance for males to encounter virgin females. Quicke (1997) suggested that the protandry is common in many parasitoids because a late-emerging male is likely to encounter already mated females and he is genetically doomed, as he cannot get any matings.

It has been reported that for some parasitic hymenopterans, a period of sexual maturation is necessary, for example, the bean weevil parasitoid, *Chryseida bennetti* Burks (Perez-Lachaud & Campan 1994) and the tortrix moth parasitoid, *Ascogaster reticulatus* Watanabe (Kainoh 1986). In the present study, newly emerged *A. ervi* females were able to mate with males but males need at least 4 h to become sexually mature. Therefore, the early emergence of *A. ervi* males may be a selective strategy for higher reproductive fitness. For example, early emerged males have a better chance to encounter virgin females (Nadel & Luck 1985, 1992), inseminate a greater number of the females they encounter (Waage & Ng 1984), and reduce the risk of females' death before oviposition (Fagerström & Wiklund 1982). Moreover, since virgin females start to lay eggs within 30 min after emergence (X.Z. He, unpubl. data), early emerged males are able to mate with females before their oviposition.

Diel periodicities of insect activity are often determined by a combination of endogenous and exogenous rhythms (Beck 1980). The decreasing oviposition and parasitism of *A. ervi* during the photophase may be the result of a decreasing load of mature eggs, as reported in the sycamore aphid parasitoid, *Monoctonus pseudoplatani*

(Marshall) (Collins & Dixon 1986). Furthermore, visual cues play an important role in host finding and attacking by *A. ervi* (Michaud & Mackauer 1994). In this study, *A. ervi* females laid fewer eggs and attacked fewer aphids during the scotophase even though they had sufficient eggs. This suggests that the oviposition pattern of *A. ervi* is determined by an exogenous factor, the light regime.

In conclusion, light appears to entrain movement, oviposition and emergence rhythms of *A. ervi*. Emergence of the adults in the morning probably coincides with more favourable conditions, such as the light, in which parasitoids may increase the chance for host habitat location, searching for mates and hosts, and oviposition. The findings of this study have implications for laboratory mass rearing and field release of *A. ervi*. For example, newly emerged parasitoids should be held for 12 h for copulation to occur before release, while the parasitoids should be released the following morning to achieve the higher reproductive output.

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EFFECT OF PARASITISM BY *APHIDIUS EADYI* (HYMENOPTERA: APHIDIIDAE) ON REPRODUCTION OF PEA APHID, *ACYRTHOSIPHON PISUM* (HEMIPTERA: APHIDIDAE)

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ABSTRACT

The effect of parasitism by *Aphidius eadyi* Starý, González & Hall on reproduction of pea aphid, *Acyrthosiphon pisum* (Harris), was studied in the laboratory. Aphids attacked as 1st and 2nd instars became mummies in the 4th instar; parasitised 3rd instar nymphs became mummies in the adult stage without producing any progeny. Parasitised 4th instar nymphs and adults produced progeny but had a shorter reproductive period and produced fewer ($P<0.0001$) progeny than unparasitised aphids. Parasitised 4th instar nymphs and adults had significantly lower intrinsic rates of increase (r_m), net reproductive rates (R_0), shorter generation time (T) and longer doubling time (DT) than unparasitised aphids. The potential impact of the parasitoid on host population growth is discussed.

Keywords: pea aphid, *Acyrthosiphon pisum*, *Aphidius eadyi*, reproduction, population growth.

INTRODUCTION

Parasitism by entomophagous parasitoids increases mortality and reduces growth, longevity and reproductive potential of the hosts. Many studies have reported that aphids parasitised by hymenopteran parasitoids in an early life stage die before reproducing, but those parasitised in older life stages may produce a limited number of progeny before mummification (Fox et al. 1967; Campbell & Mackauer 1975; Lui & Hughes 1984; Mackauer & Kambhampati 1984; Sequeira & Mackauer 1988).

Pea aphid, *Acyrthosiphon pisum* (Harris), is an important pest of lucerne in New Zealand. *Aphidius eadyi* Starý, González & Hall is a specific parasitoid of pea aphid, and an important agent for biological control of this pest. Both species occur throughout New Zealand (Cameron & Walker 1989). A lack of information makes it difficult to understand the effect of *A. eadyi* on pea aphid populations in the field. Here, we report the effect of parasitism by *A. eadyi* on pea aphid survival, reproduction and population growth.

MATERIALS AND METHODS

Healthy pea aphids and those parasitised by *A. eadyi* were collected from red clover, *Trifolium pratense* L., on an AgResearch farm at Aorangi near Palmerston North in early January 2002. Aphids were reared in the laboratory on potted broad beans, *Vicia faba* L. cv. Pride, in an aluminium framed cage (64 cm length × 45 cm width × 40 cm height) with fine metal mesh on the back and both sides and perspex and aluminium alloy on the top and bottom, respectively. The colony was maintained and experiments were conducted at 20 ± 1°C and RH 60-70% with a photoperiod of 16:8 h light:dark.

In this study, parasitised individuals (n = 20) of five life stages (4 nymphal instars and adults) were established. To obtain pea aphids of known life stage, adults were introduced onto a bean plant and removed after 5 h and the resulting progeny reared. To obtain

parasitised aphids, we released a mated *A. eadyi* female into an enclosed Petri dish (8.5 cm diameter × 1.3 cm height) where 20 newly moulted aphids of the same life stage were maintained. Aphids that received one ovipositioning strike from the female were removed until 20 individuals were collected. Each attacked aphid was individually introduced onto a bean plant held in a transparent plastic cylinder (8.5 cm diameter × 12 cm height, with three gauze-covered holes for ventilation) and observed once a day at 1200 h. We recorded the survival period of aphids and the period they started reproduction after being parasitised. Offspring were counted and removed. At the same time, 10 unparasitised adults reared individually in cylinders served as controls to compare progeny production and reproductive period.

The daily survival rate of each stage and fecundity were compiled into a life table according to the method of Birch (1948) and Jervis & Kidd (1996). We estimated the intrinsic rate of increase, r_m, by solving the Lotka-Euler equation ($\sum e^{-r_m x} l_x m_x = 1$), and calculated the net reproductive rate ($R_0 = \sum l_x m_x$), mean generation time (days) ($T = \log_e(R_0)/r_m$) and doubling time (days) ($DT = \log_e(2)/r_m$), where x is the pivotal age, l_x the proportion of the females surviving to age x and m_x the number of offspring produced per female at age x. A jackknife method (Caswell 1989) was used to estimate standard errors of above parameters.

Aphids that did not become mummies were considered unparasitised and were not included in statistical analysis. Data were analysed using ANOVA. When significant differences in variables occurred, means were separated using Tukey's studentised range (HSD) test (P=0.05). All analyses were conducted on SAS STAT 8.1.

RESULTS

Survival

Aphids parasitised as 1st and 2nd instars became mummies in the 4th instar; all parasitised 3rd and 4th instar nymphs reached adult stage. Survival periods of parasitised aphids were similar regardless of the stage attacked (P>0.05) (mean ± SE: 7.40 ± 0.13, 7.32 ± 0.11, 7.15 ± 0.08, 7.05 ± 0.05 and 7.05 ± 0.05 days, for parasitised 1st, 2nd, 3rd, 4th instars and adults, respectively; Tukey test, q<3.93). Survival of parasitised aphids was significantly shorter than that of unparasitised adults (20.8 ± 1.10 days) (Tukey test, q>4.11; P<0.0001)

Reproduction

Aphids parasitised as 4th instars and adults started reproduction about 5 and 1 days, respectively, after attack (Fig. 1). Aphids parasitised as 4th instars had a shorter reproductive period and produced fewer progeny than those parasitised as adults, and parasitised adults had a shorter reproductive period and produced fewer progeny than unparasitised adults (P<0.0001) (Table 1).

TABLE 1: Effect of parasitism by *Aphidius eadyi* on reproductive period and progeny production of pea aphid[1].

Aphid	Reproductive period	No. of progeny
Parasitised 4th instar	2.05 (0.12) c	9.79 (0.80) c
Parasitised adult	3.95 (0.09) b	27.80 (0.91) b
Unparasitised adult	16.00 (0.47) a	113.40 (3.57) a

[1]Means (± SE) followed by the same letters in columns were not significantly different (P>0.05).

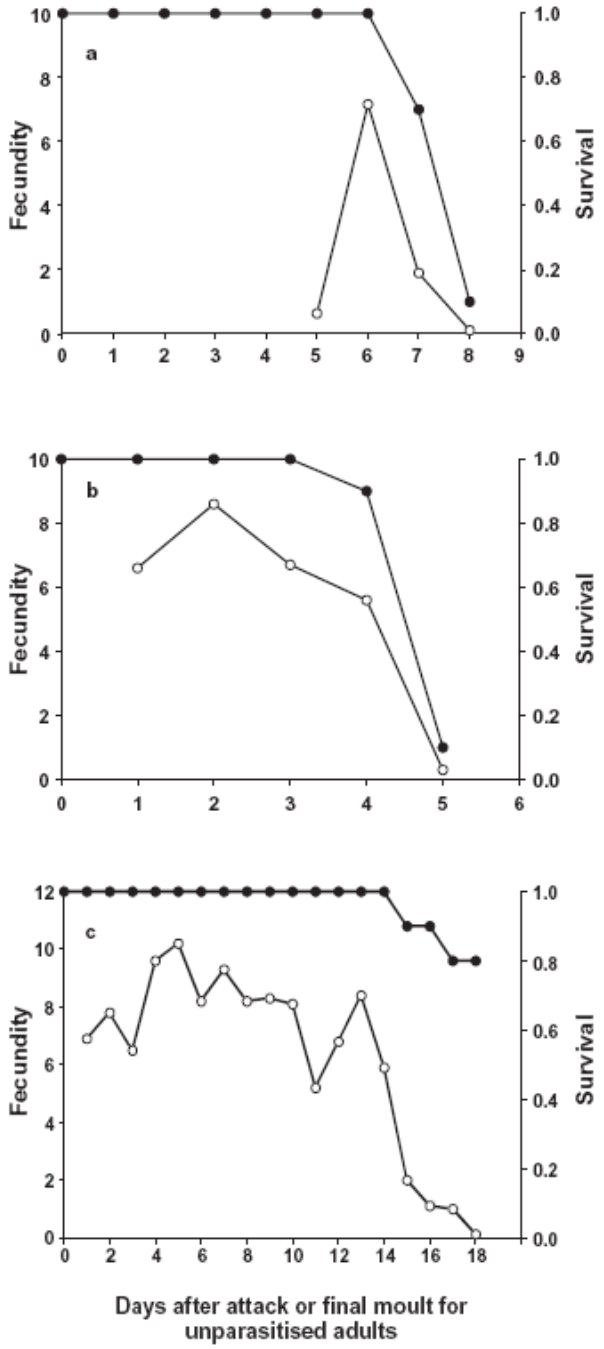

FIGURE 1: **Influence of parasitism by *Aphidius eadyi* on fecundity (no. progeny/day) (open circles) and survival (%) (closed circles) of pea aphids, *Acyrthosiphon pisum*. (a) parasitised 4th instars, (b) parasitised adults and (c) unparasitised adults.**

Life table

Parasitism by *A. eadyi* severely affected the population growth of pea aphids (Table 2). Aphids parasitised as 4th instars had a lower intrinsic rate of increase (r_m) and net reproductive rate (R_0), a shorter generation time (T), and a longer doubling time (DT) than those parasitised as adults ($P<0.0001$). Parasitism in adults led to a lower r_m and R_0, a shorter T and a longer DT when compared to unparasitised aphids ($P<0.0001$) (Table 2).

TABLE 2: Effect of parasitism by *Aphidius eadyi* on life table parameters of pea aphid[1].

Aphid	r_m	R_0	T	DT
Parasitised 4th instar	0.2494 c	9.2000 c	8.8966 c	2.7793 a
	(0.0001)	(0.0494)	(0.0217)	(0.0075)
Parasitised adult	0.3636 b	27.5000 b	9.1159 b	1.9066 b
	(0.0010)	(0.0482)	(0.0212)	(0.0050)
Unparasitised adult	0.4005 a	113.4000 a	11.8119 a	1.7306
	(0.0001)	(0.3969)	(0.0084)	(0.0001) c

[1]Means (± SEs) followed by the same letters in columns were not significantly different (P>0.05).

DISCUSSION

The efficiency of parasitoids in the control of aphids depends on their ability to reduce aphids' reproductive potential by lowering the intrinsic rate of increase (r_m) or net reproductive rate (R_0), shortening the generation time (T) or extending the doubling time (DT). We found that parasitism of early instars prevented any reproduction and that parasitism of late instars and adults significantly reduced numbers of progeny compared with unparasitised aphids.

In the field, the success of *A. eadyi* in pea aphid control will be affected by the age structure of aphid populations. Sequeira & Mackauer (1988) stated that parasitoids that attack young host stages are likely to be more effective at suppressing aphid populations than those that attack the older hosts. Growing populations generally have a high proportion of individuals in young age classes (Schowalter 2000) and this occurs early in the season. In this study, pea aphids that were parasitised by *A. eadyi* in the 1st, 2nd and 3rd instars did not contribute to population growth, with r_m being zero. Thus, attack by *A. eadyi* early in the season should significantly suppress populations of pea aphid in the field, inhibiting its dispersal and population buildup later in the season.

Parasitism by *A. eadyi* shortened the aphids' reproductive period and reduced the number of progeny due to the destructive feeding by the developing parasitoid larvae. Similar results have been reported for pea aphid–*A. smithi* Sharma & Subba Rao (Campbell & Mackauer 1975) and pea aphid–*Praon pequodorum* Viereck (Sequeira & Mackauer 1988) systems. Aphids parasitised as 4th instars and adults could still produce some progeny. Our results indicated that aphids parasitised as 4th instars achieved a net reproductive rate three times lower than aphids parasitised as adults, and parasitised adults yielded four times less than unparasitised aphids. It is predicted that parasitism by *A. eadyi* would decrease the overwintering population of pea aphid late in the season.

The effect of parasitism on pea aphid population growth is reflected in changes in the intrinsic rate of natural increase (r_m) (Campbell & Mackauer 1975). When the age structure of aphid populations and parasitism distribution among aphid ages are stable in an unlimited environment, r_m can be used to predict the population growth of pea aphid (Carey 1993; Schowalter 2000). However, the effect of *A. eadyi* on biological control of the pea aphid also greatly depends on the population growth of the parasitoid itself. Variation in the stability of the parasitoid population, especially the sex ratio of parasitoids and distribution of parasitism between host ages, may mean that aphid population growth can only be predicted over a short period. Other parasitoid species such as *A. ervi* Haliday can also attack the pea aphid in New Zealand. Sequeira & Mackauer (1988) stated that the changes in reproductive pattern of pea aphid reflect a general response to parasitism rather than that induced by different parasitoid species. Therefore, if the effect of parasitism by *A. ervi* on pea aphid reproduction is similar to that by *A. eadyi*, this model could be used to predict the population growth of pea aphid regardless of the parasitoid species. Modelling parasitism adds to the understanding of the pea aphid population dynamics in the field.

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Operational Sex Ratio and Population Density Influence Partial Local Mating Behaviour in *Aphidius ervi* (Hymenoptera: Aphidiidae)

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Aphidius ervi Haliday is a cosmopolitan, solitary, endophagous parasitoid and a major biological control agent of several aphid species on economically important crops such as legumes and cereals. *A. ervi* is also a quasi-gregarious species (i.e. solitary species that develop in hosts that are aggregated), and thus partial local mating should be common. This study was to investigate how operational sex ratio (OSR, ♂:♀) and population density influenced the mating behaviour in *A. ervi*. Results indicate that the frequency of male homosexual behaviour and competition for mates significantly increased with increasing OSR and male density. Mating success significantly increased with increasing male or female density. Mating period was not affected by female density but it significantly increased with increasing OSR and male density. The prolonged mating in male-biased OSR and high male density may be a strategy serving as mate-guarding to reduce the probability of female remating and thus sperm competition. Females could remate < 1 min after the first mating but no remating occurred > 1 min after the first mating. The frequency of female remating was significantly higher in male-biased than in female-biased OSR, and the mating period in the second mating was significantly shorter than in the first mating. Results suggest that *A. ervi* females accept the second mating or remain attractive to males within only a brief period after the first mating.

Mate age at mating and male mating history affect mate choice and reproduction in *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae)

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Hymenopteran parasitoids are usually arrhenotokously parthenogenetic, where fertilized eggs produce diploid females and unfertilized eggs give rise to haploid males; the reproductive fitness of males depends on the number of daughters they father. The effect of mate age at mating and male mating history on mate choice and reproductive fitness of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae), was studied in the laboratory. Both sexes prefer 1-d-old to 3-d-old partners for mating if given a choice, and 1 or 2 d mating delay of any sex reduces the proportion of daughters produced. Furthermore, mating delay affects females more severely than males in production of daughters. Once-mated males have significantly greater mating success than virgin males, suggesting that males learn from previous mating(s). If allowed 24-h recovery time, the proportion of daughters produced only slowly decreases with the increase of the number of matings males have had but males can still supply enough sperm to produce female-biased progeny after seven matings. This suggests that males suffer little sperm depletion if allowed 24-h recovery time between matings and that the decline of daughter production with increasing matings may be caused by the increasing male age. If only allowed 1 h between matings, males would experience significant sperm depletion after three matings. The implications of these results in the biological control are discussed.

Keywords: *Aphidius ervi*, age, mating history, sperm, proportion of female progeny

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Research Summary – Word Limit 750 Words

Introduction and Aims

Body size of insects has usually been considered to be a key trait potentially affecting reproductive fitness in many ways. For example, in hymenopteran parasitoids, large females usually lay more eggs and parasitised more hosts, and large males usually have better genes and more sperm supply.

Aphidius ervi Haliday is a cosmopolitan solitary endophagous parasitoid and a major biological control agent of several aphid species on economically important crops such as legumes and cereals in New Zealand. A positive relationship was found between body size of *A. ervi* and pea aphids at parasitization However, no study has addressed whether and how body size of both *A. ervi* males and females affects reproductive fitness. The parasitoid body size-fitness relationship is important in mass-rearing programs as body size is commonly monitored as an indicator of parasitoids' quality. This study was to investigate whether and how body size of both sexes affected the number of eggs laid and progeny produced and progeny's sex ratio.

Materials and Methods

Parents of different body size (head width) were paired and allowed to mate once: small female (SF) x small male (SM), large female (LF) x large male (LM), SF x LM, and LF x SM. The small parasitoids emerged from aphids parasitised at the first instar and large ones from aphids parasitised at fourth instar. The mean body size used in the experiment was 0.55, 0.59, 0.61 and 0.66 mm for SM, SF, LM and LF, respectively. Each mated female was offered daily 50 healthy 3rd instar pea aphids feeding on a broad bean plant until she died. Ten of the 50 aphids were randomly selected and dissected daily to detect the number of eggs laid. The number and sex of progeny emerged from remained aphid mummies were recorded.

Statistic Analysis

The data on the number of eggs laid and progeny produced were analysed using ANOVA followed by a Tukey's studentized range (HSD) test. The central composite design (i.e., response surface) was used to analyse the relationship between parasitoid body size and reproductive fitness in terms of number and proportion of female progeny. The relationship is given by the polynomial equation: fitness = $\mathrm{Exp}(\beta_0 + \beta_1 f + \beta_2 m + \beta_{11} f^2 + \beta_{22} m^2 + \beta_{12} fm)$, where β_0, β_1, β_2, β_{12}, β_{11} and β_{22} are model parameters, and f and m are female and male body size, respectively. Only significant terms, after running the full regression models, were kept in the final models.

Results

Large females laid significantly more eggs and produced significantly greater number of progeny than small females. Unlike females, male body size had no significant effect on eggs laid and progeny produced.

The number of female progeny significantly increased with the body size of both sexes but with the increasing body size, male size had significantly more effect on the number of female progeny produced than females. Unlike males, the female size had negative effect on the proportion of female progeny.

Conclusions and Discussion

The results indicate that large females had greater ability to generate eggs when needed and parasitised more aphids, and thus produced more progeny than small females. Male size had no effect on the number of eggs laid and progeny produced suggests males did not provide any nutrition to females during copulation.

The higher number of female progeny produced by large males and females may be due to more sperm supplied by those males and more eggs produced by those females, while the lower proportion of female progeny given by large females may be because large females have more eggs than males can fertilised.

2006_Research_Summary

Reproductive response of *Aphidius ervi* (Hymenoptera: Aphidiidae) to pea aphid density

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The reproductive response of *Aphidius ervi* Haliday to the density of pea aphid, *Acyrthosiphon pisum* (Harris) was investigated. Six aphid densities (15, 25, 50, 75, 100 and 125 aphids), each kept on a broad bean cutting in a plastic cylinder (6.5 × 8.5 cm), were examined. Mean number of aphids parasitised and eggs laid by *A. ervi* significantly increased with the increase of host density from 15 to 100 aphids, after which no further increase occurred. However, the number of eggs laid in each aphid significantly decreased with the increase of host density from 15 to 75 aphids, after which no further decrease occurred. These results suggest that the parasitoid quickly adjusts oviposition strategy in response to increasing host density through increasing parasitism and decreasing superparasitism, and has high ability to suppress the increasing aphid population. The proportion of female progeny quickly increased (up to 70%) with the increase of host density from 15 to 50 or 75 aphids, after which it gradually declined, suggesting that the sperm limit occurs when host density reaches 50 to 75 aphids. When the host density was low (such as 15 or 25 aphids), female parasitoids lowered the allocation of fertilized eggs. This suggests that the female parasitoids can predict the immediate future need of sex ratio, i.e. the need of the number of female progeny.

Key words: *Aphidius ervi*, host density, reproduction, sex ratio

Effect of Body Size on Reproductive Fitness in Aphidius ervi (Hymenoptera: Aphidiidae)

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Aphidius ervi Haliday is an important parasitoid of several aphid species on economically important crops such as legumes and cereals. To improve its mass rearing and field release efficiency, we studied the relationships between body size and reproductive fitness of A. ervi on pea aphid, Acyrthosiphon pisum (Harris), in the laboratory. Large females lived significantly longer (14.10 ± 0.72 d), had significantly higher searching efficiency (1.50 ± 0.37 10^{-4}min), fecundity (574.25 ± 23.76 eggs) and fertility (338.74 ± 19.41 eggs), and parasitized significantly more aphids (410.70 ± 17.74) than did small females (11.60 ± 0.57 d, 0.97 ± 0.09 10^{-4}min, 302.74 ± 17.61 eggs, 213.39 ± 15.17 eggs and 257.07 ± 14.18 aphids for longevity, searching efficiency, fecundity, fertility and parasitism, respectively). However, fertility rate was significantly higher for small females (0.6923 ± 0.0238) than large females (0.5819 ± 0.0252). Male size was positively correlated with fertility ranging from 246.60 ± 20.43 eggs for small males to 305.53 ± 20.02 eggs for large males, and with fertility rate ranging from 0.5771 ± 0.0276 for small males to 0.6971 ± 0.0199 for large males. Male size had no effect on fecundity and parasitism. Implications from this study were discussed.

EFFECT OF APHID LIFE STAGE AND PARASITOID ADULT AGE ON PARASITISM AND SEX RATIO OF *APHIDIUS EADYI*

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Aphidius eadyi (Hymenoptera: Aphidiidae) is a specific parasitoid of pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae). The effect of *A. eadyi* adult age and *A. pisum* life stage on *A. eadyi* parasitism and sex ratio was investigated in transparent plastic cylinders (85 mm diameter by 120 mm high), each having a potted broad bean plant. Both choice (100 cylinders, each holding a mixture of 5 life stages with 10 aphids in each stage) and non-choice (500 cylinders, each containing 50 aphids of one of the five stages) experiments were carried out. Ten parasitoids of 10 ages (1-10 d old) were used for each experiment. Each parasitoid of a certain age was allowed to be in a cylinder for 24 h. With the increase of parasitoid age, parasitism rate significantly decreased and the proportion of male progeny significantly increased in both experiments. With the increase of aphid life stage, parasitism rate significantly increased in the choice experiment but significantly decreased in the non-choice experiment. The preference of older aphids by parasitoids in the choice experiment may be interpreted as that the older aphids have more nutrition for their offspring. The decline in parasitism rate on older aphids in the non-choice experiment may be explained as that (1) older aphids have greater defense ability so that the parasitoids need more time and energy to parasitize them, and (2) the number of aphids of a particular life stage was 5 times greater than that in the choice experiment so that the parasitoids attacked fewer older aphids within 24 h. In both experiments sex ratio of the parasitoid progeny was female-biased except for those from 1st instar aphids, whose sex ratio was close to 1:1. Our results predicted that the parasitoid female laid about 66.3% of her eggs in the first 5 d of her lifetime when 4th instar aphids were offered, and that the sex ratio of progeny produced by 6-d-old or older parasitoids was male-biased.

Host stage preference by *Aphidius eadyi* (Hymenoptera: Aphidiidae) and its effect on reproductive fitness

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Abstract

Host stage preference by *Aphidius eadyi* Starý, González & Hall (Hymenoptera: Aphidiidae) on pea aphid, *Acyrthosiphon pisum* (Harris), and its effects on *A. eadyi* reproductive fitness, were studied in the laboratory at 20 ± 1°C and RH 60-70% with a photoperiod of 16 h light:8 h dark. *A. eadyi* accepted aphids of all life stages for oviposition. In the non-choice experiment, host stages had no significant effect on the fecundity of the parasitoids but significantly higher parasitism rate occurred on 2nd and 4th instar nymphs than on other stages. In the choice experiment, both parasitism rate and fecundity significantly increased with the increase of aphid life stages. Sex ratio of progeny from all host stages was female-biased in both experiments. Female parasitoids developed significantly faster in older aphids (from 3rd instar to adults) than in younger ones (1st and 2nd instars) and males developed significantly faster than females in all host stages. The parasitised host stage significantly affected parasitoids' body size and egg load. Larger parasitoids with higher egg loads emerged from aphids, which were parasitised later in their life cycle. It is suggested that 4th instar aphids should be used for mass rearing in biological control programs because aphids of this stage appear to yield parasitoids with the highest reproductive potential and greatest number of females. Our study also implied that the parasitoid population would build up faster when attacking older aphids.