

**HOST-RANGE EVOLUTION IN APHIDIUS  
PARASITOIDS: ECOLOGICAL AND EVOLUTIONARY  
PERSPECTIVES**

by

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## ABSTRACT

A fundamental objective in biology is to understand the evolutionary and ecological processes that lead to patterns of diversity. Specialized insect-host relationships have long been suspected of facilitating evolutionary diversification due to the intimate relationship parasites share with their hosts and the potential for disruptive selection when utilizing different host species resulting in genetic divergence. However, the vast majority of research investigating insect-host specialization has focused on herbivores, while the mechanisms of population and species divergence in insects that parasitize and kill other insects (parasitoids) have been largely ignored. Parasitoids have great potential for research in host-specialization and speciation due to their unique biological properties and the immense diversity that is characteristic of this ecologically important guild.

The goal of my Ph.D. thesis was to contribute to the greater understanding of host specialization and speciation in parasitoid wasps, with a particular focus on the processes that drive adaptive diversification and the behavioural mechanisms that maintain genetic variation. These topics were addressed through a combination of experimental biology, fieldwork and theoretical modelling using a host-parasite system consisting of a single parasitoid (*Aphidius ervi*) and two host species (the pea aphid, *Acyrtosiphon pisum* and the foxglove aphid, *Aulacorthum solani*).

Specifically, the physiological mechanism involved in overcoming host defences was investigated to determine if parasitoids adaptively evolve to overcome host defences and if this process is under directional selection. Host fidelity, host plant preference, mating preference, and host-instar selection were investigated to determine the importance of these behaviours in mediating gene flow between parasitoid populations utilizing different host species. In combination, these studies further contribute to our basic understanding of the mechanisms that lead to and maintain genetic diversity in insect parasitoids.

**Keywords:** host-specialization, speciation, biodiversity, behaviour, parasitoids

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## CHAPTER 1: GENERAL INTRODUCTION

## 1.1 General Introduction to Thesis Research

Understanding the origin of biodiversity is of fundamental importance to evolutionary biology. Despite long standing debate, many details of the patterns and processes that generate biological diversity are not well understood. Of the extraordinary diversity found within the Insecta, herbivores and parasitoids comprise the majority of species (Strong et al. 1984, Godfray 1994). Host shifts and subsequent host specialization to form what are termed “host-races” or “ecotypes” is a process that has been long been suspected of facilitating evolutionary diversification. Host shifts promote novel subpopulations through the process of disruptive selection that is associated with the unique selective pressures that are associated with utilizing different host species (Dres and Mallet 2002). Subsequent reproductive isolation due to processes such as assortative mating, host fidelity or host-plant preference may lead to the formation of host-races and eventually to speciation (Abrahamson et al. 2001).

The vast majority of research investigating insect-host specialization and the formation of host-races has focused on herbivores (e.g. Dres and Mallet 2002, Hawthorne and Via 2001, Feder et al. 2005, Nosil 2007), while the mechanisms of population and species divergence in insects that parasitize and kill other insects (i.e. parasitoids) has been largely ignored. Parasitoids have great potential for research in host-specialization and speciation due to their unique biological properties. In the major parasitoid clades extensive adaptive radiation is evident: the Tachnidae are among the most specious dipteran family with an estimated 10,000 species (Irwin et al. 2003), and the hymenopteran parasitoids account for 20% of all insect species (Godfray 1994). Parasitoids exhibit intimate associations with hosts and the majority are highly specialized (Godfray 1994). In addition, parasitoids are often extremely sensitized to cues derived from particular herbivore-plant interactions, which act as cues to locate particular host species (Vet and Dicke 1992). These characteristics suggest that host-related selection and genetic divergence could be important components contributing to parasitoid diversification.

## 1.2 Focus of Thesis Project

Most insect species that are parasitic on plants or on insect hosts tend to display narrow specialization, in that they only feed on a small fraction of the potential hosts species they encounter (Fry 1996). Even generalist insects, which can be largely polyphagous at the species level, often exhibit limited host species use at the population or community level (Fox and Morrow 1981). Host shifts in insect parasites are generally thought to be an important process that generates genetic differentiation of populations and species diversity. When a population of parasites colonizes a novel host divergent selective pressures that are distinct to each host species, and the environment in which the host resides, can promote the divergence of ecologically important traits. In system where genotypes have different fitness rankings on different hosts, the advancement of specialization is greatest when gene flow is reduced between populations (Fry 1996). Therefore, the population level outcome of processes driving diversification, such as trade-offs or selection, is dependent on the system-specific mechanisms that impact gene flow.

The goal of my Ph.D. thesis was to contribute to the greater understanding of host specialization, and speciation in parasitoid wasps, with a particular focus on the processes that drive adaptive diversification and the behavioural mechanisms that maintain genetic variation between parasitoid populations utilizing different host species. A concomitant focus of this research project was to investigate host-induced phenotypic plasticity in parasitoids and the role of behavioural plasticity in mediating gene flow between host-associated parasitoid populations. These topics were addressed using a combination of experimental biology, fieldwork and theoretical modelling. The model system that I chose to work with consisted of a single aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae), and two host species, the pea aphid, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae), and the foxglove aphid, *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae). *Aphidius ervi* is an excellent candidate of this research as it is considered a generalist species although some host range specialization has been reported. *Aphidius ervi* from Europe utilizes a variety of host species and is considered predominantly a generalist, whereas *A. ervi* from Japan primarily specializes

on pea aphids and exhibits low levels of fitness on other host species commonly used by the European variety (Hajimu and Tada 2000).

### **1.3 Overview of Thesis Chapters**

My dissertation is organized into 5 research chapters, and a concluding chapter that summarizes the major finding of the project. The dissertation is organized as an article-style thesis, corresponding with Simon Fraser University standards, as each chapter has already been published or is currently in review for publication. Therefore each chapter is presented as a complete element, containing an abstract, introduction, methodology, results, discussion, figures and tables presented at the end of each chapter in the same format as they were published and a reference list in the same citation style of the journal in which the manuscript was published. In the following section I will briefly outline each chapter.

The thesis begins with an intensive investigation into the host species itself as a potential agent generating genetic diversity in parasitoid populations utilizing different host species through the process of disruptive selection. The subsequent chapters investigate several behavioural mechanisms that function to maintain or homogenize the genetic differences generated by the aforementioned process. Host fidelity, host-instar selection and mate choice were chosen as behaviours that potentially influence species interactions and evolutionary dynamics through the movement of genes between host-associated parasitoid populations. Although this is clearly not an exhaustive list of behaviours that mediate gene flow between parasitoid populations, these behaviours represent important mechanisms that influence the success of migrant individuals and thus potentially contribute to the maintenance of biological diversity in parasitoids. Moreover, the role of these behaviours in the evolution of genetic differentiation, specialization and speciation in parasitoids has largely been unexplored.

Chapter 2 investigates the potential of different host species to act as selective agents that drive ecological diversification of parasitoid populations. The physiological mechanism involved in overcoming host defences was investigated to determine if

parasitoids adaptively evolve to host defences, and if this process is under directional selection. The genetic basis of host-associated fitness trade-offs was explored using a reciprocal transplant design to determine if antagonistic fitness costs were associated with adaptation to a novel host species. This chapter also includes an experiment that investigates the influence of host fidelity on host-range expansion in *Aphidius* parasitoids.

Chapter 3 is the beginning of a series of experiments investigating the adaptive significance of host-instar selection in parasitoids. This study demonstrates that host instar selection can be maladaptive in that parasitoids were found to select instars that produce few offspring when a parasitoid is initially exposed to a novel host species.

Chapter 4 is a continuation of the previous chapter that extends the concept of adaptive host selection to include the role of phenotypic plasticity in behaviour and adult parasitoid body size using parasitoids reared on two different host species. The work presented here describes a correlation between a behavioural response and a highly plastic developmental strategy, which results in an adaptive shift in host selection. This chapter also describes a novel hypothesis for the evolutionary pathway of these co-adapted traits in parasitoids.

Chapter 5 presents an experimentally parameterized foraging model that utilized the theory developed in the previous chapter on adaptive host-size selection to investigate the influence of parasitoid body-size on the range of acceptable host instar classes. We then compare the efficiency of optimal host selection strategy against an indiscriminate host selection strategy over a range of different parasitoid body sizes foraging on different size structured aphid populations using a demographic model. This chapter discusses the potential of within-generation behavioural interactions to impact between-generation dynamics in host-parasitoid populations.

Chapter 6 investigates the role of mating preference and the influence of adult male body size on mating success, using parasitoids adapted to different host species. This chapter demonstrates the importance of assortative mating in combination with the success of migrant males in mitigating gene flow between host-associated insect populations. Additionally, the research presented in this chapter further develops the theory of how host-determinant growth influences life history decisions in parasitoids by



relating parasitoid body-size to the mating success of migrant males, and the subsequent movement of genes between host-associated parasitoid populations. The importance of this mechanism for community dynamics and the evolution of host specialization in parasitoids are discussed.

Finally I close with a concluding chapter that summarizes the major findings of this thesis work and discusses future researchers directions.

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## **CHAPTER 2: HOST-RANGE EVOLUTION IN APHIDIUS PARASITOIDS: FIDELITY, VIRULENCE AND FITNESS TRADE-OFFS ON AN ALTERNATE HOST <sup>1</sup>**

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## 2.1 Abstract

The diversity of parasitic insects remains one of the most conspicuous patterns on the planet. The principal factor thought to contribute to differentiation of populations and ultimately speciation is the intimate relationship parasites share with hosts and the potential for disruptive selection associated with utilizing different host species. Traits that generate this diversity have been an intensely debated topic of central importance to the evolution of specialization and maintenance of ecological diversity. A fundamental hypothesis surrounding the evolution of specialization is that no single genotype is uniformly superior in all environments. This “trade-off” hypothesis suggests that negative fitness correlations can lead to specialization on different hosts as alternative stable strategies. In this study we demonstrate a trade-off in the ability of the parasitoid, *Aphidius ervi*, to maintain a high level of fitness on an ancestral and novel host, which suggests a genetic basis for host utilization that may limit host range expansion in parasitoids. Furthermore, behavioural evidence suggests mechanisms that could promote specialization through induced host fidelity. Results are discussed in the context of host-affiliated ecological selection as a potential source driving diversification in parasitoid communities and the influence of host species heterogeneity on population differentiation and local adaptation.

## 2.2 Introduction

Over half of the known species on the planet live parasitic lifestyles (Windsor 1998). The extraordinary diversity of the world’s parasites is often attributed to the intimate relationship they share with a limited number of host species, and the potential for disruptive selective pressures associated with different hosts driving ecological divergence (Price 1980; Ackermann and Doebeli 2004). Most parasites are restricted to utilizing a relatively small number of the available hosts within their environment (Fox and Morrow 1981). The evolutionary mechanisms responsible for host-range limitations have been an intensely debated subject of central importance to the evolution of specialization and maintenance of ecological diversity (e.g. Dethier 1954; Krieger et al.

1971; Bernays and Graham 1988; Futuyma and Moreno 1988; Jaenike 1990; Fry 1990, 1996; Joshi and Thompson 1995; Kawecki 1998). A fundamental hypothesis surrounding the evolution of specialization is that no single genotype is uniformly superior in all environments, thereby promoting the development of locally adapted varieties (Falconer 1952). It follows that extensive specialization in the presence of trade-offs would then generate the aforementioned diversity.

Patterns of host use in parasitic arthropods have been the focus of a vast amount of research with the central goal of better understanding how the diversity of insect communities have evolved and how they are maintained in time and space (e.g. Jermy 1984; Futuyma and Moreno, 1988; Thompson 1994; Jaenike 1990; Mopper 1996; Stireman et al. 2005). Most insect species that are parasitic on plants or on insect hosts tend to display narrow specialization, in that they only feed on a small fraction of the potential hosts species they encounter (Fry 1996). Even generalist insects, which can be largely polyphagous at the species level, often exhibit limited host species use at the population or community level (Fox and Morrow 1981; Smith et al. 2007).

Host range is influenced by localized genetic variation in both parasite and host populations and whether selection or trade-offs lead to specialization on different host species (Dres and Mallet 2002; Lajeunesse and Forbes 2002; Kawecki and Ebert 2004). Divergent selection associated with the utilization of particular hosts can result in localized genetic structuring of parasite populations that may impede host shifts to alternate species or restrict an expansion in host range. A “host shift” refers to a population forming an association with a novel host, which differs from the standard definition of host range expansion, which involves a population colonizing a new host with the continued utilization of the previous host (Agosta 2006). Barriers to the integration of novel hosts can be behavioural, or physiological in nature, with adaptations to one host species potentially resulting in trade-offs in the ability to utilize alternate hosts. Behavioural barriers may include the ability to accurately locate a host in the environment or microhabitat, recognition of potential hosts, and coping with external host defenses (Althoff et al. 2001; De Moraes et al. 1998). Physiological barriers, including biochemical or morphological adaptations, can include digestive enzymes necessary to

overcome a host's internal defense, or specialized structures required to access a host (Dethier 1954; Vinson and Iwantsch 1980).

A popular theory to explain some of the patterns of host affiliation in phytophagous insects is that traits leading to an increased fitness on one host are detrimental on others (Krieger et al. 1971; Rausher 1983; Futuyma and Moreno 1988; Jaenike 1990; Via 1990; Fry 1996). Referred to as the "trade-off" hypothesis, this theory suggests that negative fitness correlations, caused by the antagonistic pleiotropic action of one or more genes, can lead to specialization on different hosts as alternative stable strategies (Castillo-Chavez et al. 1988; Futuyama and Moreno 1988; Jaenike 1990). Although conceptually appealing, evidence of direct genetic trade-offs (i.e. negative cross-host fitness correlations) using quantitative genetics techniques have been found in only a few studies (Gould 1978; Karban 1989; Fry 1990; Karowe 1990; Via 1991; MacKenzie 1996; Agrawal 2000) and more frequently have not been found (e.g. Rausher 1984; Hare and Kennedy 1986; Bernays & Graham 1988; Fox 1993; Thompson 1996). However, evidence of negative genetic cross-host fitness correlations does not preclude the existence of trade-offs. As Fry (1996) illustrates, specialization is promoted whenever fitness norms cross, indicating that genotypes have different fitness rankings on different host species. This pattern is observed in many phytophagous insects and is a plausible yet controversial explanation for why there is so much diversification and specialization in host use by herbivorous insects.

In a system where genotypes have different fitness rankings on different hosts, the advancement of specialization is greatest when gene flow is reduced between populations (Fry 1996). The population level outcome of processes driving diversification, such as trade-offs or selection, is therefore dependent on the system-specific mechanisms that impact gene flow. Host fidelity, is one such mechanism that can greatly reduce gene flow between populations because many insects feed, mate and oviposit on or near their hosts (Hawthorne and Via 2001; Funk et al. 2002). Locally adapted gene pools are therefore degraded when gene flow is high between populations and conserved when gene flow is limited. The conservation of gene pools thus preserves the differences generated by trade-offs or selection and is an important factor in the maintenance of genetic differentiation in insect populations.

Although there has been great progress in understanding the ecology and evolution of host use in phytophagous insects, little is known about the processes that mediate host range evolution in another diverse and highly specialized group, the insect parasitoids. Parasitoid lifestyles are predominantly found in the orders Hymenoptera and Diptera, but are also found in many other holometabolous orders of Insecta. Parasitoids have a particularly intimate relationship with hosts because a single host harbours the parasitoid's offspring until maturity (Godfray 1994). Within families, parasitoids have undergone extensive adaptive radiation as evidenced by the vast number of species in the major parasitoid clades (Irwin et al. 2003; Godfray 1994; Godfray and Shimada 1999). Although little is known about the mechanisms that mediate population and species divergence in parasitoids there is evidence that suggests host-affiliation may be responsible for driving differentiation of lineages (Fellowes and Kraaijeveld 1998; Dupas et al. 2003; Powell and Wright 1988; Pike et al. 1999; Morehead et al. 2001; Aldrich and Zhang 2002; Stireman et al. 2006). Many parasitoids have specialized traits designed to cope with host defenses and are highly sensitized to chemical cues that they use to locate particular host species (Vet and Dicke 1992; De Moraes et al. 1998). The intimate nature of this relationship promotes co-evolutionary dynamics, while generating disruptive selective pressures associated with specialization on different host species (Thompson 1994). These factors suggest that local host species utilization may play an important role in the differentiation and diversification of parasitoid communities.

In the following experiments we investigate the potential for adaptation to a novel host by an insect parasitoid, as well as the costs associated with the host shift, using a replicated quasi-natural selection experiment under controlled laboratory conditions. The aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) is considered a generalist species although some host range specialization has been reported. *Aphidius ervi* from Europe utilizes a variety of host species and is considered predominantly a generalist, whereas *A. ervi* from Japan primarily specializes on pea aphids and exhibits low levels of fitness on other host species commonly used by the European variety (Hajimu and Tada 2000). Our experiments were designed to address the following questions:

1. Are host species potential agents of directional selection in *Aphidius* parasitoids? Furthermore, can a population of parasitoids adapt to a novel, initially low quality host as demonstrated through an increase in population fitness?
2. What costs are associated with adaptation to a novel host and are fitness trade-offs evident in the ability to utilize an ancestral host species?
3. What are the behavioural and physiological mechanisms that contribute to, or confine, host range expansion? Further, are these traits selected over successive generations or are they plastically induced within a generation?

## 2.3 Materials and Methods

### 2.3.1 Insect stock and selection lines

The colony of *Aphidius ervi* was originally collected from *Acyrtosiphon pisum* (Harris), (Hemiptera: Aphididae) (i.e. the “ancestral” host) in alfalfa fields at London, Ontario and maintained at the Southern Crop Protection and Food Research Centre, Agriculture Canada for several generations, on broad beans *Vicia faba* L. (cv. ‘Broad Windsor’), before being transferred to Simon Fraser University (Burnaby, British Columbia). The source parasitoids were arbitrarily assigned to 9 colonies (>300 individuals each), and were maintained on *A. pisum*, on broad beans at Simon Fraser University for approximately 3-4 parasitoid generations to allow the parasitoids to acclimate to laboratory conditions prior to the start of the experiment. All insect colonies were maintained at  $19\pm 2.0^{\circ}$  C daytime,  $17\pm 2.0^{\circ}$  C nighttime temperature, 50-60% RH and a L16:D8 photoperiod. These environmental conditions maintained the aphids in a continual parthenogenetic cycle throughout the experiment. Replicate selection lines were initiated by haphazardly removing approximately 1000 parasitized aphids as mummies from the 9 stock colonies and placing them in a common emergence cage. Each mummy harboured a single *Aphidius* parasitoid in pupal form. Upon emergence, adults were allowed to mate. The mated females were then sub-divided into replicate populations maintained on either of two different host species; pea aphids, *A. pisum* (the “ancestral” host) or foxglove aphids, *Aulacorthum solani* (Kaltenbach) (Hemiptera:



Aphididae), (the “novel” host). These are hereafter called P-line and F-line for the pea and foxglove selection lines, respectively. The original population of *A. solani* was established via collections from commercial pepper greenhouses in Abbotsford, British Columbia. These were transferred to broad bean plants and maintained for 2 months prior to the start of the experiment to allow the aphids to adapt to the new host plant and laboratory conditions. *A. pisum* was collected from alfalfa fields in Southern British Columbia and maintained in 12 separate colonies at Simon Fraser University. *A. pisum* are a large, high quality host, and are a primary host of *A. ervi* in many regions (Hajimu and Tada 2000) whereas *A. solani* are a small, low quality host; in that *A. ervi* generally produces substantially fewer mummies when initially exposed to this host species (Henry et al. 2005). Both aphid species in experimental and stock colonies were maintained on broad beans to remove plant effects on parasitoid behaviour or reproductive performance. Replicate selection populations were initiated sequentially over 8 weeks as parasitoid females became available from the stock colonies. Eight populations were maintained on *A. pisum* and 14 populations were maintained on *A. solani*. Replicate populations were used for each host species in order to reduce the stochastic effects of genetic drift on the overall mean values. Each replicate population was initiated using approximately 50 mated female parasitoids, which were exposed to 24 broad bean plants infested with several thousand host aphids. Dilute honey (10% solution), water (nutrition for parasitoids) and fresh bean plants (food plants for aphids) were added each week. Aphids were added to each replicate population on a per cage basis if aphid populations dropped below a specified density determined by sub-sampling several leaves in each population per week ( $\bar{x}=50\pm10$  aphids per apical bean leaf). Furthermore, no aphids were ever removed from the cages, which prevented mixing of aphids and parasitoids between replicates, and allowed aphids to co-evolve to some degree with the parasitoid populations. Parasitoid populations were maintained in isolation on each host species for 2 years (~50 parasitoid generations).

### 2.3.2 Assay insects

Assay parasitoids, reared on *A. pisum* or *A. solani*, were always 2-3 day-old females that had been given continuous access to dilute honey (10% solution), water and males from their replicate cage for fertilization. Parasitoid females were naïve at testing meaning that they had no contact with any hosts except the mummy casings from which they emerged. Each parasitoid female was used only once then discarded.

### 2.3.3 Experiment 1 - Parasitoid fitness and host-affiliated trade-offs

F-line and P-line parasitoids were assayed for fitness on their natal host (i.e. selection line host species) and non-natal host every generation for the first 4 parasitoid generations to determine the initial response in trait values to the hosts, then again after 40 and 50 generations. Therefore, the four treatment groups sampled at each parasitoid generation were: F-line parasitoids assayed on pea aphids; F-line parasitoids assayed on foxglove aphids; P-line parasitoids assayed on pea aphids and P-line parasitoids assayed on foxglove aphids. For each of the parasitoid generations assayed, a sub-sample of 60-80 mummies was removed from each population and isolated in glass emergence containers as a group. Upon emergence, parasitoids were allowed to mate with individuals from their own replicate population and fed on honey and water for 24 h, after which 20 females were randomly selected from each cohort and individually assayed on natal and non-natal hosts (10 females on foxglove and 10 on pea). Parasitoids that were not assayed were returned to their appropriate replicate populations.

Individual parasitoid females were allowed to forage on 40 2<sup>nd</sup> instar (46-48 hours old), *A. pisum* or *A. solani* on a bean leaf for 4 h. Aphid instar was determined by age, which correlates strongly with size for each species (mean volume at 46-48 hours is  $0.031 \pm 0.006 \text{ mm}^3$  for *A. solani* and  $0.109 \pm 0.011 \text{ mm}^3$  for *A. pisum*). Second instar aphids were chosen for this assay because they represent a relatively high quality host that does not display behavioural defenses that would affect parasitoid handling time. After 4 hours, parasitoids were removed and the aphids were carefully transferred to an excised bean leaf, which was kept fresh by inserting the leaf petiole into a water-filled glass vial capped with Parafilm™. Excised leaves with aphids were then sealed in Petri dishes where they remained until mummies formed.

Proxies chosen to represent parasitoid fitness for each selection line were mean number of hosts parasitized (mummies formed per 40 aphids), and mean proportion of parasitoid adults eclosing from mummies (Roitberg et al. 2001). The mean number of hosts parasitized was analyzed using all of the above noted generations. Proportion eclosing from mummies was compared at parasitoids generations 1 and 50 only. Traits were compared for each assay host species separately (i.e. F-line vs. P-line fitness on foxglove and F-line vs. P-line fitness on pea) in order to reduce the complexity of the statistical model. The P-line was always used as the control population, with deviations in trait values indicating a response to selection in the F-line parasitoids.

A generalized linear model (GLM) was used to analyze the results from Experiment 1 with an overdispersion parameter applied to account for non-independence of sampling units (McCullagh and Nelder 1989). GLM Model effects included: parasitoid host selection line, parasitoid generation and parasitoid generation\*selection line cross. All model effects were treated as fixed. For mean hosts parasitized, a Poisson distribution was used with a log link function. Proportion eclosion was analyzed using a logistic regression with a binomial distribution. Data were analyzed using JMP 6.0 statistical software (SAS Institute, Cary, NC, USA).

#### **2.3.4 Experiment 2 - Host fidelity and parasitoid virulence**

Factors influencing host range evolution in insect parasitoids generally involve both behavioural and physiological components that work in conjunction to mediate acceptance and subsequent reproductive success on a given host. The following experiments were used to explore the influence of host fidelity of the female parasitoid (i.e. willingness to accept a host species) and the physiological virulence of the parasitoid larvae, when adapting to a novel host species. In the following experiments equal numbers of individual females were sampled from each replicate population. Individuals used for each experiment were pooled for the analyses.

##### **2.3.4.1 Host fidelity**

Host species fidelity was investigated using parasitoids from each host selection regime (F-line and P-line) assayed on either a natal or non-natal host species at generations 1 and 50. Individual parasitoids were allowed access to a single 2<sup>nd</sup> instar host in a gel capsule. Parasitoids were allowed to sting each host only once, and the behaviours leading to the initial sting were recorded for each individual. Host fidelity was assessed by measuring oviposition latency, and host rejection. Oviposition latency was defined as the time from the parasitoid's first inspection of the host (i.e. antennal contact with host) until the host was accepted, indicated by a successful oviposition. Parasitoids that did not oviposit in the host after 5 min were considered to have rejected the host. Host fidelity was investigated using parasitoids which had remained on a given host species for 50 generations. In order to determine whether the parasitoid's host fidelity had evolved under the host selection regime or was a plastic response to the current natal host, latency was assessed at generation 1 and compared to generation 50 using F-line parasitoids only. Oviposition latency was analyzed using an ANOVA for all selection and assay host combinations. Differences in the number of hosts rejected and accepted between assay hosts were compared separately for each selection line using a ChiSquare analysis.

#### **2.3.4.2 Parasitoid virulence**

The physiological component of adaptation to a novel host was explored by assessing the performance of the developing larvae for each combination of host selection regime and assay host. Individual parasitoids were given access to a single host from either species in a gel capsule as described above. After a single sting, the aphid was removed and transferred to an excised bean leaf. Aphids were monitored daily to determine parasitoid larval development. Parasitoid virulence and host resistance were used to determine the overall success rate of larval development until pupation. Parasitoid virulence was defined as the loss of fitness of a parasitized host (i.e. aphid mortality due to infection with the parasitoid), because the death of the host is required for successful parasitism. Virulence was therefore ascertained by comparing the number of aphids that died due to parasitism, as indicated by the formation of a parasitoid

mummy compared with the aphids that resisted parasitism. This measure was tested for each parasitoid selection line on each assay host species using a ChiSquare analysis (i.e. F-line assayed on pea vs. foxglove and P-line assayed on pea vs. foxglove). A parasitoid's fitness was therefore determined by its success in transmitting offspring to a particular host species.

To establish that a sting was equivalent to an egg being laid, sub-samples of hosts (~40 per selection/assay host cross) from this experiment were dissected and inspected for parasitoid eggs. Stung aphids from both species were injected with methyl blue, which made the parasitoid eggs easier to identify. Egg presence was compared for each assay host and host selection line combination using a ChiSquare analysis.

## **2.4 Results**

### **2.4.1 Experiment 1 - Parasitoid fitness and host-affiliated trade-offs**

There was significant variation in the number of hosts successfully parasitized from P-line and F-line parasitoids assayed on foxglove aphids (GLM,  $\chi^2_{(1)} = 175.42$ ,  $P < 0.0001$ , overdispersion = 4.71). Selection line influenced the number of hosts successfully parasitized ( $\chi^2_{(1)} = 23.50$ ,  $df = 1$ ,  $P < 0.001$ ). F-line females parasitized an overall greater number of hosts when assayed on foxglove aphids ( $\bar{x} = 8.43 \pm 0.46$ ) than P-line females ( $\bar{x} = 5.03 \pm 0.36$ ). There was a significant effect of generation ( $\chi^2_{(5)} = 94.84$ ,  $P < 0.0001$ ). There was no interaction between generation and treatment ( $\chi^2_{(5)} = 3.94$ ,  $P > 0.05$ ). This latter result is most likely due to the large variance created by sampling the mean hosts parasitized at 6 different parasitoid generations. To determine if the F-line parasitoids increased their mummy production over the 2 years on foxglove aphids, a contrast analysis was applied to compare the difference in the number of hosts parasitized at generation 1 versus generation 50. This analysis demonstrated that the selection lines differed in the number of hosts parasitized when assayed on foxglove aphids at generations 1 and 50 (Contrast analysis,  $\chi^2_{(1)} = 15.52$ ,  $P < 0.0001$ ). F-line females produced significantly more mummies when assayed on foxglove at the end of the 50-generation period than they did at generation 1 (Fig. 2.1).

The number of hosts parasitized differed significantly when comparing P-line and F-line females assayed on pea aphids (GLM,  $\chi^2_{(9)} = 68.09$ ,  $P < 0.0001$ , overdispersion 3.67). Individual effects indicated that selection line affected the number of hosts parasitized ( $\chi^2_{(1)} = 24.32$ ,  $P < 0.0001$ ). The P-line produced a greater number of mummies over all generations combined (hosts parasitized: F-line  $\bar{x} = 13.5 \pm 0.6$ ; P-line  $\bar{x} = 17.7 \pm 0.6$ ). There was an effect of generation ( $\chi^2_{(4)} = 25.56$ ,  $P < 0.0001$ ) and a selection line by generation interaction, with the F-line parasitizing fewer hosts over the generational period than P-line females (Fig. 2.2) ( $\chi^2_{(4)} = 17.12$ ,  $P < 0.005$ ). Generation 3 was not included in the analysis and figure due to missing data.

When comparing the proportion eclosion from mummies for the F and P-line parasitoids assayed on pea aphids a whole model effect was detected (GLM,  $\chi^2_{(3)} = 12.89$ ,  $P < 0.005$ , overdispersion 2.26). Individual model effects indicated that there was no effect of selection line ( $\chi^2_{(1)} = 1.64$ ,  $P > 0.05$ ), or of selection line within generations ( $\chi^2_{(1)} = 1.93$ ,  $P > 0.05$ ). However, an effect was detected between generations 1 and 50 in that generation 50 had fewer parasitoids successfully eclosing from pea aphid mummies ( $\chi^2_{(1)} = 10.07$ ,  $P < 0.005$ ) (Fig. 2.3A). The proportion eclosion from mummies was not different between the two selection lines assayed on foxglove aphids (GLM,  $\chi^2_{(3)} = 2.90$ ,  $P > 0.05$ , overdispersion 1.51). Furthermore, no individual model effects were detected for the proportion eclosion from foxglove mummies (selection line,  $\chi^2_{(5)} = 0.01$ ,  $P > 0.05$ ; generation,  $\chi^2_{(1)} = 0.93$ ,  $P > 0.05$ ; generation\*selection line,  $\chi^2_{(1)} = 0.73$ ,  $P > 0.05$ ) (Fig. 2.3B). A general lack of variation in eclosion between selection lines over the generational period indicates that this trait is most likely not under selection within this system.

## 2.4.2 Experiment 2 - Host fidelity and parasitoid virulence

### 2.4.2.1 Host fidelity

A similar pattern in host fidelity arose for both selection lines when parasitoids were assayed on natal versus non-natal hosts. There was a significant difference between natal and non-natal assay groups in the latency period before a host was accepted

(ANOVA,  $F_{3,285} = 12.80$ ,  $P < 0.0001$ ). No effects of selection host ( $p > 0.05$ ) or assay host ( $p > 0.05$ ) were detected; however there was an interaction between selection line and assay host ( $F_1 = 41.99$ ,  $P < 0.0001$ ). A Tukey HSD analysis revealed that parasitoids accepted a natal host (i.e. selection regime and assayed on the same host species) more quickly than when offered a non-natal host (Table 2.1). A similar pattern arose for the difference in the number of hosts accepted or rejected, with a greater number of non-natal host's being rejected. There was a significant difference in the number of accepts and rejects for F-line parasitoids ( $\chi^2_{(1,162)} = 10.60$ ,  $P < 0.005$ ) with females rejecting more pea (21.7%) than foxglove aphids (4.9%). P-line females showed a similar pattern with more foxglove aphids being rejected (14.6%) than natal pea aphids (5.5%), and the analysis only marginally failed to demonstrate significance ( $\chi^2_{(1,159)} = 3.7$ ,  $P = 0.055$ ).

The increase in the number of hosts parasitized observed in the F-line parasitoids assayed on foxglove aphids over the selection period indicated that a trait influencing parasitism, such as host fidelity or virulence, was under selection. In order to determine if a behaviour that mediated host fidelity was under selection, oviposition latency was compared at generations 1 and 50 on the two host species using F-line parasitoids. The analysis revealed that there was no change in oviposition latency for the F-line parasitoids over the 2-year selection period (generation\*assay;  $F_{3,171} = 9.55$ ,  $P > 0.05$ ). The F-line parasitoids took significantly longer to oviposit in pea aphids than in foxglove aphids, and this response did not change over the selection period indicating that the behaviour was plastically induced after developing in a host for a single generation.

#### **2.4.2.2 Parasitoid virulence**

The differential survival of single-stung aphids was assessed using parasitoids from both host selection lines to determine if different host species act as selective agents on parasitoid larvae. In order to eliminate the potential confound of parasitoids not laying eggs when they sting a sub-sample of stung aphids from the selection line/assay crosses were dissected, stained and inspected for a parasitoid egg. There were no differences in the number of eggs found in any of the hosts, ( $\chi^2_{(3,113)} = 1.16$ ,  $P > 0.05$ )

demonstrating that a sting was equivalent to an egg being laid regardless of selection line or assay host species.

Larval virulence was compared between selection lines separately from each assay host species. Early aphid mortality was recorded but not used in the analysis because mortality occurred, in almost all cases, within the first 24 hours after oviposition due to aphids walking off the leaves and refusing to feed, prior to parasitoid egg hatching. F-line parasitoids had a greater virulence when assayed on foxglove aphids than P-line parasitoids in that they successfully parasitized a natal host species more often than a non-natal host ( $\chi^2_{(1,123)} = 5.74, P < 0.05$ ). A similar pattern emerged from the P-line parasitoids, which also had a greater virulence on their natal host than the F-line parasitoids ( $\chi^2_{(1,102)} = 8.06, P < 0.005$ ) (Fig. 2.4).

## 2.5 Discussion

Selection experiments are a powerful tool in the study of evolutionary biology because they allow the investigator to vary the environmental context in a controlled manner. This approach is particularly useful in the study of local adaptation when exploring trade-offs associated with niche breadth, providing an alternative method to classic quantitative genetics techniques (Fry 2003). In the present study, a trade-off in the ability to maintain a high level of fitness on more than one host suggests a genetic basis for host utilization that may limit host range expansion in *Aphidius* parasitoids. Trade-offs associated with utilization of different hosts are thought to be an important mechanism generating genetic diversity among populations of insects and encourages local adaptation when combined with limited gene flow (Kawecki 2004).

In this study, adaptive phenotypic evolution was investigated using a single population of parasitoids exposed to pea aphids (*A. pisum*), a high quality host, and to foxglove aphids (*A. solani*), a low quality novel host. Reciprocal trials were performed on both hosts in each of several generations in order to determine if adaptation to the novel host resulted in a trade-off in fitness on the ancestral host. When initially exposed to the novel host, parasitoid fitness was low as shown through the modest mummy



production in experiment 1 at generation 0. However, mummy production increased substantially after a single generation on the novel host species. This change in fitness is most likely due to a plastic shift in host fidelity that conditions parasitoids towards the host species in which they developed. Evidence for this behavioural plasticity demonstrates that *A. ervi* maintains a greater affinity for a host after developing in it for a single generation, which decreases oviposition latency and host rejections, thereby facilitating elevated mummy production through increased host exploitation (see also Henry et al. 2006). If under selection, host fidelity could be responsible for changes in mummy production over a generational period as parasitoids become more willing to accept natal than non-natal host species. If host fidelity was under selection, oviposition latency should have changed over the selection period. The induced response in host fidelity did not change over the 2-year selection period, indicating that the mechanism is plastically induced and not under direct selection. A similar plastic response has been documented for host plant preference in *Aphidius* parasitoids in that parasitoids have a greater attraction for the host-plant complex in which they developed previously, regardless of their population of origin (Poppy et al. 1997; De Moraes et al. 1998; Daza-Bustamante et al. 2002). The following 3 generations did not show a significant change in fitness on either host, however the initial fitness increase was maintained on foxglove aphids in the F-line parasitoids. Behaviours that encourage continual use of the same host species, such as the increase in host fidelity observed in *Aphidius* parasitoids, promote further adaptation through natural selection and the conservation of locally adapted gene pools. Where behavioural modifiers cause avoidance of feeding or oviposition on a host, mean population fitness has the potential to increase by adapting to one host free of countervailing selection from the other (Fry 1996). Therefore trade-offs in the presence of a trait that limits gene flow have the potential to stabilize specialization and destabilize generalization under idealized environmental conditions.

After remaining on foxglove aphids for 2 years, parasitoid fitness on foxglove aphids increased dramatically compared to P-line parasitoids (Fig. 2.1). A concurrent decrease in fitness was observed in the performance of the F-line parasitoids on the ancestral host, the pea aphid (Fig. 2.2). This pattern suggests that simultaneous maximization of fitness on pea and foxglove aphids was constrained within the initial

population of parasitoids used in this experiment, possibly due to the antagonistic pleiotropic action of one or more genes. A local maximum appears to have been reached in the F-line parasitoids at generation 40 as subsequent sampling revealed a plateau in mummy production on both host species. When controlling for parasitoid behaviour, parasitoids similarly retained greater virulence (i.e. more parasitoids successfully completed development) developing in a natal as opposed to a non-natal host (Fig. 2.4). These results provide strong evidence that there is a physiological mechanism involved in overcoming host defenses and that this process is under directional selection, indicated by the increase in virulence beyond the initial plastic shift, over the selection period. After an initial plastic response to a novel environment, there is often a period of latency before a subsequent fitness increase is a pattern consistent with genetic change through the rearrangement of allele frequencies, with noticeable phenotypic change occurring only as the population moves towards homozygosity in the genes under selection. This pattern is consistent with our data in that noticeable fitness trade-offs did not occur until parasitoids spent 4 generations on foxglove aphids. Although our design did not take into account the possibility of maternal effects such as viruses, virus-like particles, endosymbionts and other substances known to be maternally transmitted and that increase virulence in ichneumonoid parasitoids or resistance in hosts (reviewed in Vinson 1990; Oliver et al. 2005), the existence of fitness trade-offs on the ancestral host supports the notion of a genotypic response to a selective agent.

Genetic variation in virulence within natural populations of parasitoids has been measured in several systems. *Leptopilina boulardi*, a parasitoid of *Drosophila simulans* provided evidence that a single population contained significant heritable variation in the ability to parasitize their hosts (Carton et al. 1989). Similarly, a selection study using laboratory populations of parasitoids showed an increase in their ability to overcome *Drosophila* encapsulation (Kraaijeveld et al. 2001). Considerable genetic variation in parasitoid virulence and host resistance has been demonstrated in several well-studied endoparasitoid systems (Henter and Via 1995; Hufbauer 2002; Kraaijeveld et al. 2002; Dupas et al. 2003). Within aphid parasitoid systems Henter (1995) found significant additive heritable variation in the ability to parasitize pea aphids within a single population of *Aphidius ervi*. In a related study, Henter and Via (1995) found significant

genetic variability within a population of pea aphids in their susceptibility to parasitization by *A. ervi*. The presence of additive genetic variation in aphid resistance and parasitoid virulence indicates that these populations have the potential to co-evolve in response to selection (Henter 1995). Variation in the ability to parasitize natal and non-natal host species has been recorded for several aphid parasitoids suggesting a genetic basis for virulence and resistance across a variety of systems (Powell and Wright 1998; Pennacchio et al. 1994; Pike et al. 1999; Antolin et al. 2006). Research by Oliver and associates (2005, 2006) on the *A. ervi* - pea aphid system has revealed that aphid endosymbionts confer varying degrees of resistance to parasitism. This discovery indicates that we may need to broaden our outlook on the co-evolutionary dynamics of host-parasitoid systems to include the function and frequency of endosymbionts, or other organisms influencing parasitism rates, within populations of both hosts and parasitoids.

Host range evolution in insect parasitoids is typically governed by both behavioural and physiological components. We investigated the traits of adults and offspring, as both can potentially influence overall fitness levels and the detection of trade-offs associated with utilizing different hosts (Scheirs et al. 2005). Our work demonstrates that adult behaviour remains plastic within this system but larval virulence is genetically determined, as indicated by the accrued fitness of F-line parasitoids and inability of parasitoids to simultaneously maximize fitness on more than one host, a pattern suggested to occur in other aphid-parasitoid systems (Powell and Wright 1988; Antolin et al. 2006). Theory predicts that in a system where genotypes have different fitness on different hosts, selection should promote specialization as long as parasites are capable of host choice and the cost of being “choosy” is low (Fry 1996). The logic behind this theory is that specialization is favoured because parasites that remain on a single host can evolve faster in response to the evolution of a single host’s defenses (Kawecki 1998). Our research, as well as research in several other *Aphidius* systems has demonstrated that parasitoids are “choosy” in that they have evolved a tendency to prefer the same host type in which they developed (Pennacchio et al. 1994; Poppy et al. 1997; Daza-Bustamante et al. 2002). This process is thought to inherently destabilize generalization and promote specialization, even if trade-offs are absent (Balkau and Feldman 1973). When combined with a trait under hard selection, such as parasitoid

virulence, fitness trade-offs associated with the utilization of different host species have the potential to rapidly progress populations towards specialization on different hosts as alternate stable states (Fry 1996). However, it should be noted that intraspecific genetic variation for performance on different hosts is just one mechanism that can potentially lead to differentiation and diversification of insect populations. The evolution of a specialist or generalist strategy is mediated not only by mechanisms generating and maintaining genetic variation, but also how these traits interplay with the many different factors that are specific to each environment, such as competition for hosts with other organisms and the abundance and distribution of host species that parasitoids are exposed to at both a spatial and temporal scale. Aphid populations follow classic meta-population dynamics and are prone to successions of colonization and extinction of local populations. This process is thought to inherently destabilize specialization through the obliteration of locally adapted gene pools (Kawecki 2004). However, *Aphidius* parasitoids may counteract this process by the use a highly sensitized host location mechanism or through the “hitchhiking” of parasitoid larvae within colonizing aphids. Furthermore, local adaptation could be diluted if constant migration from other hosts occurs, although migration between host species could slow evolution but not prevent a response to selection or diffuse coevolution between species (Henter 1995).

Host-associated genetic divergence has been reported in several parasitoid species (Morehead et al. 2001; Stireman et al. 2005; Hayward and Stone 2006), although few studies have addressed host-based genetic divergence in aphid parasitoids. Vaughn and Antolin (1998) found that *Diaeretiella rapae* from two adjacent fields containing Russian wheat aphids and cabbage aphids had extensive genetic differentiation between six host-associated populations tested (using heritable RAPD markers). Two subsequent studies on *D. rapae* populations concluded that while genetic differentiation and local adaptation does occur by host species, analyses of mitochondrial DNA suggested that sufficient gene flow prevents populations from becoming completely isolated and that local selection rather than isolation creates genetic subdivisions between populations on different hosts (Baer et al. 2004; Antolin et al. 2006). Our work supports the notion that local host-affiliated selective pressures could potentially generate genetically distinct sub-

populations that correspond to different host species with fitness trade-offs limiting the number of hosts a single populations could optimally utilize at a given time.

In conclusion, models of genetic differentiation associated with ecological specialization emphasize divergence as a consequence of different selective pressures between differing environments (Schluter 2001). Empirical evidence is growing in support of the many proposed mechanisms thought to contribute to the widespread patterns of host affiliation in herbivore communities (for reviews see: Bush 1975; Price 1980; Mopper 1996; Poulin and Morand 2000; Berlocher and Feder 2002). However, far less research has been directed at the insect parasitoids, which are also an immensely diverse, potentially rich taxa for studying host affiliated ecological divergence. Our results suggest that a number of behavioural and physiological mechanisms could promote specialization in *Aphidius* parasitoids as well as limit the number of hosts a single population can effectively utilize at any one time. These mechanisms are characteristic of traits thought to contribute to host-associated differentiation in parasite populations. However, local adaptation has not been detected in a number of natural populations of other, widely studied parasitoid systems exhibiting similar traits (Hufbauer 2001; Kraaijeveld et al. 2002; Dupas et al. 2003) but has been detected in others (Vaughn and Antolin 1998; Althoff and Thompson 2001; Morehead et al. 2001; Stireman et al. 2005; Antolin et al. 2006; Hayward and Stone 2006). It should be noted that the insects and hosts studied to date represent only a fraction of the tremendous diversity of parasitic relationships that exist. Only now are we acquiring evidence that suggests that host-associated differentiation and cryptic speciation may be more common than previously thought (Stireman 2005), especially true in the insect parasitoids (Stireman 2006; Smith et al. 2007). Although traits facilitating directional selection and specialization exist in many parasite systems as a means to constantly improve fitness or counteract host defenses, the evolution and maintenance of differentiation between populations requires specific environmental conditions, such as stable host populations and limited gene flow between host-affiliated populations. Host-parasite systems that are prone to disturbances or those that experience extensive gene flow may have selection slowed or disrupted. Our work has demonstrated the potential for a single population of *Aphidius* parasitoids to differentiate based strictly on host species utilization. However, in order to gain a

better understanding of how these traits function in nature, detailed studies are required that link mechanisms that drive differentiation to the genetic structure of natural parasitoid populations.

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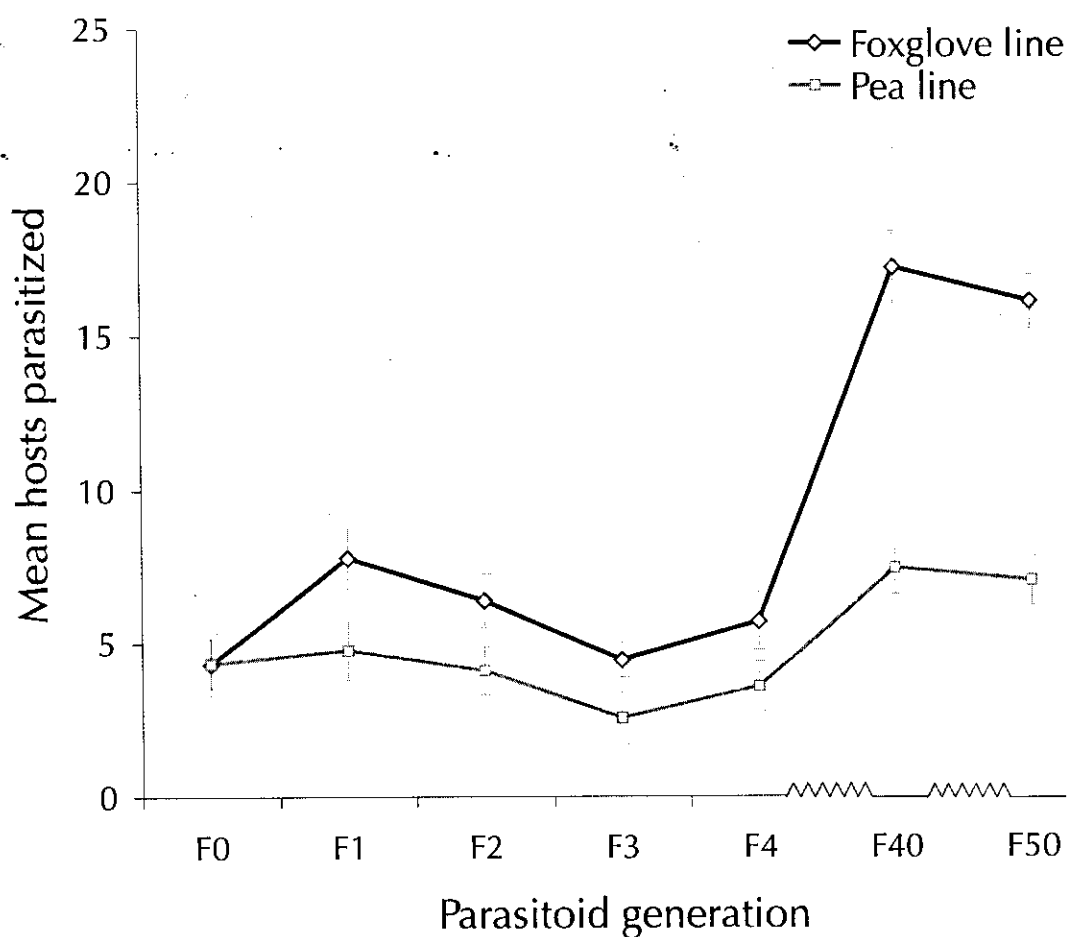
Windsor, D. A. 1998. Most of the species on earth are parasites. *Int. J. Parasitology.* 28:1939-1941.

**Table 2.1** Mean latency period (seconds) prior to oviposition for parasitoid females from each selection line assayed on natal and non-natal host species.

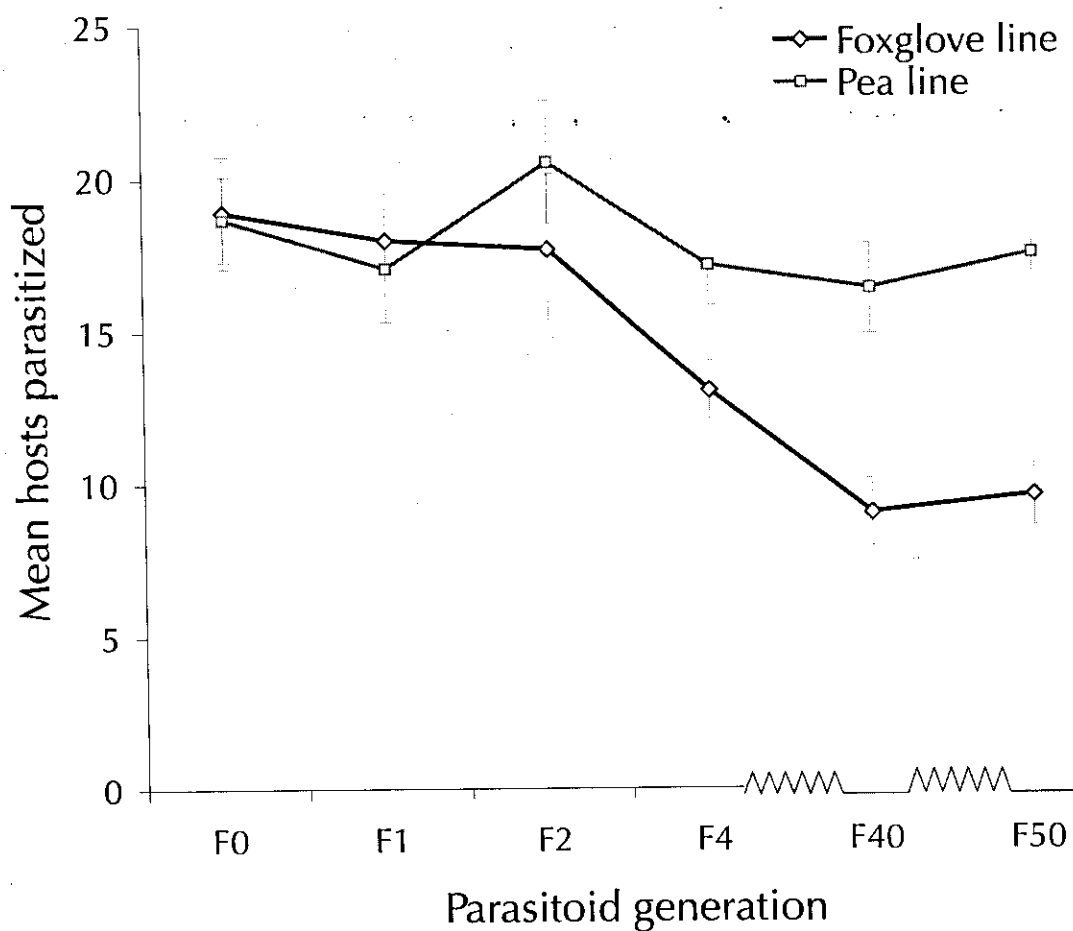
SELECTION HOST	ASSAY HOST	
	Foxglove	Pea
Foxglove	18.67±6.87	66.61±7.47
Pea	55.08±8.41	17.47±4.29



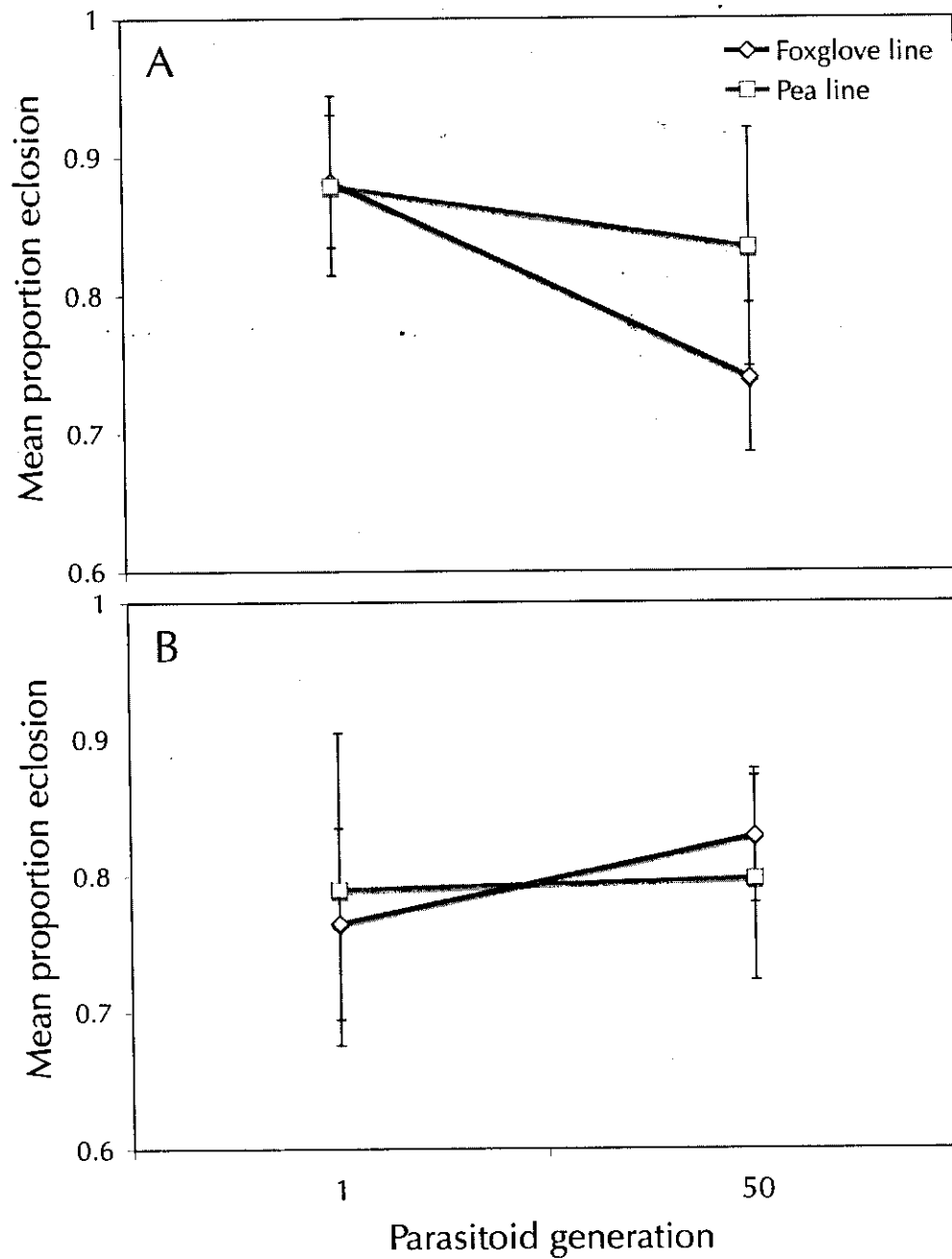
**Figure 2.1** Mean hosts parasitized per parasitoid generation for parasitoids assayed on 40 2<sup>nd</sup> instar foxglove aphids, *A. solani*. Black line with diamond markers represents foxglove selection line (F-line) and grey line with square markers represents pea aphids, *A. pisum*, selection line (P-line) parasitoids. Black bars signify standard error for each parasitoid generation.



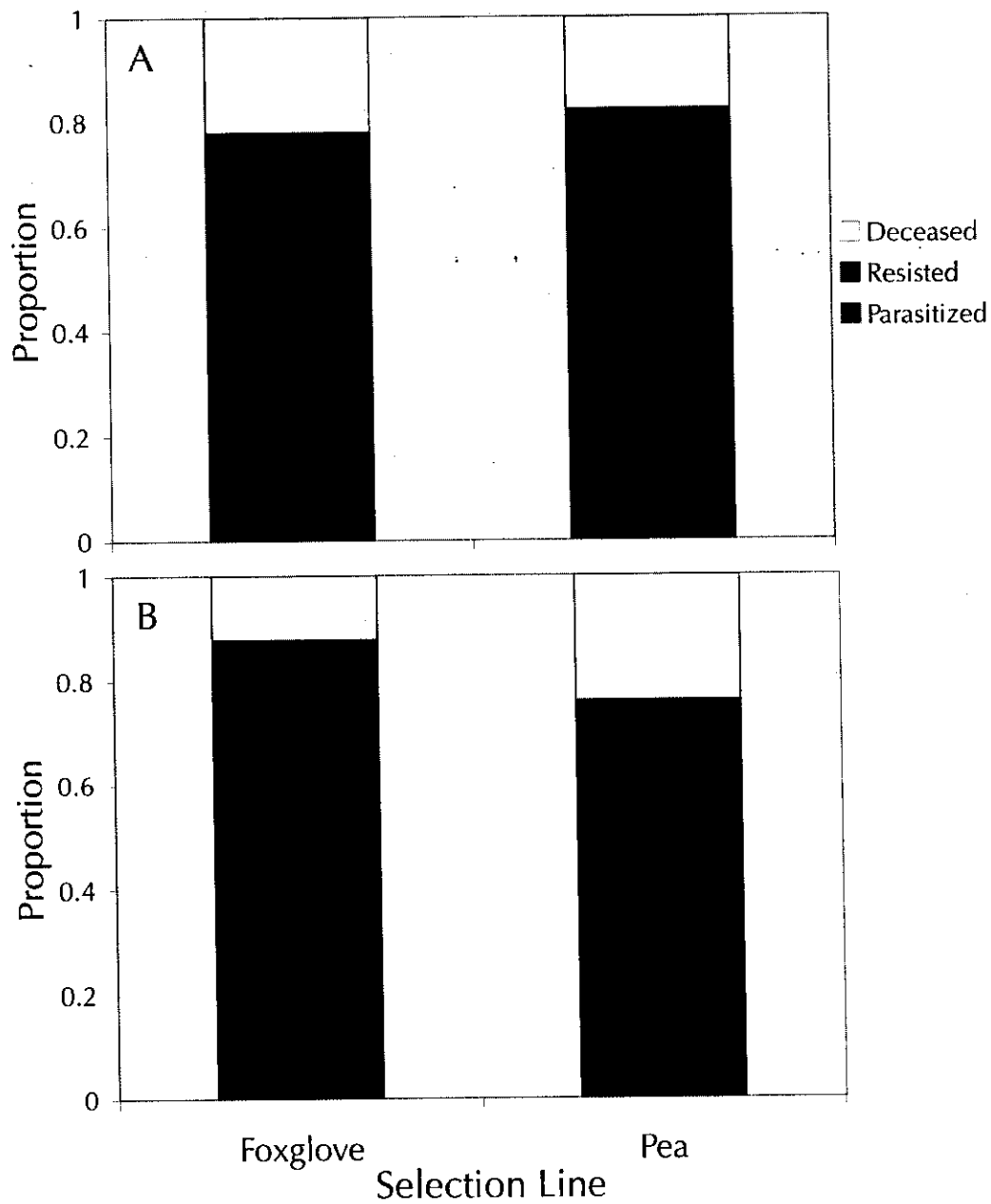
**Figure 2.2** Mean hosts parasitized per parasitoid generation for parasitoids assayed on 40 2<sup>nd</sup> instar pea aphids, *A. pisum*. Black line with diamond markers represents foxglove aphid, *A. solani*, selection line (F-line) and grey line with square markers represents pea aphid selection line (P-line) parasitoids. Black bars signify standard error for each parasitoid generation.



**Figure 2.3** Mean proportion eclosion from parasitized pea (A) and foxglove (B) aphids at generations 1 and 50 for F-line (black diamonds) and P-line (grey squares) parasitoids. Black bars signify standard error.



**Figure 2.4** Proportion of pea (A) and foxglove (B) aphids parasitized (grey), resisted (black) or deceased (white) at 24 hours after receiving a single oviposition from either F-line or P-line parasitoids.



# CHAPTER 3: OVIPOSITION PREFERENCE REDUCES REPRODUCTIVE PERFORMANCE IN THE GENERALIST PARASITOID *APHIDIUS ERVI*<sup>1</sup>

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### 3.1 Abstract

The reproductive success of female parasitoids is dependent on their ability to accurately assess the suitability of a host for larval development. For generalist parasitoids, which utilize a broad range of species and instars as hosts, a set of assessment criteria determines whether a host is accepted or rejected. The suitability of a host, however, can only be imperfectly assessed by the female parasitoid, which can result in the selection of lesser quality hosts for oviposition. In this study we explored the disparity between host quality and host preference using the generalist koinobiotic parasitoid *Aphidius ervi* and the host *Aulacorthum solani*, the foxglove aphid. The second instar hosts produced the highest level of reproductive success while third and fourth instars resulted in a substantially reduced reproductive performance. When given a choice of host instars, parasitoids preferred the older hosts for oviposition disregarding their reduced suitability for larval development. Results are discussed in context of mechanisms involved in *A. ervi* host selection and biases in the criteria used to assess hosts that may arise when parasitoids transfer host species between generations.

### 3.2 Introduction

Parasitoid larvae rely on their mother's ability to accurately assess the suitability of hosts for progeny development. This is because the immature stages of hymenopteran parasitoids are completely dependent on the host insect for nutrients (Mackauer and Sequeira 1992a). Although all insects that are considered to be hosts must be suitable for parasitoid development (Salt 1940; Mackauer 1973), host species and developmental stages vary in the quality and quantity of resources they provide to the immature parasitoid (Vinson and Iwantsch 1980; Sequeira and Mackauer 1992b). Where parasitoids attack multiple host species and several life stages of each host, the nutritive quality for larval development varies with host size and species (Vinson and Iwantsch 1980). Therefore, all else being equal, female parasitoids should select the host stage or host species that maximizes their reproductive performance (Charnov and Skinner 1985; Sequeira and Mackauer 1994).

Parasitoids commonly use host size as an indicator of host quality (van Alphen and Jervis 1996). In koinobiotic species, whose larvae feed while hosts develop, host growth during parasitoid larval development produces a size-fitness relationship that varies with host instar, rather than by size at parasitization (Sequeria and Mackauer 1992b). Studies on koinobiont parasitoids have shown that host instar selection strategies commonly differ from idiobiotic species and that the largest host may not necessarily be the host that confers the greatest fitness return for the ovipositing female parasitoid (Sequeria and Mackauer 1994; Harvey et al. 1994; Chau and Mackauer 2001a).

Host value can be defined as the perceptual assessment of a host's quality by a parasitoid female (Mackauer et al. 1996). After a female has encountered a potential host, she evaluates its suitability and nutritive quality for offspring development by antennation and ovipositor probing (Mackauer et al. 1996). An erroneous value may be assigned to a host if the parasitoid is inexperienced with that particular species or when ranking hosts with imperceptible physiological defenses against parasitism, in which case a decision to oviposit can significantly reduce the offspring's chance of survival (Henter and Via 1995; Kraaijeveld et al. 2002; Harvey and Strand 2002). If the host is perceived to exceed a female's response threshold and is deemed suitable for larval development it is accepted and an egg is deposited. For koinobiotic parasitoids, hosts continue to grow and molt normally until the larval parasitoid reaches a critical size where upon host growth decreases due to destructive feeding and competition for resources. Growth rates of parasitized hosts and parasitoid larvae are highly dependent on the relative size of the host species and the developmental stage of the host at the time of oviposition (Sequeira and Mackauer 1992a).

*Aphidius ervi* is a generalist koinobiotic parasitoid that occupies a large continuous range throughout Europe and Asia where it has been reported to parasitize over 15 species of aphids with different degrees of success (Mackauer and Stary 1967; Cameron et al. 1984; Vinson et al. 1998). The foxglove aphid, *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae), is readily attacked by *A. ervi* in both natural (Dunn 1949) and agricultural settings but produces few offspring (Hajimu and Eri 2000). The reasons for this reduced fitness were investigated using three separate experiments that explored the attack preference and reproductive fitness returns for each instar attacked.

More specifically, we asked if the preferred instar for oviposition reflects the optimal host for maximizing reproductive success.

### **3.3 Materials and Methods**

#### **3.3.1 Insect cultures**

All insect colonies were maintained at  $20 \pm 2.0^\circ \text{C}$ , 50-60% RH and a L16:D8 photoperiod for approximately four months prior to experimentation. Colonies of *A. ervi* were generated from colonies at the Southern Crop Protection and Food Research Centre, Agriculture Canada (London). Parasitoids were reared in 30 cm square plexiglass cages on *A. pisum*, from stock colonies at Simon Fraser University, feeding on *Vicia faba* L. (cv. 'Broad Windsor'). Plants and aphids were added on a weekly basis to sustain the parasitoid population. Mummies were clipped from plant material and left to develop in separate containers. Test parasitoids were always 2-3 day-old females that had been given continuous access to dilute honey, water and males. Two days was determined to be sufficient time to ensure that mating occurred. Colonies of *A. solani* were transferred from colonies that were maintained on pepper at Pacific Agri-food Research Centre (Agassiz) and reared on *V. faba* L. approximately eight months prior to their use in experiments. The aphid life-cycle consists of 4 instars, which correspond to 24, 48, 75 and  $120 (\pm 4)$  h for *A. solani*. Experiments were conducted between 9am-1pm under the same conditions used for rearing. Each parasitoid was used only once then discarded.

#### **3.3.2 Survivorship following oviposition and frequency of egg laying event**

This experiment served as a control to determine how frequently stings resulted in an oviposition and to establish whether eggs were laid at the same rate in second versus fourth instar aphids. The experiment also assessed the effect of a single sting on the two host's mortality. Naïve, mated female parasitoids were given access to a single second or a single fourth instar aphid inside a gelatin capsule. Females were allowed to attack and sting the aphid only once, then the aphid was removed and replaced by the other instar.



Each female alternated through as many second and fourth instar aphids to a maximum of 10 second and 10 fourth instar aphids. Trials were stopped if two minutes elapsed without an attack. Only parasitoids that stung a minimum of one second and one fourth instar were included in the analysis. The experiments were repeated with 34 parasitoids over two weeks, using two successive generations of parasitoids. The first aphid presented to the parasitoid was alternately a second or fourth instar. In this way, 122 second and 126 fourth instar aphids were stung in total by the 34 parasitoids. Stung aphids were placed on broad bean leaves to develop until mummies formed. Survivorship of each instar group was compared over a 72 hour period using a repeat analysis MANOVA test, where the proportion remaining alive was recorded at 24, 48 and 72 hour periods. The survivorship of stung second and fourth instars was compared using a MANOVA test. Groups of 10 second (n=12) and 10 fourth (n=12) instar *A. solani* that had not been subject to an attack were used as an internal control to determine any differences in survivorship between the two instars caused by handling (MANOVA).

Due to the difficulty of accurately locating the parasitoid eggs within each host, the presence of *A. ervi* larvae at 72 hours was used to determine if eggs were laid equally in both second and fourth instars. This did not take into account the possibility of eggs being resisted by either host instar prior to hatching, however due to the relatively high levels of larvae in both hosts it was used as a rough estimate for the frequency of oviposition. Preliminary dissections revealed that *A. ervi* eggs hatched approximately 72 hours after being laid inside *A. solani* nymphs. Dissections were carried out on both second and fourth instar *A. solani* that had been subject to a single oviposition by *A. ervi*. Approximately half of the 59 second and 57 fourth instar aphids that were alive at 72 hours were dissected. A contingency table was used to determine heterogeneity of larval presence between instars. The remaining aphids were left to develop on broad bean leaves. Differences in the proportion of aphids mummified and in survivorship and mortality of second and fourth instar *A. solani* were compared using a two by three contingency table.

### 3.3.3 Instar Preference

The preference of *A. ervi* for different instar nymphs of *A. solani* was investigated by allowing naïve, mated females access to second, third and fourth instar aphids at a 10:10:10 ratio. In order to ensure that the preference was not frequency dependent the same experiment was repeated with two different ratios of host instars that the parasitoids might encounter in a natural setting. The additional ratios were 14:8:8 and 18:6:6 second, third and fourth instar *A. solani* respectively. Aphids were placed on a broad bean leaf disk on a damp piece of filter paper in a 3 cm Petri dish. Each female *A. ervi* was given a fifteen-minute foraging period on a group of aphids, during which the number of successful and unsuccessful attacks on each instar was recorded. An attack was recorded as successful if the aphid was grappled and probed with the female's ovipositor. Attacked aphids were not replaced and superparasitism was permitted. Trials were repeated on five different days using three separate generations of parasitoids. Trials were replicated 35, 29 and 28 times for the 10:10:10, 14:8:8 and 18:6:6 ratios, respectively. Instar preference was established by comparing the actual number of each instar probed against the null hypothesis of no preference, for each ratio of second third and fourth instars tested using a Chi-square Goodness-of-Fit test. To justify pooling the attack counts across parasitoids for each ratio, a contingency table was used to determine if there was an effect of parasitoid individual on preference.

#### **3.3.4 Impact of *A. solani* host instar on reproductive fitness of *A. ervi***

The effect of *A. solani* instar on the reproductive fitness of *A. ervi* was tested by allowing a single, mated, naïve female access to thirty second, third or fourth instar aphids for 1h. First instar *A. solani* were not included in the trials as they were typically ignored by *A. ervi* females (Lee Henry pers. obs.). The 30 *A. solani* were presented on an excised bean leaf in a 9 cm Petri dish. To keep the leaf material fresh, excised petioles were inserted into a small water-filled glass vial capped with parafilm to prevent leakage. After one hour in the Petri dish the parasitoid was removed and aphids were left on the leaf for parasitoid development and emergence. A 1cm<sup>3</sup> damp cotton wick was placed in each Petri dish to keep the relative humidity levels at approximately 50-60%. The number of hosts parasitized (mummies formed per thirty aphids), days until

mummification (checked twice daily), proportion of emergence from mummies, and the sex ratio, weight and hind tibia length of offspring were determined, and served as proxies for reproductive fitness (Roitberg et al. 2001). Parasitoids were dried at 60°C for 48 hours. Whole insects were weighed individually on a Cahn<sup>TM</sup> microbalance. Hind tibia were measured by removing the whole hind leg, mounting it on glass slides and measuring the tibia with a calibrated ocular micrometer on a dissecting microscope at 20X magnification. Thirty replicates were performed in total for each instar treatment on three separate dates. Sample populations for proportions mummified, days until mummification, proportion emergence and proportion female were not normally distributed. Therefore, variation among instars for the response variables noted above were analyzed using a non-parametric analysis of variance by ranks Kruskal-Wallis test. Hind tibia length and dry weight were analyzed using an ANOVA test. Data were analyzed using JMP 5.0 statistical software (SAS Institute, Cary, NC, USA).

### 3.4 Results

#### 3.4.1 Survivorship following oviposition and frequency of egg laying event

The proportion of aphids surviving decreased over time in both treatment and control groups (MANOVA,  $F_{3, 80} = 38.91$ ,  $p < 0.0001$ ). There was no difference in survivorship between second and fourth instar hosts in the control group (MANOVA,  $F_{3, 17} = 0.22$ ,  $p = 0.88$ ). The controls were therefore not included in the subsequent analysis. Mortality of second and fourth instar *A. solani* that had been subject to a single sting differed over time (MANOVA,  $F_{3, 61} = 2.81$ ,  $p = 0.046$ ) (Fig. 3.1). Dissections of the 27 second and 26 fourth instar *A. solani* that had been subject to a single sting revealed 16 and 19 *A. ervi* larvae in the second and fourth instar hosts respectively. There was no difference in the presence of *A. ervi* larvae between host instars ( $\chi^2$  test:  $df = 1$ ,  $\chi^2 = 1.13$ ,  $p = 0.29$ ). However, aphids that had been stung once and left to develop on broad bean leaves differed between instars in the proportion of mummies formed, aphid mortality and survivorship (2 X 3 contingency table:  $df = 2$ ,  $\chi^2 = 11.51$ ,  $p = 0.003$ ) (Fig. 3.2). Parasitoid larval arrestment occurred in most fourth instar aphids in the larval

developmental phase, prior to spinning a cocoon. Second instar aphids produced 13 mummies out of 32 stung aphids and fourth instars produced 2 mummies out of 31 aphids attacked (Fig. 3.2). Approximately 13 d after oviposition 10 adult wasps emerged from the second instar mummies. No adult parasitoids emerged from the fourth instar hosts.

### 3.4.2 Instar preference

It was determined that there was no difference in the preference of individual parasitoid females within each ratio tested (Contingency table: 10:10:10,  $\chi^2 = 52.92$ , df = 64,  $p = 0.83$ ; 14:8:8,  $\chi^2 = 71.65$  df = 56  $p = 0.08$ ; 18:6:6,  $\chi^2 = 57.56$ , df = 56,  $p = 0.42$ ). Therefore the individual counts were pooled for all parasitoids within each ratio to determine overall instar preference. When attacking *A. solani*, *A. ervi* demonstrated a preference for the older and larger aphid instars (Fig. 3.3). The number of probes for each instar was different from the null hypothesis of no preference for all three ratios of second, third and fourth instar aphids that were presented to the parasitoids ( $\chi^2$  analysis: 10:10:10,  $\chi^2 = 60.71$ , df = 2,  $p < 0.0001$ ; 14:8:8,  $\chi^2 = 111.47$  df = 2  $p < 0.0001$ ; 18:6:6,  $\chi^2 = 88.15$ , df = 2,  $p < 0.0001$ ). Superparasitism of preferred host instars was occasionally observed in this experiment, which added to the conservative nature of the test as some parasitoids preferentially attacked larger aphids multiple times rather than accepting smaller hosts.

### 3.4.3 Impact of *A. solani* host instar on *A. ervi* reproductive fitness

A greater number of mummies formed in second than in third or fourth instar *A. solani* ( $\chi^2_{0.05, 2} = 29.42$ ,  $p < 0.0001$ ) (Table 3.1). Mummies formed approximately 8 to 9d following attack with second instar hosts taking longer to mummify than third and fourths ( $\chi^2_{0.05, 2} = 24.89$ ,  $p < 0.0001$ ) (Table 3.1). Approximately 14 to 15d after attack adult parasitoids emerged from the mummies. There was no difference in the proportion of adults that emerged between instars ( $\chi^2_{0.05, 2} = 4.99$ ,  $p = 0.08$ ) (Table 3.1). The mean proportion of females differed between instars ( $\chi^2_{0.05, 2} = 8.75$ ,  $p = 0.01$ ), with younger instar hosts producing fewer females (Table 3.1). However, these results should be

viewed with caution as only a few individuals hatched out of fourth instar hosts, all of which were females. Dry weight of emerging parasitoids was not different for males or females between host instars (males; ANOVA;  $F_{1,48} = 0.14$ ,  $p = 0.71$ , females; ANOVA;  $F_{2,61} = 3.06$ ,  $p = 0.054$ ). There was however, a trend for female weight to increase with host instar which approached significance. A similar trend was observed for the hind tibia lengths, which was not significant for males or females (males; ANOVA;  $F_{1,48} = 0.31$ ,  $p = 0.58$ , females; ANOVA;  $F_{2,61} = 2.96$ ,  $p = 0.059$ ), but did approach significance for females.

### 3.5 Discussion

Over the past two decades, there has been considerable interest in the relationship between an insect mother's preference and her offspring's performance (Mayhew 2001). This is particularly true for work on herbivores where apparent maladaptive adult preference has been explained by a variety of mechanisms, including, adult survival (Sheirs et al. 2000), natural enemies, physiological and information state (Mangel and Roitberg 1989). In the case of parasitoids, much of the attention has focused on acceptance of low quality hosts via changes in acceptance thresholds as opposed to host preference for such hosts (Speirs et al. 1991). Where both preference and performance have been measured there has generally been concordance (e.g. Buitenhuis et al. 2004). Explanations for mismatch between preference and host suitability include, threat of hyperparasitoids (Ayal and Green 1993), host defense (Gerling et al. 1990) and learning (Wardle and Borden 1991). Note: a lack of discrimination (e.g. Rivero 2000) among different quality hosts requires a different explanation than host preference i.e. the former deals with an acceptance threshold whereas the latter deals with rank order. The preference phenomenon is poorly studied for parasitoids.

When given access to second, third or fourth instar *A. solani*, *A. ervi* produced the greatest proportion of mummies from the second instar hosts (0.18) and the lowest from the 4<sup>th</sup> instar hosts (0.01) (Table 3.1). These results indicate that the quality of *A. solani* nymphs for *A. ervi* development decreases with increasing host size and age. This

suggests that either the third and fourth instar hosts are better able to generate a physiological or behavioural defense response to parasitism or that the older hosts, having completed their growth phase, do not provide sufficient future resources for the developing parasitoid. An increase in parasitoid mortality in older instar hosts has been demonstrated in several aphid-parasitoid systems (Walker and Hoy 2003; Chow and Mackauer 2001a) and in other koinobiont-host interactions (Harvey and Strand 2002). Mortality in fourth instar *A. solani* increased dramatically past 72 hours once the egg had hatched and the parasitoid entered the larval phase (Fig. 3.1). The increased mortality combined with the reduced rate of mummification (Fig. 3.2) demonstrated that the fourth instar hosts were not suitable for *A. ervi* larvae to complete development through the destructive feeding stage to reach pupation.

The mean number of days until mummification differed between instars, with the parasitoids in the smaller aphid hosts developing at a slower rate. In certain koinobiotic parasitoid species, including *A. ervi*, larvae possess developmental plasticity that allows them to spend extended periods as first-instars when developing in small or poor quality hosts (Smilowitz and Iwantsch 1973; Sato 1980; Sato et al. 1986). Hosts can be consumed quickly in order to decrease parasitoid larvae development time (Harvey and Strand 2002); however, a rapid developmental time is only possible in hosts that initially exceed the minimum required nutrient reserves to support development of the parasitoid larvae. In situations where hosts fall below the minimum required nutrient level a prolonged developmental time is necessary in order to acquire additional resources as hosts develop (Sato et al. 1986).

Sequeira and Mackauer (1992b) demonstrated that the developmental rate of *A. ervi* larvae remained relatively constant among host instars, when parasitizing *A. pisum*, with the size of the emerging wasps increasing with older instar hosts. When parasitizing *A. solani*, a host that is approximately half the size of *A. pisum*, there was no effect of host instar on size of *A. ervi* offspring. There was however, a prolonged larval developmental time within second instar *A. solani* hosts, which suggests that *A. ervi* larvae may have required more time to gain additional nutrients in order to complete development. Preliminary studies demonstrated that *A. ervi* larvae required significantly more time to develop into mummies in *A. solani* ( $8.43d \pm 0.21$ ) than they do in the much

larger host, *A. pisum* ( $7.30d \pm 0.06$ ), with the emerging offspring being two to three times larger when reared on *A. pisum* ( $187\mu g \pm 16$ ). In populations of *A. ervi* in Chile, parasitoid larvae develop at a considerably slower rate and produce significantly smaller offspring when reared on the smaller wheat aphid *Sitobion avenae* (Fabricius) than from the larger host *A. pisum*, nonetheless there is no difference in reproductive rates between the two populations (Daza-Bustamante et al. 2003). A significant trend was also observed in the sex ratio of the adults produced in different host instars that corresponds with results found in other host-parasitoid system (Charnov et al. 1981; Godfray 1994; Ueno 1998), with the larger aphid hosts producing more females than the smaller hosts.

When given a choice between second, third and fourth instar *A. solani* the parasitoids showed a clear preference for attacking the older aphid instars (Fig. 3.3). This preference was exhibited in all three ratios of second, third and fourth instar *A. solani* that were presented to the parasitoids, with multiple attacks occurring on older hosts in preference to the youngest. A preference for the oldest instars of a host is uncommon in most aphid parasitoid systems (Tang et al. 1996; Hopper 1986; Sharmila and Rajendra 1999; Chau and Mackauer 2001a; Chau and Mackauer 2001b; Perdakis et al. 2004). Optimality theory (Stephens and Krebs 1986; Bell 1991) as applied to host selection by foraging parasitoids predicts that, when given a choice of hosts, female parasitoids should preferentially oviposit in the host that will result in the greatest fitness returns. In idiobiotic parasitoid systems, host size is the primary index of quality used by females when selecting hosts with females receiving greater fitness returns when ovipositing in larger hosts (Charnov et al. 1981). However, for koinobiotic parasitoids, which grow and develop with their hosts, selecting the oldest host instars limits the future resources available to the developing larvae. Aphid parasitoids are koinobionts that can attack all stages of their hosts including the adults. Therefore, by choosing to oviposit in the adult stage of a host the possibility of host death before the larvae has completed development poses a considerable risk to the parasitoid larvae. Host selection by koinobiont parasitoids should therefore favor strategies that maximize progeny (and host) survival to adulthood first and other traits secondarily (Harvey and Strand 2002).

The attack preference of a parasitoid is not the absolute value of suitability as upon insertion of the ovipositor the parasitoid may decide not to lay an egg if the host is

deemed unsuitable, through receptors found on the ovipositor (Mackauer et al. 1996). In order to validate that the preference for larger *A. solani* was equivalent to the parasitoids assessment of a high host value, the frequency of egg deposition for smallest and largest instars attacked was assessed. Dissections revealed that when given second and fourth *A. solani* instars in alternating sequence, eggs were laid at an equal rate in both host stages. This demonstrates that the host instar preference in choice settings was an accurate indicator of the parasitoids assessment of host value. Furthermore, this preference for third and fourth instars combined with a reduced survivorship in fourth instar hosts (Fig. 3.1 and 3.2) indicated that the female parasitoids were making an inaccurate assessment of the host's suitability for larval development.

The assessment of a host's value is governed by a parasitoid's recent experience, physiological state and possible trade offs between risks of her own and her offspring's mortality (Mackauer et al. 1996; Morris and Fellowes 2002). Furthermore, it has been suggested that larval conditioning may influence host selection decision to the host in which the parasitoid was reared (Powell and Wright 1988; Hastings and Godfray 1999). The parasitoid population used in these experiments was reared on *A. pisum*, and then transferred to *A. solani*. A reduced reproductive performance on a particular host size or species has been demonstrated in several host transfer studies where parasitoids switch host species between generations (Powell and Wright 1988; Daza-Bustamante et al. 2003). Enzymatic conditioning for the host in which the parasitoid developed has often been blamed for this reduced reproductive performance (Powell and Wright 1988; Hastings and Godfray 1999). We suggest another possible mechanism for reduced rates of parasitism in newly transferred hosts. Our results indicate that the wasps could increase their reproductive performance considerably if they did not preferentially attack the larger, less suitable, *A. solani* instars. It is possible that the set of criteria utilized by koinobiotic parasitoids when assessing a host's value is not specific for each host species but instead a general rule that does not function optimally in every situation. An observation that the preferred fourth instar *A. solani* are approximately the same size as the preferred second instar *A. pisum*, a host that yields a high reproductive potential, reinforces this hypothesis. Possible explanations for the observed erroneous size preference in naïve *A. ervi* may reside in contact with mummy casings or a predisposition



for a particular host size due to some aspect of the parasitoids morphology such as adult size or antennal length. *Aphidius ervi* attacks a broad range of host species of various sizes in nature (Hajimu and Eri 2000). It is interesting that in host-transfer experiments using *A. ervi*, low levels of mummification are observed when transferring to a smaller host species, such as with *A. pisum* to *S. avenae*, but normal levels of parasitism are seen when transferring from the smaller to the larger species (Powell and Wright 1988; Daza-Bustamante et al. 2003). This suggests that host transfer in parasitoids may be governed by more than just enzyme incompatibility and that the behaviour of host selection may play an important role. Future research is aimed at addressing the mechanisms behind host size preference for koinobiotic parasitoids in successive generations to determine whether rates of parasitism will change due to a shift in behavioural responses or through selection of parasitoids that are more enzymatically adapted to develop in alternate hosts.

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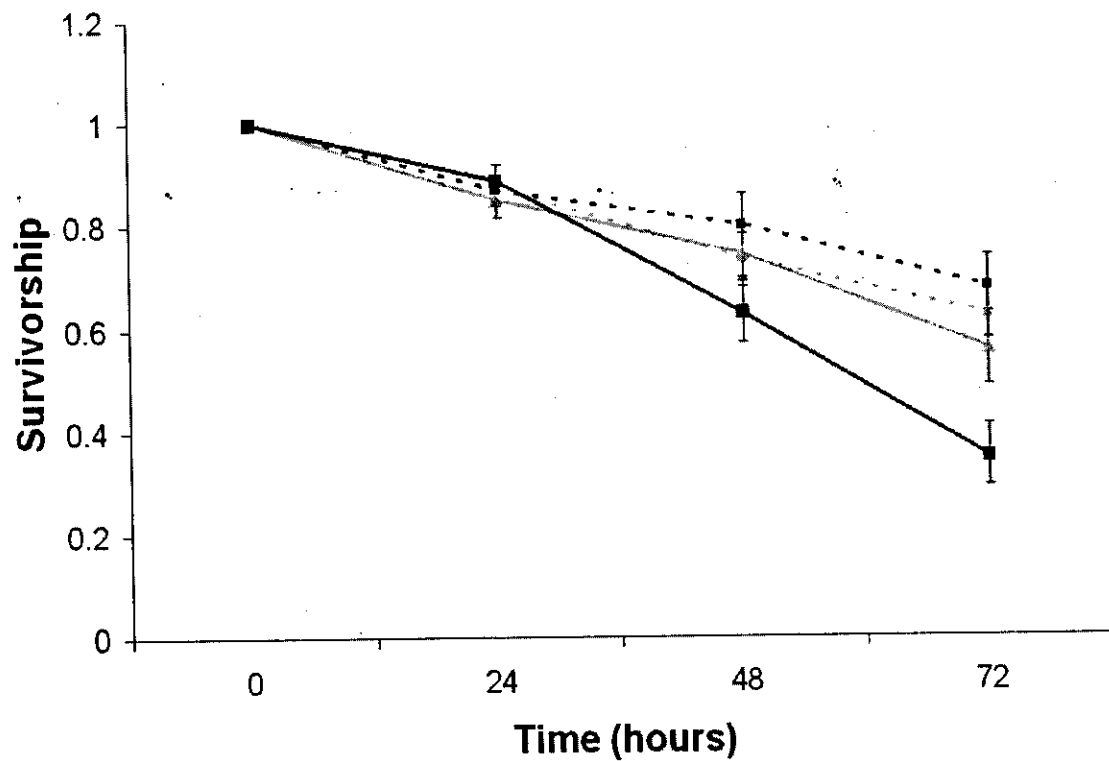
**Table 3.1** Proportion mummification, days until mummification, proportion emergence from mummies and sex ratio (mean  $\pm$  SE) of *A. ervi* progeny emerging from thirty 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> instar *A. solani*

Host instar	N	Mean proportion Mummification	Mean days until Mummification	Mean proportion emergence	Mean proportion females
2	30	0.18 $\pm$ 0.03 <sup>a</sup>	8.87 $\pm$ 0.09 <sup>a</sup>	0.87 $\pm$ 0.03 <sup>a</sup>	0.46 $\pm$ 0.07 <sup>a</sup>
3	30	0.09 $\pm$ 0.02 <sup>b</sup>	8.22 $\pm$ 0.08 <sup>b</sup>	0.76 $\pm$ 0.05 <sup>a</sup>	0.70 $\pm$ 0.08 <sup>b</sup>
4	30	0.01 $\pm$ 0.01 <sup>c</sup>	8.20 $\pm$ 0.45 <sup>b</sup>	0.50 $\pm$ 0.00 <sup>a</sup>	1.0 $\pm$ 0.00 <sup>b</sup>

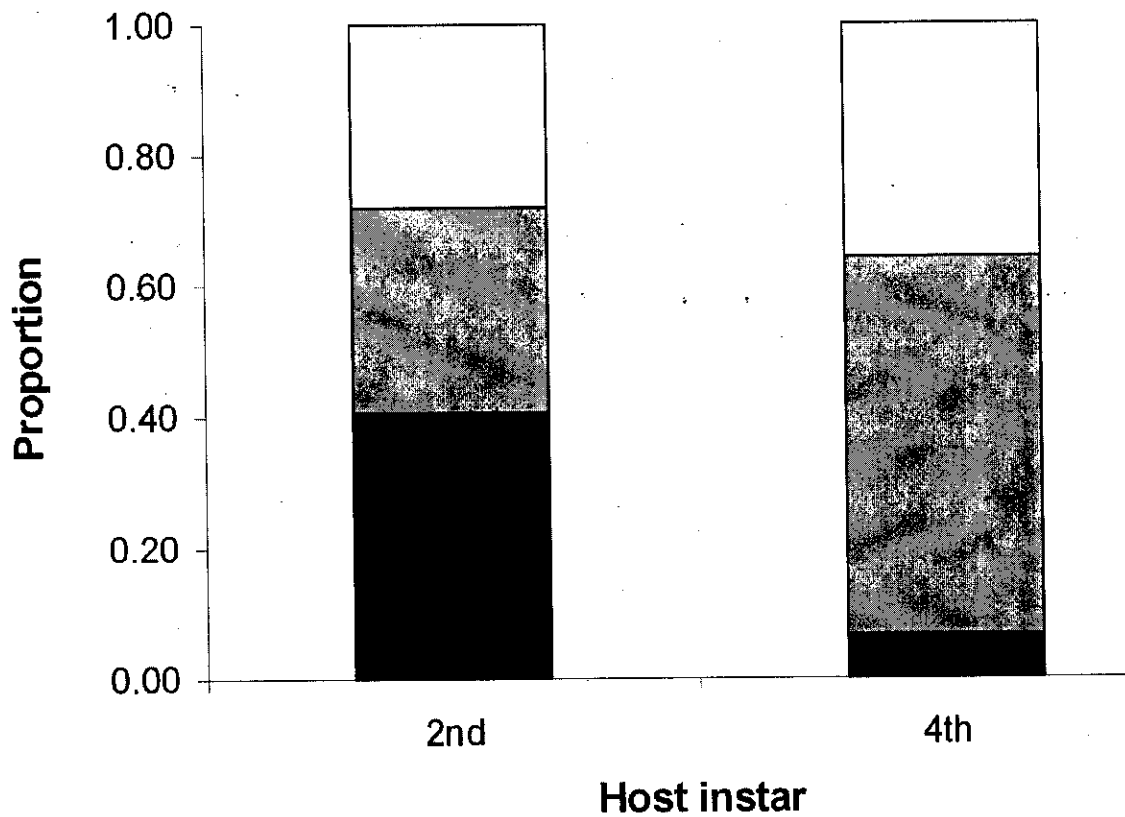
Mean results followed by the same letter are not statistically significant  
(Student's t test:  $\alpha=0.05$ )



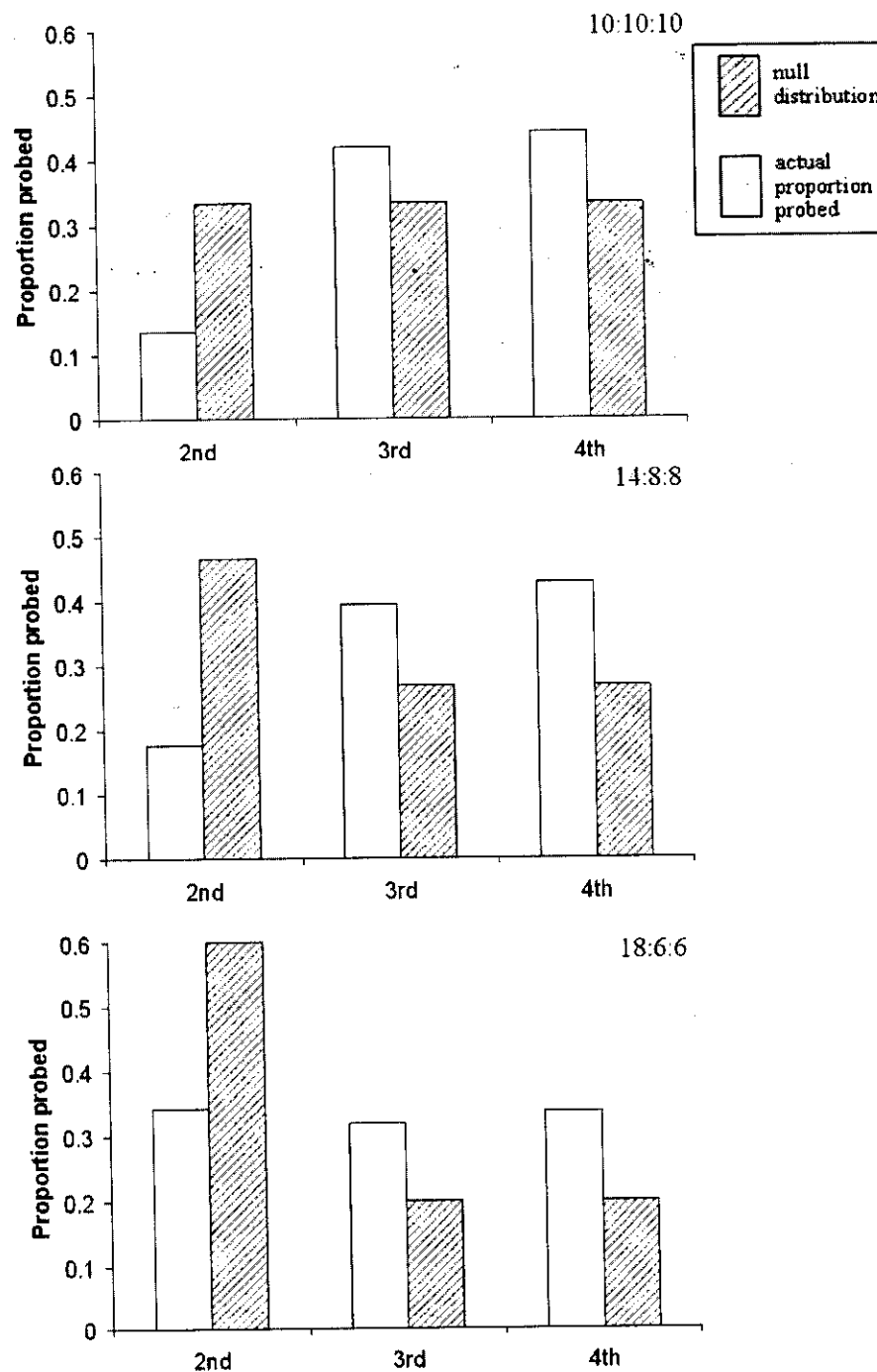
**Figure 3.1** Survivorship, displayed as proportion remaining, of 2<sup>nd</sup> (grey) and 4<sup>th</sup> (black) instar *A. solani* over a 72 hour period which had been subjected to a single oviposition from *A. ervi*. Hatched line represent control populations of 2<sup>nd</sup> and 4<sup>th</sup> instar *A. solani* that had not been subject to attack.



**Figure 3.2** Status of 32, 2<sup>nd</sup> and 31, 4<sup>th</sup> instar *A. solani* left to develop for 9 days on broad bean leaves after receiving a single oviposition by *A. ervi*. Black represents proportion of aphids mummified, grey represent aphid mortality and white represent aphid survivorship.



**Figure 3.3** Instar oviposition preference of *A. ervi* when attacking 10:10:10, 14:8:8 and 18:6:6, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar *A. solani* individuals respectively. White bars represent actual proportion of each instar probed, hatched bars represent theoretical null distribution of probes if no preference exhibited.



## **CHAPTER 4: COVARIANCE OF PHENOTYPICALLY PLASTIC TRAITS INDUCES AN ADAPTIVE SHIFT IN HOST SELECTION BEHAVIOUR <sup>4</sup>**

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## 4.1 Abstract

Flexibility in adult body size allows generalist parasitoids to utilize many host species at a cost of producing a range of adult sizes. Consequently, host selection behaviour must also maintain a level of flexibility as adult size is related to capture efficiency. In the present study we investigated co-variance of two plastic traits – size at pupation and host size selection behaviour – using *Aphidius ervi* reared on either *Acyrtosiphon pisum* or *Aulacorthum solani*, generating females of disparate sizes. Natal host was shown to change the ranking of perceived host quality with relation to host size. Parasitoids preferentially attacked hosts that corresponded to the size of the second instar of their natal host species. This resulted in optimal host selection behaviour when parasitoids were exposed to the same host species that they emerged from. Parasitoid size was positively correlated with host size preference indicating females use relative measurements when selecting suitable hosts. These co-adapted gene complexes allow generalist parasitoids to effectively utilize multiple host species over several generations. However, the fixed nature of the behavioural response, within a parasitoid's lifetime, suggests that these traits may have evolved in a patchy host species environment.

## 4.2 Introduction

Phenotypic plasticity is defined as the ability of an organism to change its phenotype in response to varying biotic and abiotic conditions (Agrawal 2001). Although, phenotypic plasticity refers to changes in chemistry, physiology, development, morphology or behaviour, the main focus of studies involving phenotypic plasticity have tended to concentrate on changes in morphology, physiology and life history traits (DeWitt and Scheiner 2004). Conceptually, plasticity in behaviour is very different from other attributes in that behaviour is a labile trait that can change in expression several to many times within an organism's lifetime. By contrast, developmental plasticity is much more rigid in that once a form has been adopted it can be irreversible, or at least, slow to revert (Tufto 2000). However, variation in behaviour due to adaptive differences between populations may restrict behavioural flexibility, within a given environment,

resulting in a mosaic of fixed policies across ecosystems (Foster 1999). Plastic traits often react in conjunction forming a correlated response to environmental change. When the performance of a trait is conditional on the response of another, phenotypic linkages may form that connect trait responses through adaptive evolution.

The adaptive plasticity hypothesis refers to the evolution of plastic phenotypes that maximize fitness in a variable environment (Dudley and Schmitt 1996). Maintenance of fitness upon entering into a novel environment is almost always a result of purely phenotypic change, reflecting a species' plasticity, which translates into genetic differences later by natural selection (Price et al. 2003). High levels of plasticity often coincide with generalism, given that generalist species are thought to have evolved to tolerate a greater breadth of environmental heterogeneity (reviewed in Kassen 2002). When a single species occurs over a range of ecosystems, diverged populations may exhibit very different phenotypes that are adapted to a discrete set of environmental conditions. This is a phenomenon that has been well documented in generalist herbivores locally adapted to different host plants (Simon et al. 2003; Funk et al. 2002). Diverged populations have also been shown to exhibit biases in host, habitat or prey preference, which are factors that contribute to reduced gene flow between populations (Vos and Vet 2004; Funk and Bernays 2001).

Host-seeking generalist parasitoids function within a complex environment where they may encounter a diverse number of host species varying in size, defensive capabilities and quality for offspring development. Furthermore, parasitoids also have to choose a suitable developmental stage within each host species as instars provide another level of resource and defensive variability (Gerling et al. 1990). When parasitoid species occur over a wide geographic range, divergent populations can form that are adapted to survive on the sympatric host species that is most prevalent in their region (Vaughn and Antolin 1996). Studies have demonstrated that the same species of parasitoid from different populations can vary in morphology (Biron et al. 2002) and behaviour (Kraaijeveld et al. 1995), which has been attributed to a combination of selective pressures on physiological and behavioural traits that over time result in local adaptation (Thompson 1994). Although, several studies have demonstrated the variability in traits

expressed by parasitoids across populations, few studies have investigated whether plasticity in these traits function in an adaptive manner.

*Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) is a generalist aphid parasitoid that inhabits a large continuous range throughout Europe and Asia where it has been reported utilizing over 15 host species (Mackauer and Sary 1967). Studies have shown that *A. ervi* expresses plasticity in several traits including developmental time and adult size (Sequeira and Mackauer 1992a). Adult size is a highly flexible trait in many generalist parasitoids that is conferred by host size (Nicol and Mackauer 1999). This allows aphid parasitoids to utilize many host species and often all instars within a particular host, at a cost of producing a range of adult sizes (Daza-Bustamante et al. 2003). The four aphid instars, or developmental stages, offer discrete levels of resources to developing parasitoid larvae. Young aphid instars have fewer available resources, which often results in female parasitoids allocating proportionately more sons to the lower quality 1<sup>st</sup> instar hosts (Charnov 1982). In contrast, adult aphids are large enough for parasitoid development, however the larva must compete with the host's embryos for resources and adult aphids are thought to possess greater physiological resistance to parasitism (Colinet et al. 2005; Walker and Hoy 2003). This can result in high larval or pupae mortality when larvae develop in older aphids (Walker and Hoy 2003; Colinet et al. 2005; Chau and Machauer 2001; Henry et al. 2005). For koinobiotic aphid parasitoids (i.e. hosts continue to grow after being parasitized), second and third instar hosts typically represent the most suitable developmental stage for harboring larvae until pupation (Ranking: 2<sup>nd</sup>>3<sup>rd</sup>>1<sup>st</sup>>4<sup>th</sup>) (Colinet et al. 2005). When attacking sympatric host species parasitoids can distinguish between high and low quality host instars and preferentially oviposit in higher quality hosts (Mackauer et al. 1996, Godfray 1994). However, when confronted with novel host species, females may make incorrect decisions by preferring lower quality host instars (Henry et al. 2005). To the authors' knowledge, it has never been demonstrated how koinobiotic parasitoids determine which instars are most suitable for oviposition. Furthermore, when confronted with multiple host species, of dissimilar sizes, it is unclear how parasitoids modify host selection behaviour (i.e. phenotypic plasticity) to accommodate the differences in the size of the optimal instar that occur between host species.

In the present study we investigated co-variance of plastic morphology (size at pupation) and host selection behaviour using parasitoids reared on two aphid species of dissimilar sizes, resulting in morphologically disparate parasitoid adults. Our objectives were to determine if natal host influenced the oviposition success rate and host size preference of females when choosing between instars of each host species, given that there is an approximate two-fold difference in the optimal sized host instar between the two aphid species. Results are discussed in the context of co-adapted plastic traits that are closely associated with productive fitness and the evolution of phenotypic linkage in variable environments.

## **4.3 Materials and Methods**

### **4.3.1 Study system and general procedures**

All insect colonies were maintained at  $22 \pm 2.0^\circ \text{C}$ , 50-60% RH and a L16:D8 photoperiod for approximately four months prior to experimentation. Colonies of *A. ervi* were generated from colonies at the Southern Crop Protection and Food Research Centre, Agriculture Canada (London). Parasitoids were reared in 30 cm square plexiglass cages on *A. pisum*, from stock colonies at Simon Fraser University, feeding on *Vicia faba* L. (cv. 'Broad Windsor'). Plants and aphids were added on a weekly basis to sustain the parasitoid population. Mummies were clipped from plant material and left to develop in separate containers. Test parasitoids were always 2-3 day-old females that had been given continuous access to dilute honey, water and males. Two days was determined to be sufficient time to ensure that mating occurred. Colonies of *A. solani* were transferred from colonies that were maintained on pepper at Pacific Agri-food Research Centre (Agassiz) and reared on *V. faba* L. approximately eight months prior to their use in experiments. The aphid life-cycle consists of four instars, which correspond to 24, 48, 75 and  $120 (\pm 4)$  h for *A. solani*. Experiments were conducted between 9am-1pm under the same conditions used for rearing. Each parasitoid was used only once then discarded.



#### 4.3.2 Host preference experimental design

Individual parasitoids from the two rearing host environments were exposed to a patch of *A. solani* or *A. pisum*. A patch consisted of 40 aphids, 10 of each of the four instars. Aphid instars were determined by age which corresponded to: 24-36h 1<sup>st</sup> instar, 48-60h 2<sup>nd</sup> instar, 72-84h 3<sup>rd</sup> instar and 96-108h 4<sup>th</sup> instar.

Parasitoids were allowed 10 minutes to forage in a Petri dish on either *A. solani* or *A. pisum*. During the foraging bout, behaviours relating to instar preference were recorded, including: antennation and oviposition attempts. Oviposition attempts were further classified as a successful oviposition, or failed oviposition. Antennations were defined as the parasitoid draping its antennae over the aphid, which often preceded the initial attack. Attempted oviposition consisted of a strike with the ovipositor regardless of whether contact was made with the aphid. Successful oviposition was defined as an attack that resulted in the ovipositor making contact with the aphid. Failed ovipositions were most commonly due to aphid defenses (aphids moving away, dropping, kicking or raising the body to avoid the ovipositor) and were defined by the parasitoid striking at but not making contact with the aphid. Differences in instar preference were determined for parasitoids from the two rearing populations and host species exposure groups using a Log Linear Type 3 GEE analysis, which takes into account pooling across females in each treatment group (SAS Statistical Package, version 8.2; SAS Institute, Cary, NC, USA). Missed attacks, by instar, were compared across rearing and exposure using a multivariate analysis with an identity response design (JMP Version 5.0; SAS Institute, Cary, NC, USA).

#### 4.3.3 Mechanism of host-size selection

To determine if adult parasitoid size influenced host size selection criteria we correlated parasitoid female body weight with preferred aphid size. This experiment was performed with parasitoids reared and exposed to pea aphids, to remove the effect of host species on size selection. In order to eliminate constraints on oviposition such as differences in instar defensive capabilities aphids were anesthetized with CO<sub>2</sub> for both the

pre-treatment and actual trials (Gerling et al. 1990). One to two minutes sealed in a dram vial with CO<sub>2</sub> anesthetized the aphids but not kill them. Individual females were given a pre-treatment of 8 aphids, 2 of each instars, in order to acclimatize the parasitoids to the range of host sizes. Upon stinging three hosts the aphids were removed from the vial and the parasitoids were then transferred to the trial arena. Parasitoids were then given access to sixteen pea aphids, selected to represent a full range of sizes, randomly placed in a circle around a hole cut in the centre of the trial arena. Parasitoids entered through the hole and were allowed to attack only 3 aphids which were removed. Test parasitoids were placed in a 60±2°C drying oven for 72 hours prior to being weighed using a Cahn<sup>TM</sup> microbalance. The three stung aphids were adhered to a glass slide and measured. Dorsal measurements of aphid length (vertex to base of cauda) and width (distance between cornicles) were taken using a dissecting scope with an ocular micrometer. Volume of a cylinder ( $L\pi r^2$ ) was used to calculate total aphid size. The mean volume of the three aphids that the wasp attacked was compared to parasitoid weight using a linear regression (JMP 5.0).

## 4.4 Results

### 4.4.1 Adult morphological plasticity

Parasitoid size was limited by host size resulting in substantially larger adults emerging from, *A. pisum*, compared to adults that developed in the smaller host species *A. solani* (Mean female parasitoid weight (mg): host - *A. pisum* 0.103±0.0016; host - *A. solani* 0.0454±0.0015) [ANOVA:  $F_{1,446} = 637.34$ ,  $p < 0.0001$ ].

### 4.4.2 Host size preference

Oviposition attempts (successful and unsuccessful) were used to determine parasitoid instar preference as this removed biases in success rate caused by constraints imposed by aphid species and instars, e.g. degrees of defensive capabilities. The aphid host species in which the parasitoid developed, had an effect on the instar that parasitoids

preferred to attack [LOG LINEAR:  $\chi^2 = 9.55$ , d.f. = 3,  $p = 0.0229$ ]. Parasitoids which had developed in pea aphids preferred larger instars on average than parasitoids reared in foxglove aphids. Exposure to different aphid species was significant [LOG LINEAR:  $\chi^2 = 7.98$ , d.f. = 3,  $p = 0.0464$ ]. There was no interaction between rearing host species, exposure species and instar preference [LOG LINEAR:  $\chi^2 = 4.19$ , d.f. = 3,  $p = 0.2414$ ]. When comparing the instar preference regimes of each rearing-exposure combination, parasitoids demonstrated a consistent instar preference only when reared and then exposed to the same aphid species. Furthermore, parasitoids demonstrated a parallel deviation in instar ranking when reared and then exposed to the alternate host in that parasitoids reared on foxglove aphids preferred smaller pea aphids and parasitoids reared on pea aphids preferred larger foxglove aphids (Fig. 4.1).

#### 4.4.3 Missed attacks due to aphid defense

Mean number of missed attacks increases with host age across all rearing-exposure groups due to the superior defensive capabilities of the mature 3<sup>rd</sup> and 4<sup>th</sup> instar aphids (Fig. 4.2). The interaction between rearing and exposure groups indicates a divergence in the number of missed attacks across instars [MANOVA:  $F_{4,106} = 3.649$ ,  $p = 0.008$ ]. This deviation is most likely due to the foxglove-aphid-reared parasitoids that were exposed to pea aphids, which resulted in a much greater number of missed attacks when attempting to attack 3<sup>rd</sup> and 4<sup>th</sup> instars pea aphids. Pea aphids, being a large, well-defended aphid species, generated a substantially greater number of missed attacks by parasitoids [MANOVA:  $F_{4,106} = 5.1445$ ,  $p = 0.0008$ ]. The superior defenses of pea aphids compounded with a marginal increase in the number of missed attacks due to being reared in foxglove aphids [MANOVA:  $F_{4,106} = 2.47$ ,  $p = 0.0489$ ] resulted in the substantial increase in missed attacks in the foxglove-reared, pea-aphid-exposed group.

#### 4.4.4 Mechanism of host size selection

Parasitoid weight was positively correlated with aphid size preference (LINEAR REGRESSION:  $F_{1,33} = 22.19$ ,  $p < 0.0001$ ) (Fig. 4.3). The coefficient of variation

determination indicated that parasitoid weight accounts for 40% of the variation in the observed aphid size preference ( $R = 0.402$ ).

## 4.5 Discussion

Parasitoids provide an exceptional system for examining questions about adaptive behaviour (Godfray and Shimada 1999). A substantial body of research has focused on causes and consequences of parasitoid host-choice behaviour, and the influence of natal host on adult traits (reviewed in Godfray 1994). Natal host has been shown to influence odour preference, handling time, sex allocation behaviour and modify adult parasitoid morphology (Fellowes et al. 2005; Morris and Fellowes 2002). Adult size is a highly flexible trait in many parasitoids that is determined by host size (Harvey et al. 1994; Sequeira and Mackauer 1992b). This allows generalist parasitoids to utilize a broad range of host species. Variability in adult size has been demonstrated in several Aphidiinae wasps utilizing a broad range of host species, of diverse sizes (Ode et al. 2005; Nicol and Mackauer 1999; Honek et al. 1998; Elliot et al. 1994) and in other koinobiont parasitoid systems (Abe 2006; Harvey and Vet 1997; Mohamed 2003). The maintenance of growth plasticity has been demonstrated with *A. ervi* from two host species populations where adult parasitoid size correlated with host species size irrespective of natal host population (Daza-Bustamante et al. 2003; Lee Henry unpublished data). Body size has been shown to influence foraging behaviour, which in turn affects niche partitioning (Wang and Messing 2004b). In predator-prey systems, predator to prey size ratio has been shown to influence handling time, foraging success and subsequent prey preference (Lafferty and Kuris 2002). However, the consequences and co-variance of adult size on host selection behaviour is a topic that has received little attention in host parasitoid systems.

When considering the attempted attacks on each instar, that is, the biological preference excluding factors such as host defense, the results of our experimental manipulations indicated that natal host affects the way females choose between host size classes. Assuming that the parasitoids are still attempting to select the highest quality

host for their offspring we can infer that natal host influences a female's host assessment criteria in that it changes the ranking of perceived host quality with relation to host size. For aphid parasitoids, which attack all stages of their hosts, selecting the highest quality host for larval development involves selecting an instar that is allocating all of its resources towards growth, yet can handle the damage ensued by an oviposition (Vinson and Iwantsch 1980). This typically results in intermediate hosts being the most suitable for larval development, due to young hosts being limited in resources and adult aphids declining in quality through superior physiological defenses against parasitism or competition for host resources with embryos (Colinet et al. 2005). Intermediate instars have been reported yielding the highest fitness returns in many aphid parasitoid systems. Instar quality for *A. ervi* larval development was shown to drop dramatically from 18% to 1% for 2<sup>nd</sup> to 4<sup>th</sup> instar *A. solani* (Henry et al. 2005). Percent parasitism of *A. ervi* exposed to instars of *A. pisum* (20 individuals) resulted in 2<sup>nd</sup> instar hosts producing the highest reproductive returns with declining returns from younger and older instars (McBrien, PhD Thesis). Similar patterns have been demonstrated with *Lipolexis oregmae* and *Lysiphlebia mizari* utilizing *Toxoptera citricida* (Walker and Hoy 2003; Tsai and Wang 2002); *Monoctonus paulensis* utilizing *A. pisum* (Chau and Mackauer 2001); and *A. ervi* utilizing *Myzus persicae* (Colinet et al. 2005). Intermediate instars represent a high quality pool of future resources for koinobiotic parasitoids because the larva continues to grow and develop with their hosts after parasitization. Therefore, the developmental stage of a host becomes far more important for larval survival than the actual size of the host at parasitization. Generalist parasitoids that attack a wide range of species, of variable sizes, can have their host size selection criteria complicated, given that the optimal 2<sup>nd</sup> and 3<sup>rd</sup> instars may have a two or three fold deviation in size between host species. The two hosts used in this experiment presented such a contrast in that *A. pisum*, the pea aphid, is on average twice as large as *A. solani*, the foxglove aphid.

Parasitoids reared and then exposed to instars of the same host were able to accurately select the highest quality instar for larval development. This was true for both natal host treatments with the distributions of attacks resulting in 2<sup>nd</sup> then 3<sup>rd</sup> instar aphids being preferred in both rearing, exposure groups (Fig. 4.1). However, when parasitoids were exposed to the alternate host from which they were reared, there was a deviation

from the optimal behaviour. When the larger phenotype parasitoids (*A. pisum* reared) were exposed to *A. solani*, the smaller host species, they preferentially selected the larger, lower quality, *A. solani* instars for oviposition. A consistent deviation from the optimal behaviour was demonstrated by parasitoids reared on *A. solani* then exposed to the larger host *A. pisum*, which preferentially selected smaller instars of this host species. This pattern suggests that adult parasitoid size is influencing instar preference behaviour in *A. ervi*. However, we could not rule out that some aspect of the natal host other than the changes in adult size may have contributed to the observed preference.

In order to examine the mechanism of host size selection and to determine if adult parasitoid size was the primary factor influencing host size selection we investigated the relationship between adult size and host size preference within a single rearing/exposure host species, *A. pisum*. Aphids were anesthetized to remove any factors that may have influenced the parasitoids foraging behaviour. Parasitoid size correlated with host size preference (Fig. 4.2). This suggests that the plasticity in host size selection behaviour between natal host species groups is most likely caused by the size differentiation that results from parasitoids developing in different hosts. Even when hosts were anesthetized removing many constraints on oviposition success, such as accessibility and aphid defenses, parasitoids still preferred to oviposit in hosts that correlated with their own body size. This suggests a behavioural trait that has been evolutionarily conditioned to perform in conjunction with the size of the adult.

Size and fitness are generally correlated in the parasitic Hymenoptera and as a result, a female's choice of hosts directly affects the fitness of her offspring (Morris and Fellows 2002; Lampson et al. 1996). Larger female parasitoids often have higher egg loads, live longer, and have higher dispersal capabilities (Eijs and van Alphen 1999). Consequently, selecting the largest host in order to maximize offspring size is a trend that is commonly seen among many parasitoids, especially idiobiotic parasitoids (Wang and Messing 2004a). However, to benefit from having a large-bodied offspring there must exist a greater benefit than cost in achieving a large body (Leather 1988). Factors that may decrease the overall returns of selecting a larger host include: a prolonged developmental time which increases the chance of offspring predation and increased

juvenile mortality which is commonly observed in koinobiotic parasitoids selecting the largest host instars (Sequeira and Mackauer 1992b; Harvey et al. 1994).

Possibly the most important factors influencing overall reproductive returns are demonstrated by the mother parasitoid in terms of foraging success and capture efficiency. Research using *D. giffardii*, a generalist ectoparasitoid of many tephritid flies, has demonstrated that variation in female parasitoid body size, as a result of natal host development, influences host selection behaviour in that parasitoid size is positively correlated with host puparia size selection (Wang and Messing 2004a). This is thought to be due to a trade-off between attempting to select the largest host for offspring development countered by an increased handling time in smaller wasps drilling through the thick puparia of larger host (Wang and Messing 2004b; Morris and Fellowes 2002). Consequently smaller wasps are thought to gain greater reproductive returns from selecting a greater number of smaller hosts. A similar pattern is seen in instar-specific defenses of many koinobiotic parasitoids such as caterpillar and aphid parasitoids. Host instar specific physical defenses typically increases with age and female parasitoids must make a choice between an increased handling time and risking injury in attempting to oviposit in a larger host (Gerling et al. 1990). Our study suggests that success rate is further influenced by parasitoid size given that small parasitoids (i.e. foxglove reared) miss more attacks thereby wasting more time and energy when attacking larger aphids (i.e. pea aphids) (Fig. 4.3). Accordingly, if handling time and capture efficiency are influenced by the ratio between parasitoid to host size this may have contributed to the evolution of a size-based preference policy.

The relationship between body size and capture efficiency of natural enemies is important in understanding the evolution of prey preference. The attack success of many predators is predicated on having a substantial size advantage over prey, although this is not always the case in highly specialized solitary killers and facultative social predators (Lafferty and Kuris 2002). For aphid parasitoids that must gain control over hosts and cope with their defenses there is clearly a parasitoid to host size threshold that dictates attack success. Consequently, it is not surprising that host size selection co-varies with body size in aphid parasitoids given the existence of host-determined body growth. An asymmetry that exists within this system is that aphid defenses impede the attack success

of small parasitoids to a greater extent than large parasitoids. Therefore, although the small-phenotype parasitoids exhibited a sub-optimal instar selection regime, by selecting small instars of the larger host species, they may in fact be optimizing their reproductive returns when aphid defensive behaviour is included. However, size selection behaviour in small-phenotype parasitoids was not provisionally based on host defensive capabilities as a sub-optimal instar regime was still chosen when aphids were anesthetized, removing instar-specific defenses (Fig. 4.2). In contrast, larger sized, pea-aphid-reared parasitoids preferentially attacking 3<sup>rd</sup> and 4<sup>th</sup> instar foxglove aphids is indeed maladaptive given that they thus preferred the lowest quality instars within that host species (Henry et al. 2005). Our work demonstrates how a behavioural policy may be required to maintain a certain level of flexibility when an organism has a plastic developmental strategy, in order to increase oviposition accuracy, thereby optimizing reproductive returns.

Evaluation of host size in relation to one's own body size involves relative measurements that can correlate parasitoid to host size ratios. The ability to measure absolute host size has been demonstrated using *Trichogramma* egg parasitoids that utilize short time intervals when crossing a host's surface to determine host volume and subsequent egg deposition (Schmidt and Smith 1987). The aforementioned experiment demonstrated that parasitoids did not use relative measures given that small and large parasitoids laid the same number of eggs per host volume. Our results show that *A. ervi* and possibly aphid parasitoids rely on some aspect of their own body size to dictate what host is most profitable to attack. It is possible that the antennation process that often precedes initial attacks is a method of comparatively measuring, given that antennal length generally correlates with overall parasitoid size.

The adaptive nature of a trait depends on the trait's flexibility, and the variability of the environment in which the organism lives (Kawecki and Stearns 1993). A behavioural policy that is fixed upon emergence can lead to erroneous decisions when a parasitoid is confronted by an alternate host species (Henry et al. 2005). This brings up interesting questions about the evolution of such linked plastic traits given that a maladaptive policy should be selected against in a non-adapted environment (Ernande and Dieckmann 2004). Having traits fixed by the natal host for an individual's life but flexible between generations would suggest that this policy evolved under conditions



where the parasitoid would have a higher probability of encountering the same host species within an individual lifetime but possibly encounter several host species across generations. As relatively small-sized and short-lived animals, with a life expectancy in nature that may be hours rather than days, parasitoids may only sample a few host patches within their lifetime (Ellers and Jervi 2003). Due to the parthenogenetic nature of aphid reproduction it is quite probable that a parasitoid will emerge within or at least at a short distance from the same host species patch. Consequently, a fixed behavioural policy within an aphid parasitoid's lifetime may optimize the individual's reproductive strategy but still allow for population movement between host species suffering only a one-generation lag in performance.

In conclusion, adaptation through phenotypic evolution often involves trade-offs between traits in order to maximize fitness returns. This is especially true when traits are directly associated with reproductive success. Host selection behaviour and adult body size are both correlated with reproductive fitness in parasitoids yet are not mutually exclusive traits. Flexibility in adult body size allows generalist parasitoids to utilize many host species at a cost of producing a range of adult sizes. Consequently, host selection behaviour must also maintain a level of flexibility as adult size is related to capture efficiency. These types of co-adapted gene complexes allow generalist parasitoids to effectively use multiple host species over several generations. However, the fixed nature of the behavioural response, within a parasitoid's lifetime, suggests that these traits may have evolved in a patchy heterogeneous host species environment.

## **4.6 Acknowledgements**

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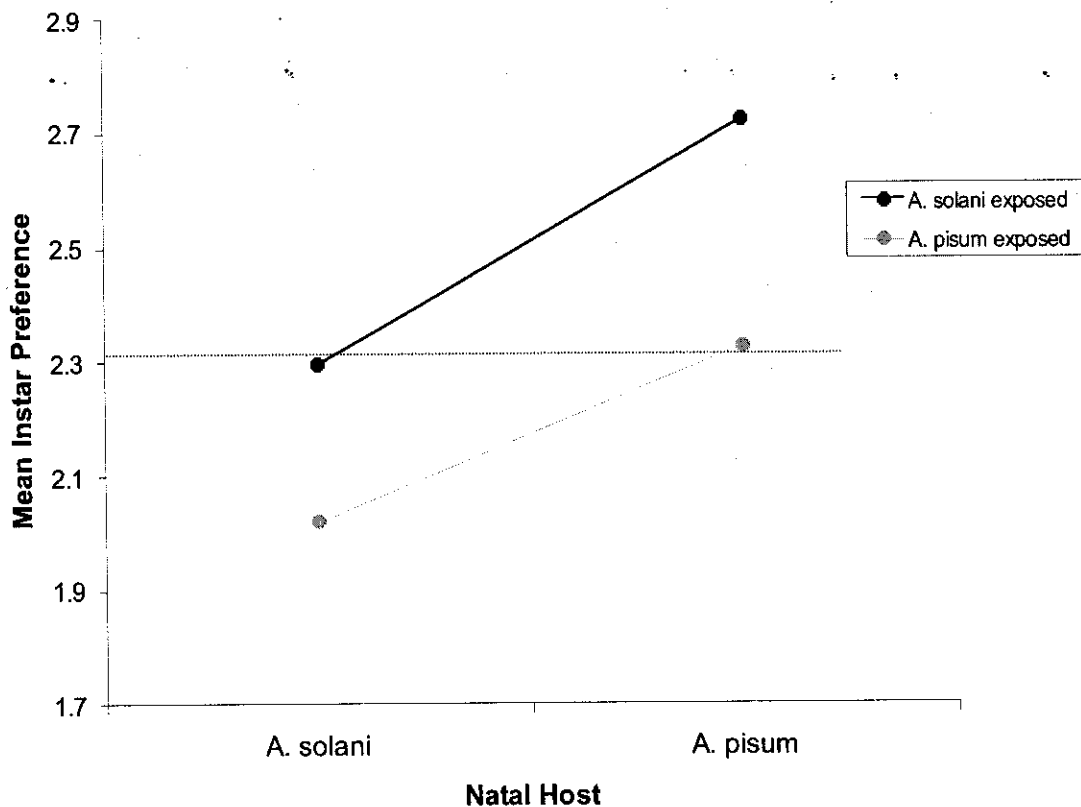
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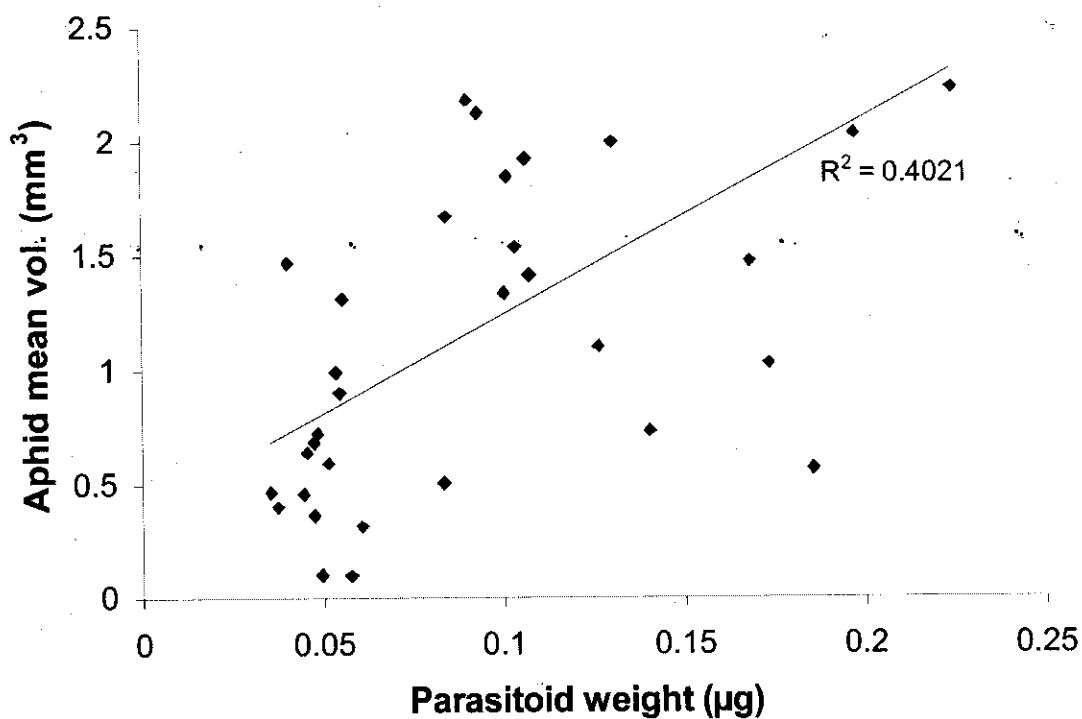
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**Figure 4.1** Host size preference of *A. ervi* after developing in two different natal hosts, displayed as the mean instar preferred per female when exposed to the foxglove aphid, *A. solani* or the pea aphid, *A. pisum*. Dashed line represents the optimal instar preference for highest reproductive returns. Elevations of the exposure lines demonstrate a general preference for larger *A. solani* and smaller *A. pisum*. Natal host development induces a shift in overall host size preference.

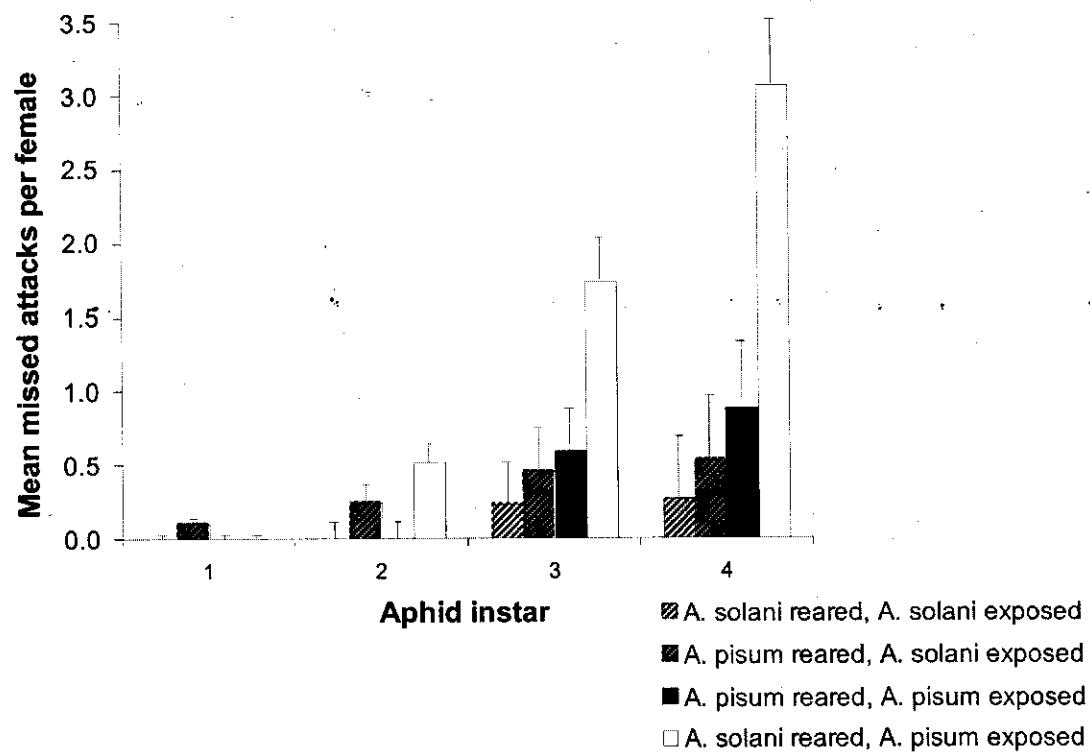




**Figure 4.2** Preferred aphid size correlated to parasitoid weight when parasitoids were reared and exposed to *A. pisum*. Aphid size displayed as the mean volume of the first three aphids selected by each parasitoid female.



**Figure 4.3** Instar specific mean missed attacks per female parasitoid after developing in two different natal hosts then exposed to either pea aphids, *A. pisum*, or foxglove aphids, *A. solani*.



## **CHAPTER 5: SIZE-MEDIATED ADAPTIVE FORAGING: A HOST-SELECTION STRATEGY FOR INSECT PARASITIDS<sup>5</sup>**

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## 5.1 Abstract

Foraging models are useful to generate predictions about predator-prey interactions such as habitat or diet choice. However, the majority of studies attempting to explain adaptive behaviour using optimality criteria have assumed there is no trait variation among individual consumers, such as the relative difference in the body-size of predators and prey. Hymenopteran parasitoids that attack the free-living stages of their host are an ideal system for studying the influence of body size on host selection, due to the wide range of adult parasitoid sizes coupled with the defensive capabilities of their hosts. In this article we use an experimentally parameterized host selection model to investigate the influence of parasitoid body-size on the range of acceptable host instar classes. We then compare the efficiency of parasitoids employing an optimal host selection strategy against parasitoids employing an indiscriminate host selection strategy over a range of different parasitoid body sizes using a demographic model. Net fitness accrual of parasitoids and the impact of host instar selection on aphid recruitment are assessed on different stage-structured aphid populations. Our results demonstrate that optimal host selection allows larger parasitoids to utilize a wider range of hosts. However, smaller parasitoids receive the greatest benefits from selecting hosts optimally by selectively utilizing a restricted range of small, poorly defended hosts when they are abundant. We argue that the correlation between flexible host selection behaviour and adult body-size may be a general phenomenon that applies to the majority of hymenopteran parasitoids that attack free-living, well-defended hosts. Furthermore, we discuss the potential of within-generation behavioural interactions to impact between-generation dynamics in host-parasitoid populations.

## 5.2 Introduction

The importance of individual behaviour for population and community level processes has been demonstrated in a number of studies (e.g. Werner et al. 1983; Beckerman et al. 1997; Luttbegg and Schmitz 2000; Peacor and Werner 2001). Extensive theory has been developed that extends individual-level behaviour into a community

ecology framework (Dill 1987; Fryxell and Lundberg 1994; Ma et al. 2003; Persson and De Roos 2003). In host-parasitoid systems, a parasitoid's choice of habitat, host species or host size may influence parasitoid-host dynamics (Mangel and Roitberg 1992), which in turn can produce cascading effects on populations that shape the structure of communities (Petchey et al. 2008). However, much of the work on the impact of individual behaviour on population dynamics has centered on between-generation processes (Bolker et al. 2003), and this is especially true in host-parasitoid systems (see Mangel and Roitberg (1992) for alternative approaches). Thus, features of biological systems that are within-generation processes, such as adaptive host selection, have largely been ignored. We argue that determining the dynamics of within-generation processes is a necessary first step to measuring the impact of adaptive host selection on population dynamics.

The majority of studies attempting to explain foraging or host selection behaviour have assumed that there is no trait variation (e.g. size) among individuals within populations (exceptions see: Mittelbach 1981; Persson and De Roos 2003). This is surprising given the majority of empirical studies investigating the effects of adaptive prey selection are characterized by having a high degree of size variation within populations (e.g. Werner et al. 1983; Persson and Greenberg 1990; Beckerman et al. 1997; Peacor and Werner 2001). A positive correlation between a predator's size and the size of its prey is ubiquitous throughout natural systems (e.g. Fisher and Dickman 1993; Vezina 1985; Roger et al. 2000), yet few studies have addressed the impact of adaptive behaviour as a function of the relative difference in predator to prey body size on the growth of prey and predator populations. Furthermore, the functional relationship between body-size and foraging efficiency within different types of size- or age-structured prey environments has received little attention, even though it is a fundamental characteristic of predator-prey dynamics (Hivonen and Ranta 1996).

In this study, we design a model of within-generation dynamics of a host-parasitoid system that incorporates parasitoid size-mediated decisions. We first use a series of experiments to determine the fitness benefits and costs associated with size-mediated parasitoids host choice, by varying the size of the parasitoid and the size of the host they are attacking. Secondly, we frame our host selection decisions in the context of

an optimal host selection model, which is parameterized from our experiments. The optimal host selection model assumes that natural selection has resulted in animals that select hosts to maximize fitness (Pyke et al. 1977), while taking into account that the rate of energy intake is dependent on the parasitoid's ability to detect, capture, and handle each host (reviewed in: Krebs 1978). A female parasitoid selecting hosts optimally therefore makes decisions that maximize her net rate of fitness gain (i.e., maximize the number and size of her offspring per unit time) based on the profitability of each host, where profitability is defined as the fitness of the offspring produced per unit time spent handling. Finally, we develop a cohort-based model that examines the impact that a parasitoid of a fixed size that selects hosts optimally has on three different stage-structured aphid populations of different stage structure. We argue that this cohort-based model on adaptive host selection will help us understand within-generation dynamics, and that this understanding is crucial to further development on the impact of host size selection on between-generation dynamics in parasitoid systems (Bolker et al. 2003).

The system we have chosen is the aphid parasitoid *Aphidius ervi* Haliday, and the pea aphid, *Acyrthosiphon pisum* (Harris). Parasitoid wasps and their aphid hosts are an ideal system for studying size mediated adaptive host selection in within-generation studies. Parasitoids have been shown to adaptively alter their host-selection behaviour in relation to their own body-size (Henry et al. 2006). This size-based host selection behaviour can be thought of within an optimal foraging scenario because the likely benefits of increased energy gain is positively correlated with an increased handling time as host classes increase in size. Hosts represent a pool of resources for developing parasitoid larvae; large hosts have more resources and therefore produce larger parasitoids, which is a proxy positively correlated with fitness (Roitberg et al. 2001; Sequeira and Mackauer 1994). This specific taxon of aphid parasitoids must contact its host in order to oviposit, which puts them at risk of host defensive mechanisms. The pea aphids have a mosaic of effective defenses to avoid parasitization, such as kicking, dropping, running away and alarm pheromone dispersal, which develop exponentially as aphids mature (Gerling et al. 1990; Gentry and Dyer 2002). Furthermore, some hosts (e.g. mature aphid or caterpillar instars) can easily exceed the size and handling

capabilities of smaller parasitoids. Therefore, when choosing suitable hosts, a parasitoid must take into account the parasitoid-host size relationship.

Previous studies using a variety of aphid parasitoid species have shown that many species typically prefer the small and intermediate host instars for oviposition even though the larger host instars have more resources for developing larvae (Kouame and Mackauer 1991; Mackauer et al. 1996; Chau and Mackauer 2001; Colinet et al. 2005; Tsai and Wang 2002); however, none of these studies have considered these results in light of optimal foraging theory. Furthermore, these results have not been extended to the within-generation dynamics of a host-parasitoid system; therefore, the work we present in this study has novel implications for our understanding of host-parasitoid interactions.

## **5.3 Materials and Methods**

### **5.3.1 Experiments – model parameters**

For all of the following experiments, parasitoids were selected based on body size with the intention of creating a uniform distribution across the potential range of parasitoid sizes. After each trial, parasitoids were dried and measured. All insects were maintained at 19°C, 50% humidity, with a 16h light, 8h dark photoperiod. All experiments were run between 10am and 2pm under the same environmental conditions. Five aphid instars were used to describe the stages an aphid will progress through during normal development. We defined each instar as a size class between molting events wherein aphids increase substantially in size and ignored any within instar variation in size based on previous work that shows that between differences in size always exceeds within instar variation (Lee Henry, unpublished data). Instars were chosen to represent sizes between molting events when aphids increase substantially in size and therefore represent biologically relevant stages when aphids increase in the capability to defend themselves from parasitism as well as increase in the value of resources they represent for parasitoid larval development (i.e. fitness returns).

We performed an experiment to measure the fitness benefit for a parasitoid parasitizing an aphid host. With respect to host choice, hosts are not consumed by the

female laying the egg, but instead represent a pool of resources for the developing larva. We use the size of each offspring as the fitness benefit from choosing a particular host, as parasitoid body size has been linked with fitness in a number of studies (see review: Roitberg et al. 2001). The resources consumed by the developing larva cannot be measured directly, and we therefore use the size of the emerging larva as our proxy for the resources that a host represents. We allowed a single parasitoid female to oviposit in a single host of instar  $i$  in a gel capsule. Aphids were then transferred to an excised broad bean leaf, kept fresh by inserting the petiole in a dram vile capped with Parafilm, where the aphid remained until emergence (approximately 13 days). This methodology has been shown to produce the same positive size-fitness relationship as other studies using aphid parasitoids (Chau and Mackauer 2001), and produces highly comparable results to aphids reared on living plants (Lee Henry, unpublished data). We assume that heritability of mother size to offspring size is negligible and superseded by the effects of host instar on offspring size (Sequeira and Mackauer 1992). Hind tibia length served as the size measurement for parasitoids. Parasitoids were dried, weighed and their right hind tibia was removed, adhered to double-sided tape, and measured twice at 40X magnification, then averaged for each parasitoid. Fitness results were analyzed using JMP 6.0 statistical software (SAS Institute, Cary, NC, USA).

We performed a second experiment to determine the handling times associated with different sized parasitoids laying eggs in hosts of different instars. Handling times were determined by timing how long it took for a parasitoid female to oviposit in a single aphid after initially contacting that aphid in a bean leaf arena. Bean leaves were trimmed into circles with an area of  $9.6 \text{ cm}^2$  and placed in a 90mm petri dish. A single aphid of instar  $i$  was allowed to settle in the middle of the leaf disc for a period of 60 minutes, after which time a single parasitoid was introduced. Once the parasitoid made antennal contact with the host, the time until oviposition was recorded. Aphids were deemed escaped if the aphid crossed the edge of the leaf disc without receiving a successful oviposition. Proportion escape  $e_i$  was calculated by dividing the number of aphids that escaped by the total number of replicates per instar.



### 5.3.2 Host selection model

A parasitoid's host selection decisions are described using a variation of the optimal diet breadth model (Charnov 1976a; Stephens and Krebs 1986) where the 'diet breadth' exhibited by parasitoids is the number of different host instar classes that are accepted. In this parasitoid-host system, we use pea aphid hosts, which have 5 instar classes ( $i$ ). The class of instar  $i$  encountered in each unit of search time is denoted as  $\lambda_i$ .  $\beta_i$  is the probability of instar  $i$  being accepted and is the only variable the parasitoid can manipulate while selecting hosts. The parasitoid's handling time  $h_i(p_s)$  associated with each instar  $i$  differs based on an instar's defensive capability and a parasitoid's size ( $p_s$ ). The expected fitness value of instar  $i$  is denoted as  $w_i^\circ$ , and this is based upon the resources that it presents to a developing parasitoid larvae ( $w_i$ ) weighted by its probability of capture ( $e_i$ ). The profitability of a particular instar is thus the expected fitness over the handling time,  $w_i^\circ/h_i(p_s)$ . We rank the instars so that that the instar with the highest profitability is ranked 1 and the instar with the lowest is ranked 5; i.e.,  $w_1^\circ/h_1(p_s) > w_2^\circ/h_2(p_s) > w_3^\circ/h_3(p_s) > w_4^\circ/h_4(p_s) > w_5^\circ/h_5(p_s)$ . The parasitoid maximizes the net rate of fitness accrued through offspring biomass production ( $W_n$ ) over the entire period spent selecting hosts ( $T$ ). Thus the specific form of the equation for parasitoid fitness,  $f(\lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5)$ , is

$$f(\lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5) = \frac{W_n}{T} = \frac{\sum_{i=1}^5 \lambda_i w_i^\circ \beta_i}{1 + \sum_{i=1}^5 \lambda_i h_i(p_s) \beta_i}$$

(Equation 1)

To determine which instar classes a parasitoid should include in its attack set, we determine the conditions under which parasitoid fitness increases with the inclusion of instar class  $i$ , (i.e., where  $\partial f(\lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5)/\partial \beta_i > 0$ ). Because instar class 1 is always assumed to be the most profitable host class, it follows that it should always be accepted ( $\beta_1 = 1$ ). To determine when each of the lower profitability instar classes should be included, we solve  $\partial f/\partial \beta_i = 0$  for  $\lambda_i$ . This solution is expressed as a threshold encounter

rate of instar class 1 ( $\eta_{1,i}$ ) below which the parasitoid should include instar class  $i$ ; i.e., the conditions where  $\beta_i = 1$  (Equation 2).

$$\begin{aligned}\eta_{1,2} &= \frac{w_2^\circ}{w_1^\circ h_2 - w_2^\circ h_1} \\ \eta_{1,3} &= \frac{w_3^\circ + w_3^\circ h_2 \lambda_2 - w_2^\circ h_3 \lambda_2}{w_1^\circ h_3 - w_3^\circ h_1} \\ \eta_{1,4} &= \frac{w_4^\circ + w_4^\circ h_2 \lambda_2 - w_2^\circ h_4 \lambda_2 + w_4^\circ h_3 \lambda_3 - w_3^\circ h_4 \lambda_3}{w_1^\circ h_4 - w_4^\circ h_1} \\ \eta_{1,5} &= \frac{w_5^\circ + w_5^\circ h_2 \lambda_2 - w_2^\circ h_5 \lambda_2 + w_5^\circ h_3 \lambda_3 - w_3^\circ h_5 \lambda_3 + w_5^\circ h_4 \lambda_4 - w_4^\circ h_5 \lambda_4}{w_1^\circ h_5 - w_5^\circ h_1}\end{aligned}$$

(Equation 2)

As is the case for the simple optimal diet breadth model, this model predicts an all-or-nothing response whereby the parasitoid should either always include the host instar class or completely ignore it. Furthermore, equation 2 shows that the inclusion of the lesser quality instar classes is contingent on the encounter rates of all instar classes of higher ranking.

### 5.3.3 Mechanism of host-size selection

We simulate single parasitoids that employ the aforementioned optimal attack model while exploiting an aphid population. We use three different size classes of parasitoids representative of a small (830 $\mu$ m), mean (910 $\mu$ m), and large parasitoid (990 $\mu$ m). Each parasitoid then parasitizes one of three different simulated aphid populations: (1) an aphid population that has recently colonized a plant and has begun to reproduce, (2) a well-developed population, and (3) a senescing population (Table 5.1). We compare the parasitoid using an adaptive host selection strategy against a parasitoid that selects hosts indiscriminately (as a null model) to determine the relative difference in the two strategies.

We evaluate the impact of the host-parasitoid interaction using two measures: (1) the total fitness gain of an individual parasitoid, and (2) the impact of the parasitoid on aphid recruitment. The total fitness gain of an individual parasitoid is similar to measuring clutch size at the end of a season – a technique that Bolker et al. (2003) suggest is a valid measure of relative fitness. However, our measure of fitness also takes into account differences in offspring fitness as a result of differences in host size. Within-generation dynamics are modeled as a two-step process: (1) parasitoid attack, and (2) aphid population growth.

We use a Markov chain process to simulate a parasitoid attack on an aphid population. We assume that the parasitoid can take only one instar in a given simulation time step. A parasitoid encounters host instars of each class based on  $\lambda_i$ , which is the product of the area covered by a parasitoid ( $a$ ) and the density of instar  $i$  ( $N_i$ ).  $N_i$  is calculated as the number of that instar ( $A_i$ ) over the total area of the environment ( $x$ ). We divide space into 500 cells (i.e.,  $x=500$ ), where each cell has some probability of harboring one or no host. Thus,

$$\lambda_i = aN_i = \frac{aA_i}{x}$$

(Equation 3)

During the simulation, a parasitoid accumulates fitness each time step. We assume that the time step is large enough for an individual to handle and parasitize a host, but we weight the fitness gain based on the profitability of the host type. This also allows us to reflect the difference in fitness gained by parasitoids of different sizes. Thus, the per unit time fitness increase by a parasitoid ( $V$ ) is calculated as

$$V(i) = \sum_{i=1}^5 \lambda_i \beta_i \frac{w_i^o}{h_i(s)}$$

(Equation 4)

The aphid population in the next time step is discounted by the probability of removing each type of acceptable instar based on the host selection rules. Over the duration of the simulation, the total accumulation of fitness by a parasitoid ( $V_T$ ) is therefore

$$V_T = \sum_{i=1}^{T^*} V(i)$$

(Equation 5)

where  $T^*$  is terminal time at which the parasitism bout ends. Equation 5 will yield a monotonically decelerating curve because the parasitoid depletes the aphid population over time.

To incorporate a biologically meaningful endpoint to a bout of parasitism, we base the endpoint on the Marginal Value Theorem (MVT) (Charnov 1976b), which states that the parasitoid should leave the patch when the marginal capture rate in the patch drops to the average capture rate for the habitat; i.e., when

$$\frac{dV_T}{dT} = \frac{V(i)}{T^* + T_i}$$

(Equation 6)

where  $T_i$  is the travel time, and  $T^*$  is optimal patch residence time. The aphid population is a depleting patch; thus, the use of the MVT is valid. We use an arbitrary value of 150 time steps for  $T_i$  and solve equation 6 for  $T^*$ . It is worth noting that the value of travel time ( $T_i$ ) did not change the qualitative outcome of the model. We use the  $T^*$  value for the parasitoid adopting the optimal host selection strategy as the patch residence time for the indiscriminate parasitoid in order to compare the two strategies within a fixed time frame. This approach allows us to compare the two strategies without confounding our results because of potentially differing patch residence times due to the indiscriminate parasitoid's differing rate of expected energy gain.

Once the parasitoid has left the aphid host patch, we project the remaining aphid population forward through time to determine the effect of parasitism on aphid recruitment. We construct a stage-structured Leslie projection matrix to track the cohort of aphids (Equation 7).

$$\begin{pmatrix} 0 & 0 & 0 & 0 & g_5 \\ 0 & \alpha_{21} & 0 & 0 & 0 \\ 0 & 0 & \alpha_{32} & 0 & 0 \\ 0 & 0 & 0 & \alpha_{43} & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}^{\tau} \times \begin{pmatrix} A_{0,1} \\ A_{0,2} \\ A_{0,3} \\ A_{0,4} \\ A_{0,5} \end{pmatrix} = \begin{pmatrix} A_{t,1} \\ A_{t,2} \\ A_{t,3} \\ A_{t,4} \\ A_{t,5} \end{pmatrix}$$

(Equation 7)

where  $A_{t,i}$  is the number of instars of stage  $i$ .  $\tau$  represents the transition in an aphid class such that an individual starting at instar 1 would become a reproductive instar ( $i = 5$ ) when  $\tau = 5$ . The amount of time that it takes for a parasitism bout is much smaller than the time it takes for an instar to transition to the next stage, and is therefore assumed to be negligible. We set the predation free transition probability from stage  $i$  to stage  $j$  ( $\alpha_{ij}$ ) at 0.95 for all aphid instar classes and we set the fecundity of reproductive aphids ( $g_5$ ) to 30 (Mondor and Roitberg 2000). We progress the aphid cohort forward until all individuals are dead (i.e. until  $\tau = 5$ ) and calculate the number of offspring the aphid population is expected to produce. We calculate the aphid recruitment as

$$\sum_{\tau=0}^5 A_{\tau,5} g_5$$

(Equation 8)

We calculate the reduction in aphid population recruitment caused by the parasitoid by taking the ratio of the parasitoid-attacked aphid host population recruitment over an aphid host patch of the same starting numbers that was not attacked by a parasitoid. We apply this comparison for both the optimal and indiscriminate host selection strategies.

## 5.4 Results

### 5.4.1 Experiments – model parameters

Significant variation was found in the size of parasitoid offspring by host instar (ANOVA:  $F_{4,198} = 11.58$ ,  $P < 0.0001$ ). A post-hoc Tukey HSD indicated that 3<sup>rd</sup> and 4<sup>th</sup>

instar aphids produce the largest offspring and therefore provide the greatest fitness returns ( $w_i$ ) to the ovipositing female with offspring emerging from 1<sup>st</sup>, 2<sup>nd</sup>, and 5<sup>th</sup> (reproductive) instars declining in quality (Table 5.2). The proportion aphids escaped was positively correlated with aphid age (Table 5.2).

Our results demonstrate that the relative difference in handling time is inversely related to parasitoid size when handling aphids that have developed defensive capabilities (i.e. smaller parasitoids pay greater handling costs with larger aphids) (Fig. 5.1). The data was best described using an exponential decay function of the form

$$y = 1 + \lambda e^{\varphi x}$$

(Equation 9)

where variables  $\varphi$  and  $\rho$  define the shape of the line. The intercept represents the minimum possible handling time of 1 second.

#### 5.4.2 Host selection model

Figure 5.2 depicts three examples of the variation in instar profitability for parasitoids of the three representative size classes. The optimal range of host instars that are acceptable to a parasitoid can be predicted for parasitoids as a function of host densities using equation 2. Figure 5.3 is an example of how the range of host classes that are acceptable (upper panels) relates to the actual instars (e.g. accepting 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, instars is equivalent to 3 classes accepted) being accepted (lower panels) by parasitoid size at two fixed encounter rates ( $\lambda$ ), 4.01 (A) and 2.01 (B) (equal encounter rates across instars i.e.  $\lambda_1 = \lambda_2 \dots = \lambda_5$ ). The size of the instars accepted is positively correlated with parasitoid size for all encounter rates (i.e. larger parasitoids are predicted to accept larger instars) (Fig. 5.3 A and B lower panels). It is important to note that the range of host instars that are acceptable may remain the same for parasitoids of different sizes even though the particular instars included in that range of acceptable hosts may differ. An example of this is demonstrated by parasitoids of size class 830 and 970 in Figure 5.3. When selecting hosts optimally both parasitoids are predicted to only accept 2 instar classes (i.e. diet breadth of 2), the difference being that the smaller parasitoid (830) only

accepts 1<sup>st</sup> and 2<sup>nd</sup> instars whereas the larger parasitoid (970) only accepts 2<sup>nd</sup> and 3<sup>rd</sup> instars.

The optimal range of host classes as a function of host encounter rate and parasitoid size is represented as a landscape in figure 5.4. Figure 5.4 shows the host encounter rate at which a parasitoid of a given size should expand its host acceptance criteria to include host classes of lower profitability (for equal encounter rates across instars). Therefore, figure 5.4 describes the range of host classes that are acceptable and does not consider the actual instars being accepted. The model predicts two general outcomes: 1) parasitoids of almost all sizes should accept at least the top 2 or 3 most profitable hosts, even at very high encounter rates ( $\lambda = 4$ ), and 2) the optimal solution for larger parasitoids is to maintain a wider acceptance of host classes than smaller parasitoids. The first general conclusion demonstrates that host instar specialization within this system is not advantageous and that accepting multiple instars results in the highest fitness returns for parasitoids of almost all size at the majority of encounter rates. Although accepting more than one size class is the broad trend, the optimal number of classes accepted by the parasitoid is positively correlated with parasitoid size. This demonstrates that larger parasitoids should always accept a wider range of host instars than smaller parasitoids. The size classes accepted appears to change erratically through the intermediate range of parasitoid sizes as denoted by the ridges and valleys on the landscape. These differences are generated by the relationship between parasitoid size and the profitability of aphid instars, in that as parasitoid size increases larger instars are included in the range of acceptable hosts which can either broaden or restrict the number of hosts accepted. For example, in figure 5.3 a parasitoid of size class 905 is predicted to specialize on a single aphid instar (2<sup>nd</sup> instar) at an encounter rate of 4.01 (Fig. 5.3A), due to the net fitness returns a parasitoid of this size gains from only accepting 2<sup>nd</sup> instars at high host encounter rates. However, the same sized parasitoid should expand the instars it accepts to include three hosts (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars) at an encounter rate of 2.01 (Fig. 5.3B) because at a reduced encounter rate it is not advantageous to specialize on a single host and 1<sup>st</sup> and 2<sup>nd</sup> instar hosts are approximately the same in terms of the next most profitable instars for a parasitoid of this size.

As parasitoids encounter fewer hosts while foraging (i.e. lower host densities) the model predicts that lesser quality hosts should be accepted by parasitoids. Parasitoid size has an impact on this trend with the larger parasitoids including the lesser profitable items at an encounter rate of 0.21 and 0.11 for the 4<sup>th</sup> and 5<sup>th</sup> most profitable host's respectively. Small parasitoids never accept the 5<sup>th</sup> most profitable host and accept the 3<sup>rd</sup> and 4<sup>th</sup> most profitable hosts only at extremely low encounters rates (0.01).

#### 5.4.4 Within generation cohort model

Within a single foraging bout, parasitoids differed considerably in the amount of net fitness accrued as well as their impact on aphid population recruitment. Results are based on the expected net gain in parasitoid fitness ( $V_T$ ), whereas aphid recruitment is the proportion reduction in realized recruitment due to parasitism from the moment the parasitoid leaves the patch. Table 5.3 shows patch residence time for the three size classes of parasitoids foraging on the different stage-structured host populations. Size does not influence residence time while exploiting new populations however small parasitoids leave earlier in developed and senescing aphid populations (Table 5.3).

By definition, a parasitoid following an optimal host selection strategy maintained a higher net fitness when compared to the null model of a parasitoid following an indiscriminate host selection strategy (Fig. 5.5). However, the relative fitness gains of the strategies differ considerably depending on the interaction between host population structure and the size of the parasitoid. The relative strength of the host selection strategies differed the most with respect to parasitoid size and parasitoid fitness accrual in that smaller parasitoids benefited more from selecting hosts optimally than larger parasitoids. Parasitoids selecting hosts optimally had the greatest impact on aphid recruitment in the majority of population structures, with the exception of senescing aphid populations, where the indiscriminate host selection strategy had a greater impact on aphid recruitment. Net fitness accrued by different sized parasitoids differs qualitatively and quantitatively across and different aphid population types. It is important to note that the quantitative differences in net fitness accrued by parasitoids across populations will vary due to the number of aphids available, with parasitoids able



to generate a greater amount of net fitness in larger aphid populations (e.g. developed population).

When selecting hosts on a newly formed patch of aphids (Fig. 5.5 top panel) the two host selection strategies predict equivalent differences for small and medium sized parasitoids. Selecting hosts optimally generated a 29.7% and 30.2% increase in net fitness accrued by parasitoids of size classes 910 and 830 respectively when compared to the indiscriminate host selection strategy. In general, the optimal host selection strategy is predicted to have a greater impact on the recruitment of an aphid population, with both the small and medium sized parasitoids reducing the aphid population by 21.5% compared to a 16.5% reduction generated by a parasitoid with no instar preference. The two host selection strategies were similar for large parasitoids with only a slightly greater reduction in aphid recruitment (3.8%) as well as a slightly greater net fitness accrued (4.7%) by the indiscriminate strategy.

The different host selection strategies are predicted to have a strong influence on parasitoid fitness in well-developed aphid populations (Fig. 5.5 middle panel). This is especially true for small parasitoids, which gain a 55.3% increase in fitness when behaving optimally compared to the indiscriminate host selection strategy. However, the increase in fitness from optimal host selection is negatively correlated with parasitoid size – the rate of fitness accrued decreases from 30.6% in medium sized parasitoids and to 8.9% in large parasitoids. Medium-sized parasitoids that use optimal host selection have the greatest impact on the recruitment of well-developed aphid populations with small parasitoids producing a similar, slightly lower result. Large parasitoids that are selecting hosts optimally are predicted to have the least impact on aphid recruitment in developed aphid populations, 18% lower than what is expected from a parasitoid selecting hosts indiscriminately. Furthermore, large parasitoids that are behaving optimally are expected to obtain the lowest net fitness returns even though they spend the longest amount of time in the aphid patch before leaving (Table 5.3), with small parasitoids predicted to gain the greatest net fitness and have the shortest patch residence time.

The two host selection strategies have little influence on the net fitness accrued by parasitoids in the senescing population of aphids (Fig. 5.5 bottom panel). Large

parasitoids are predicted to have the highest net fitness prior to leaving the patch using either host selection strategy. Small parasitoids pay high costs within the senescing patch as it is dominated by large, well-defended instars and therefore gain the lowest net fitness returns. Moreover, small parasitoids are predicted to leave the aphid patch very quickly and have an extremely low impact on the recruitment of the senescing aphid population, which deviates considerably from the indiscriminate strategy (61.9% difference).

## 5.5 Discussion

Host selection strategies used by the different sized parasitoids have a significant impact on the within-generation dynamics of this host-parasitoid system. This study demonstrates that dramatically different outcomes can be generated by within-generation species interactions. Thus, understanding the impact that a parasitoid has on a population of aphids is a necessary first step to understanding how within-generation behaviour can play a role in between generation processes (Mangel and Roitberg 1992).

We develop a model that examines the within-patch behaviour of a parasitoid and the impact that it has on an aphid population. We begin by describing the size-based host selection rules that we determined from two experiments performed on the parasitoid *Aphidius ervi* and the aphid host, *Acrythosiphon pisum*. We then develop a host selection model where a parasitoid will maximize the rate of fitness gain by choosing hosts based on the ratio between the fitness increase and handling time associated with a host class. Finally, we examine the impact of a parasitoid on an aphid population in terms of parasitoid fitness gain and aphid population recruitment.

Our experiments show that parasitoid size can dramatically influence the profitability of instars due to the inverse relationship between handling time and parasitoid body size (Fig. 5.2). As aphids become larger, many species develop an increased ability to defend themselves against parasitoid attack, and therefore the relative difference in parasitoid and host body sizes becomes important in determining the success of a parasitoid's attack. Larger parasitoids pay reduced costs in terms of handling time and are therefore predicted to include larger host instars, have an expanded

range of acceptable host classes, and generally ignore small sized host instars. Small parasitoids are limited by the cost associated with handling larger instar aphids and are therefore limited to accepting a restricted range of host classes that have not developed effective defensive mechanisms (i.e. 1<sup>st</sup> and 2<sup>nd</sup> instars) (Fig. 5.3).

Numerous studies have suggested that host size selection in the parasitic Hymenoptera is based on the maximization of net fitness returns. The selection of early developmental stages, which are of less value for offspring development but are also less capable of defending themselves, is a wide spread pattern in hymenoptera that parasitize lepidopteron hosts that develop aggressive defensive behaviours (Gentry and Dyer 2002 and references therein). A positive relationship between wasp body size and host size selection has been shown in a number of spider-hunting pompilid wasps (Kurzewski and Kurzewski 1968; Field 1992 and references therein), and in a sphecid wasp (Strohm and Linsenmair 1997). Additionally, research using *D. giffardii*, a generalist ectoparasitoid of many tephritid flies, demonstrated that variation in female parasitoid body size influences host selection behaviour in that parasitoid size is positively correlated with host puparia size selection (Morris and Fellowes 2002; Wang and Messing 2004). Although the aforementioned studies suggest a relationship between host size selection and net fitness gains, none of them have explicitly considered the influence of parasitoid size on adaptive host instar selection. We therefore examined the role of size-mediated host selection by parameterizing a host selection model.

The host selection model suggests that aphid parasitoids should generally not specialize on a single instar and should accept a range of aphid classes that includes 2 or 3 host instar classes under the majority of natural host distributions, unless hosts become very rare in the environment (Fig. 5.4). When considering the rank ordering of instars, parasitoid size has a dramatic effect on instar ranking, due to the influence of body size on handling time and the subsequent profitability of each instar (Fig. 5.1 and 5.2). For example, the average size *Aphidius* parasitoid, selecting hosts from a well defended host such as the pea aphids, should preferentially select 2<sup>nd</sup>, 1<sup>st</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, then 5<sup>th</sup> instar aphids (Fig. 5.2B). This result is consistent with empirical studies on the host-instar preference of aphid parasitoids, which generally prefer a restricted range of small to intermediate sized (2<sup>nd</sup>, 1<sup>st</sup>) instars, suggesting that these parasitoids select host instars optimally in

nature (Kouame and Mackauer 1991; Weisser 1994; Mackauer et al. 1996; Colinet et al. 2005; Tsai and Wang 2002; but see Henry et al. 2005; Henry et al. 2006). This pattern of host instar selection is prevalent in many aphid parasitoid systems even though the larger instars universally produce larger offspring of greater fitness (Chau and Mackauer 2001; Sequeira and Mackauer 1994; Mackauer et al. 1996). Specifically, studies of *Aphidius* parasitoids attacking pea aphids have shown that the early and intermediate stages are often preferentially chosen (Chau and Mackauer 2001; Chau and Mackauer 2000; Henry et al. 2006). These studies suggest that aphid parasitoids do not select instars based solely on offspring quality but instead choose instars that maximize net fitness.

The breadth of acceptable host instars is predicted to change both as a function of parasitoid size and in relation to the density of hosts within the environment as dictated by encounter rates (Fig. 5.4). As host encounters become infrequent, parasitoids are expected to expand their host selection criterion to include less profitable hosts, with larger parasitoids including more instars sooner than smaller parasitoids. As a general prediction larger parasitoids are also expected to always maintain a wider range of acceptable host instars than smaller parasitoids, due to the costs associated with handling well-defended hosts. Karsai et al. (2006) found a similar body size-host selection breadth relationship in a field study using the generalist pompilid wasps, *Anoplius viaticus paganus*, in that larger females were able to handle larger host spiders and thus utilized a wider range of host species. Smaller females were limited in terms of the size of spiders they could subdue, and therefore utilized a restricted range of smaller host species. The aforementioned examples demonstrate how parasitoid body size can influence the range of acceptable host classes or host species in hymenoptera that utilize free-living well-defended hosts.

A direct test of the predictions of our size-mediated host selection model is presented by an independent study performed by Henry et al. (2006). This is one of the only studies to explicitly consider parasitoid size in relation to host-instar preference, using *A. ervi* selecting pea aphids. Our predicted host instar selection, based on body-size mediated optimal host selection criteria, generates a strong qualitative and quantitative correlation with the instars preferentially chosen in this independent experiment. In fact, 70.3% of the hosts that received an oviposition during the study by

Henry et al. (2006) were within the optimal host selection criterion as predicted by our model, which does not account for the possibility of partial preferences, learning or incomplete information that may have occurred during the actual host selection events (Krebs et al. 1977; Krebs 1978).

We further explored the impact of a parasitoid selecting host instars adaptively by implementing a within-generation model using different host densities. Host selection behaviour is an essential component of reproductive success in parasitoid life history; therefore, host size selection has likely been exposed to intense selective pressures resulting in behaviours that maximize net fitness returns. The demographic model provided a direct means of evaluating how different trait combinations (i.e. host selection behaviour and body size) perform over a range of possible host population structures a parasitoid may encounter in nature.

The within-generation model suggests that optimal host selection benefits small parasitoids more than larger parasitoids in the majority of habitats (Fig 5.5). Smaller parasitoids benefit from selecting hosts optimally by narrowing their range of acceptable hosts and selectively ovipositing in early aphid instars that are poorly defended, thereby enabling them to compete with large and medium sized parasitoids in many situations by paying substantially reducing handling costs. When an aphid population is dominated by early instars (new population) or when aphids are abundant (developed population) small parasitoids selecting hosts optimally can accrue fitness rapidly, which is due to the abundance of poorly defended hosts. However, when in a patch dominated by well-defended host, such as in the senescing population, small parasitoids are at a disadvantage, accrue very little fitness and leave the patch early. Therefore, small parasitoids that follow an optimal host selection strategy also have little impact on the recruitment of a senescing aphid population due to the reduced time spent parasitizing in this patch type. Conversely, large parasitoids have the highest performance in populations that are dominated by larger well-defended instars (senescing) due to their ability to effectively handle larger hosts. In general our model predicts that large parasitoids have less of an impact on aphid population recruitment unless large well-defended hosts dominate the populations, and intermediate and small parasitoids will generally reduce aphid population recruitment to a larger degree. Moreover, our results

demonstrate that the maintenance of size-mediated optimal host selection behaviour shifts the host selection strategy of large-bodied parasitoids to larger-sized host classes thereby freeing up small host classes for exploitation by small-bodied parasitoids. Thus, the maintenance of flexibility in host selection behaviour by parasitoids is not surprising given the intimate link between host selection and reproductive success.

Research has demonstrated that body size is positively correlated with fitness in many species of parasitoids (e.g. Visser 1994; Kazmer and Luck 1995; Peterson and Hardy 1996; Roitberg et al. 2001). Larger female parasitoids often have higher egg loads, are longer lived, and have further dispersal capabilities (Eijs and van Alphen 1999). Here we present a relatively novel component of the size-fitness relationship that argues female size is inversely related to the handling time of well-defended hosts, which enables larger females to utilize a wider range of host instars.

Our study suggests that the relationship between parasitoid size and host size can play a crucial role in determining the parasitism rate of hosts of different instar classes and potentially different host species. Furthermore, the demographic model demonstrated that this size mediated relationship also has an impact on within-generation dynamics in that it influences both fitness accrual of parasitoids and aphid population growth, which subsequently could have a profound impact on between-generation population dynamics. Parasitoid populations are often characterized by having a wide range of adult body sizes, especially the solitary endoparasitoids, due to the fixed body size of the adult being determined by the size of their natal host (Harvey et al. 1994; Sequeira and Mackauer 1992). This flexible developmental policy allows generalist parasitoids to utilize a broad range of host species thereby generating a wide size distribution of parasitoids in the next generation that must then compete within the same environment (Sequeira and Mackauer 1993). In size structured host-parasitoid systems individual behaviour can have a significant impact on resource competition between different consumer groups generating resource or habitat partitioning (Persson et al. 1998) and has the capacity to generate non-linear population dynamics through the preferential selection of specific instar or host classes (Mangel and Roitberg 1992).

The work presented here highlights the complexity of behavioural (within-generation) dynamics that can be lost in (between-generation) population dynamic models (Roitberg and Mangel 1992; Bolker et al. 2003; Ma et al. 2003; Persson and De Roos 2003). Adaptive host selection (or in the case of predator-prey interactions, adaptive diet choice) is thought to be a stabilizing process (Comins and Hassell 1973; Fryxell and Lundberg 1994). However, in our study, it is unclear how this interaction between parasitoids and hosts will influence population dynamics because of the size-mediated nature of host selection. Larger parasitoids will generally select larger hosts thereby decreasing the risk of parasitization that smaller hosts face. This creates a 'temporal refuge' for hosts (that the smaller instars leave as they develop into larger instars). However, the presence of smaller parasitoids may mitigate this effect, as they will generally select smaller hosts. Temporal refuges will create time-lagged responses by parasitoids (or predators), which may destabilize population dynamics (Gurney and Nisbet 1985; Ma et al. 2003; Pachevsky et al. 2008). The influence of this trait-mediated interaction on between-generation dynamics has yet to be fully explored.

The use of empirically derived data in combination with models presented in this article has great potential to generate testable hypotheses on the dynamics of size-structured populations in host-parasitoid communities. However, we have only considered the host-parasitoid interaction within a single generation to demonstrate the importance of within-generation trait interactions on population dynamics. To obtain a greater understanding of how variation in host-selection behaviour impacts lifetime fitness and host-parasitoid population dynamics the theoretical framework generated by our within-generation model should be projected to between-generation population dynamics and must be extended to a multi-patch universe.

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**Table 5.1** Stage structuring of host populations described as the number of individuals of each instar for three different types of aphid population.

POPULATION TYPE		POPULATION STRUCTURE				
	Instar	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
<b>New</b>	Number of	50	5	0	0	5
<b>Developed</b>	Individuals	150	80	50	40	20
<b>Senescing</b>		10	10	20	40	30

**Table 5.2** Size (hind tibia length,  $\mu\text{m}$ ) as a proxy for fitness of parasitoid offspring developing in the 5 instar classes of the pea aphids. Letters signify results that are significant at a 0.95 level.

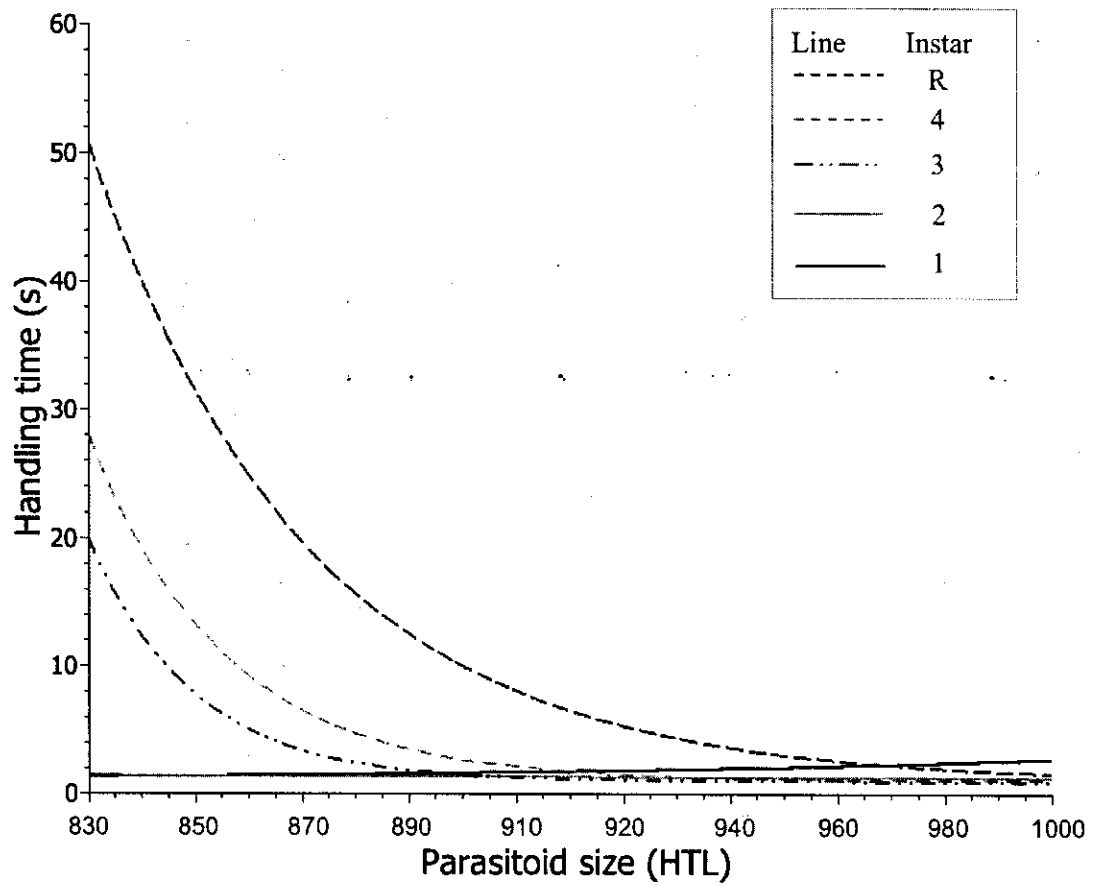
<i>I</i>	$w_i$	$1 - e_i$	$w_i^\circ$
1	822.99 <sup>a</sup>	1	822.99
2	830.37 <sup>a</sup>	1	830.37
3	895.02 <sup>b</sup>	0.86	769.72
4	946.03 <sup>b</sup>	0.84	794.66
5	888.44 <sup>ab</sup>	0.44	390.91

**Table 5.3** Total number of time steps spent foraging before leaving a patch (patch residence times) for a small (830 $\mu$ m), mean (910 $\mu$ m), and large (990 $\mu$ m) parasitoid on three different aphid population structures.

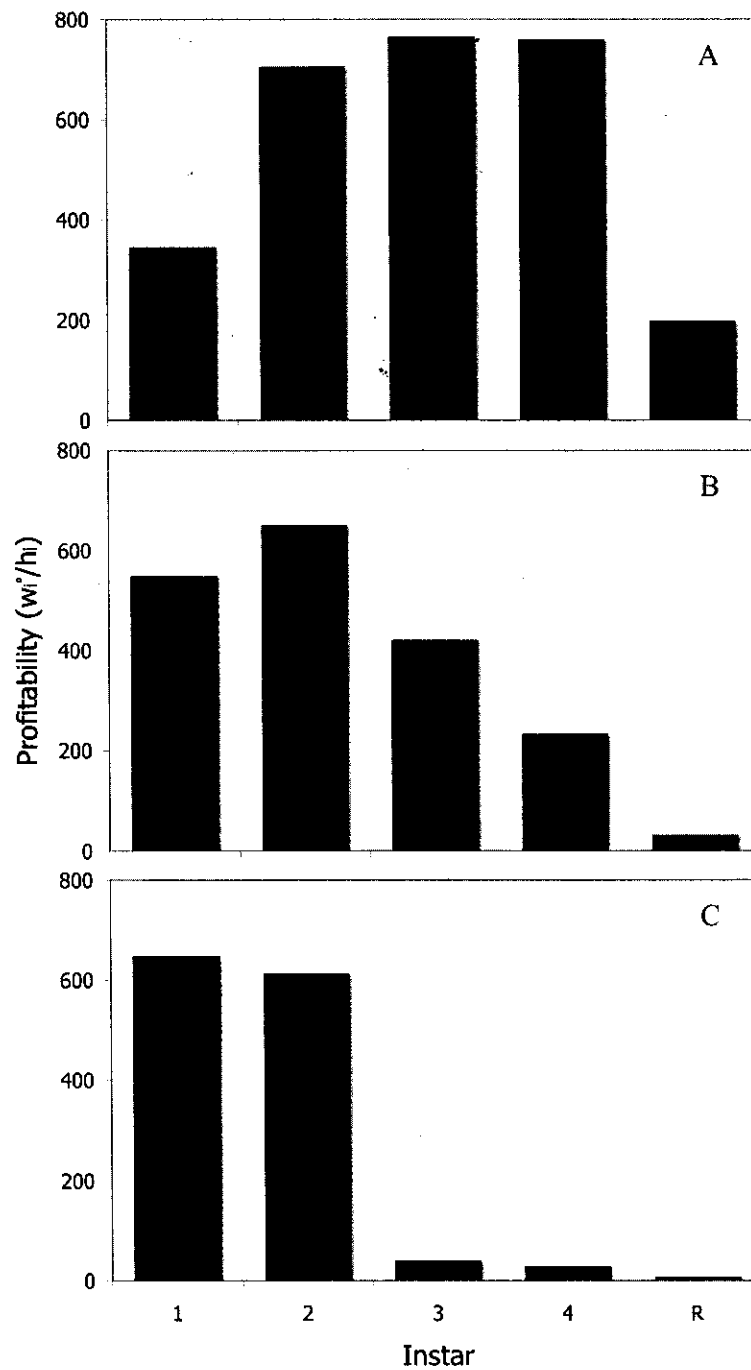
PARASITOID SIZE	POPULATION STRUCTURE		
	NEW	DEVELOPED	SENESCING
<b>830</b>	273	304	233
<b>910</b>	272	372	389
<b>990</b>	274	376	403



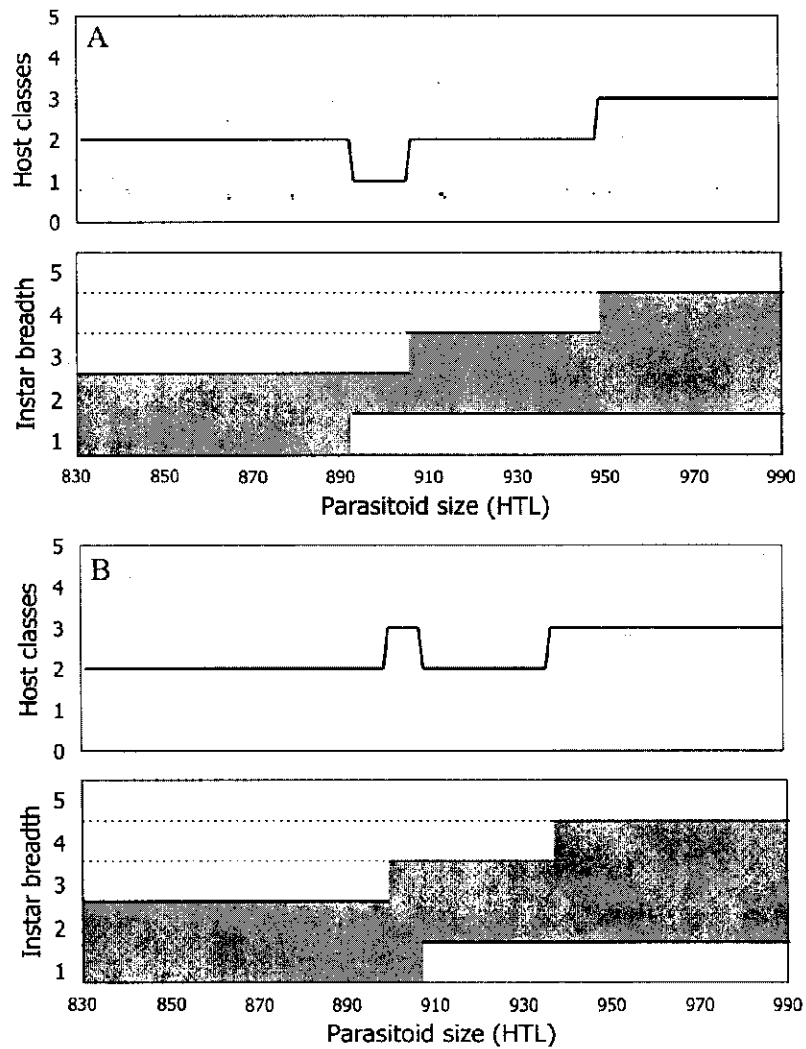
**Figure 5.1** Time required to handle and successfully oviposit in the 5 pea aphid instars over a range of parasitoid sizes.



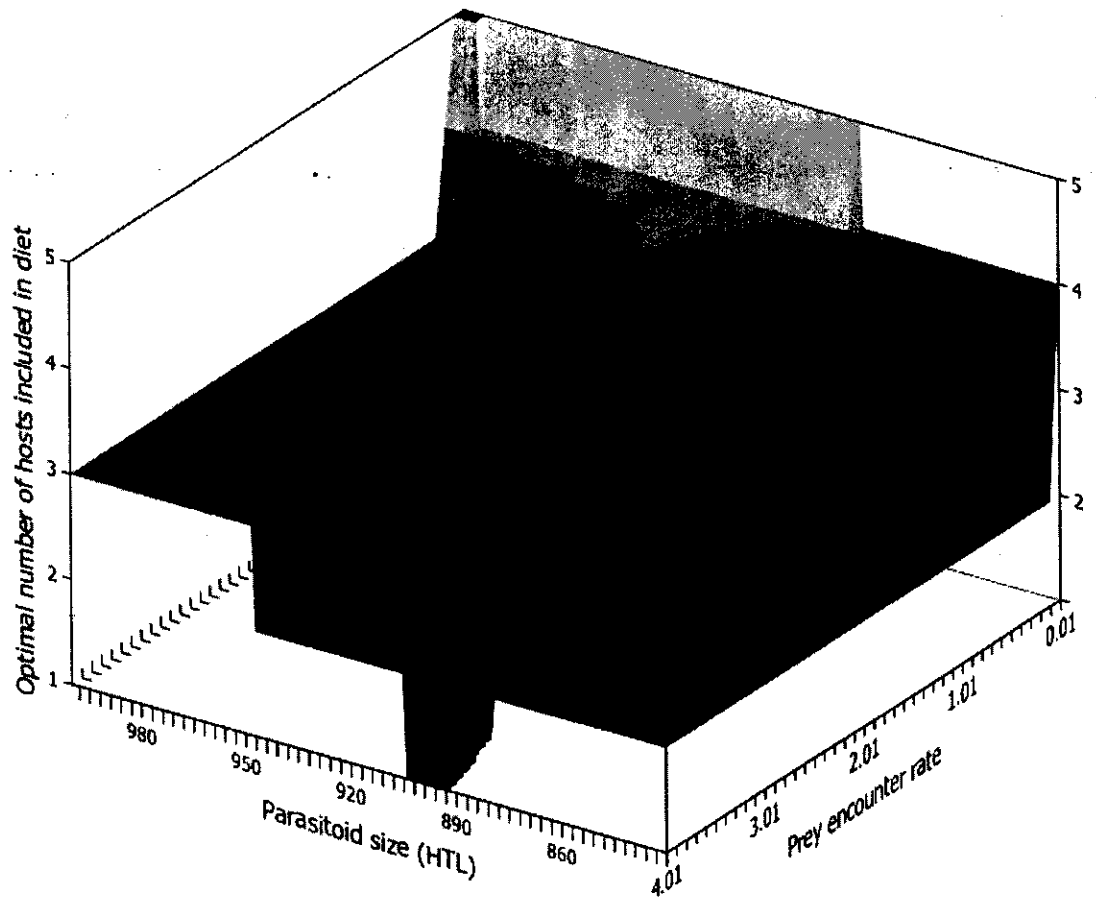
**Figure 5.2** Profitability of the 5 pea aphid instars for three different parasitoid size classes. Letters represent parasitoids of different size classes (hind tibia lengths): A) 830 $\mu$ m, B) 910 $\mu$ m and C) 990 $\mu$ m.



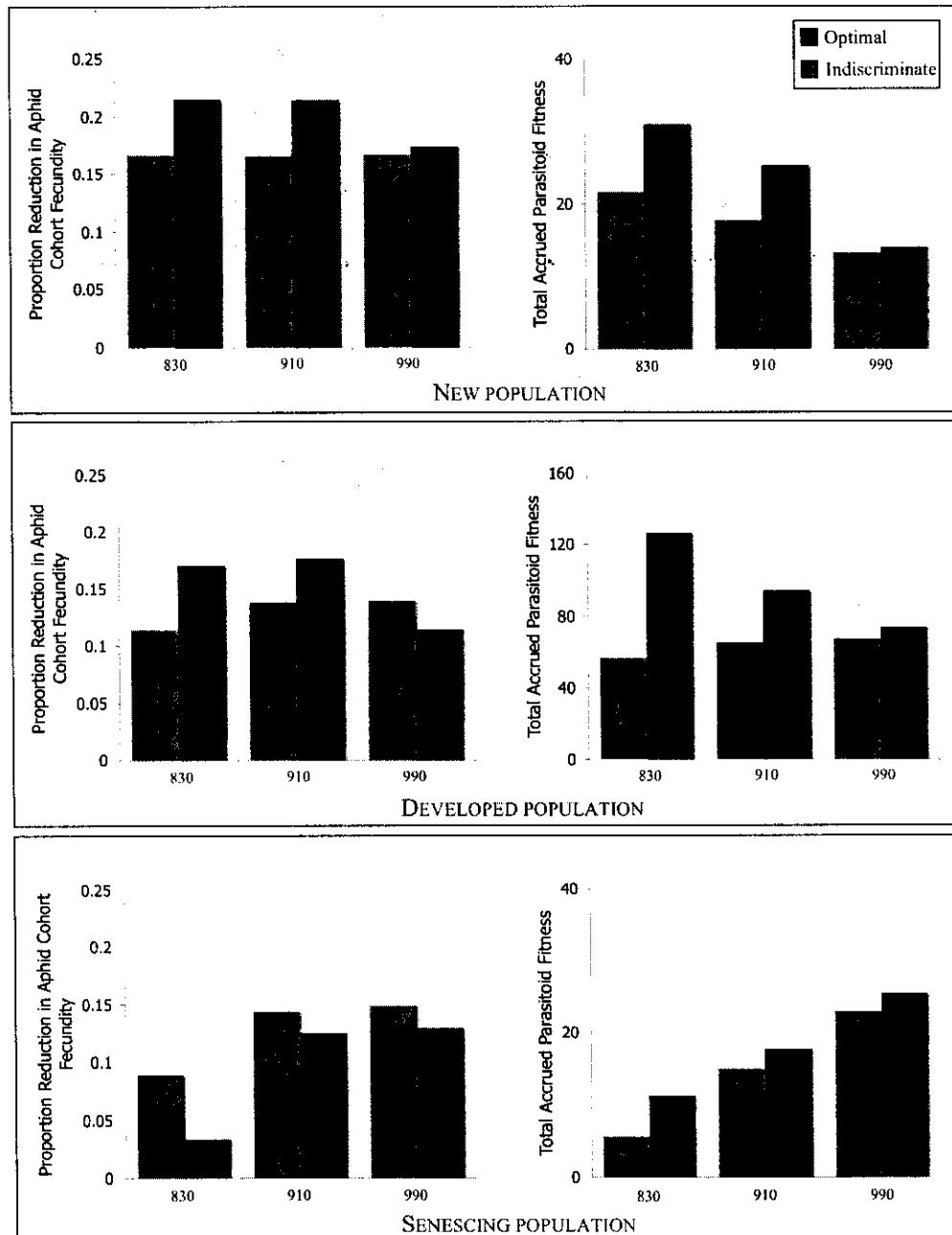
**Figure 5.3** The range of instars accepted by a parasitoid using an optimal host selection strategy in relations to the number of host classes accepted at two different encounter rates, 4.01 (A) and 2.01 (B). X-axis depicts the size of the parasitoid, with the Y-axis demonstrating how the number of host classes accepted (upper panels) relates to the actual instars (lower panels) accepted (grey region) for a parasitoid of a particular size (hind tibia length  $\mu\text{m}$ ).



**Figure 5.4** Predicted influence of parasitoid size (x-axis) on optimal diet breadth (z-axis) over a range of host-encounter rates (y-axis). Encounter rates are equal across instars (i.e.  $\lambda_1 = \lambda_2 = \dots = \lambda_5$ ). Number of hosts included in diet refers to the breadth of hosts a parasitoid should include in order to optimize fitness returns and does not specifically refer to instars accepted. Parasitoid size is measured in hind tibia length ( $\mu\text{m}$ ).



**Figure 5.5** The proportion reduction in aphid recruitment and the net fitness accrued by a single parasitoid foraging on an aphid patch. The optimal foraging model is compared to the null model of an indiscriminate forager on three different stage-structured aphid populations representing stages of aphid population development, from a newly formed population to a senescing population.



# **CHAPTER 6: ASSORTATIVE MATING AND THE ROLE OF PHENOTYPIC PLASTICITY IN MALE COMPETITION: IMPLICATIONS FOR GENE FLOW AMONG HOST- ASSOCIATED PARASITOID POPULATIONS<sup>6</sup>**

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## **6.1 Abstract**

Habitat or mate preference generates non-random gene flow between populations that can promote local adaptation. However, gene flow is not only a function of dispersal but also of the success of the migrants in their new habitat. In this study I investigated mating preference in conjunction with phenotypic plasticity using parasitoids adapted to different host species. Males actively attempted to assortatively mate but actual mating outcomes were strongly influenced by the relative size of the adult males. Results are discussed in the context of the importance of assortative mating in combination with the success of migrant males in mitigating gene flow between host-associated parasitoid populations.

## **6.2 Introduction**

The diversity of parasitic insects is often attributed to the intimate relationship they share with host species, and the potential for disruptive selective pressures associated with different hosts to drive ecological divergence. Population divergence is further promoted if selection on traits between host-associated populations leads directly or indirectly to a reduction in gene flow between populations, thereby facilitating reproductive isolation. Reproductive isolation can evolve indirectly as a by-product of selection on individual traits, or through direct selection on premating isolation if adaptation to one host is maladaptive with respect to fitness on another in which case host specialization, on patch mating or assortative mating may be favoured (Schluter 2001).

Development in contrasting environments can lead to phenotypic differences that influence the acquisition of resources, such as food or mates. For example, divergence in body size and subsequent differences in mate acquisition has been shown to contribute to premating isolation through mechanisms such as size-assortative mating (Nagel and Schluter 1998). Body size is positively correlated with mating success and is a predictor of mate quality in a wide range of organisms (Peters 1983). In insects, body size is an indicator of fecundity in females and is positively correlated with mating success in males (Bonduriansky 2001).

In this article I investigate the influence of adaptation to different host species on the development of mating outcomes in an aphid parasitoid. Laboratory reared populations of *Aphidius ervi* (Haliday) were maintained on pea *Acyrtosiphon pisum* (Harris) or foxglove aphids *Aulacorthum solani* (Kaltenbach) for a period of 2 years (~50 parasitoid generations), by which time the populations diverged in several traits including reproductive fitness (Henry et al. 2008). Furthermore, parasitoids maintained plasticity in traits such as adult body size. Adult body size is a highly flexible trait in *Aphidius* parasitoids that is determined by the size of their natal host. Male size influences mating success in parasitoids systems (Teder 2005), however few studies have addressed the influence of determinant growth on mating success between host-associated populations. The objectives of the following study were to determine:

1. Does adaptation to different host species influence mating preference in *Aphidius* parasitoids?
2. What is the influence of adult body size on mating success between host-associated parasitoid populations?

## **6.3 Materials and Methods**

### **6.3.1 Insect stock and selection lines**

*Aphidius ervi* were maintained in isolation on, foxglove or pea aphids, hereby denoted F-line and P-line parasitoids, respectively. Eight F-line populations were maintained on *A. solani* feeding on *Capiscum annuum* (Solanaceae) and 12 P-line populations were maintained on *A. pisum* on *Vicia fabae* (Fabaceae). For more information on the initiation and maintenance of host selection lines please refer to Henry et al. 2008.

### **6.3.2 Mating experiment**

Parasitoids were 1-2 days-old virgins, isolated in emergence containers, given access to only dilute honey and water. Individual females were placed in an arena with 1



natal host male and 1 non-natal host male. The mating arena consisted of a 40mm aerated Petri dish with the floor cut out, resting on a leaf corresponding to the female's natal host plant. Females only ever mated once within the 15-minute mating trial.

Mating events were partitioned into, female individual interactions with a single male (female receptiveness), male preference for female by host type and male's competing for the mating event when the female had arrested and remained passive (male competition). I measured female receptiveness as the latency from initial mount to female arrestment (pre-copulatory struggle) (ANOVA) and the probability of a male type being rejected (CHI-SQUARE). Male preference was measured as the number of attempted and successful mounts combined (CHI-SQUARE). Male competition occurred after the female had arrested, at which time both males frequently compete for copulatory privileges. To account for the sizes of the two competing males the difference in their dry weights was compared to the probability of mating successfully using a logistic regression. The probability of a male usurping another previously mounted male to win a mating contest was investigated (CHI-SQUARE). Overall mating outcomes were compared using a log-linear analysis with female host, male host and female male interaction as factors influencing the outcome. All Chi-square analyses included the four possible host population mating combinations.

One possible confound in my experimental analysis of body size is host species effect *per se*. Thus, to determine the influence of male size on competition, without the influence of different natal host species, the above experiment was repeated using parasitoids reared in pea aphids only.

### **6.3.3 Insemination**

Production of female offspring was used to confirm that insemination was successful and that females would utilize sperm from males that inseminated them. *Aphidius* parasitoids are haplodiploid thus female wasps can choose whether or not to fertilize an egg. Females develop from fertilized eggs, males from unfertilized. Post-copulation females foraged on 40 natal host aphids (3 h). Proportion insemination was

analyzed for the four possible mate pairings (CHI-SQUARE). JMP 6.0 statistical software (SAS Institute, Cary, NC, USA).

## 6.4 Results

### 6.4.1 Mating experiment

The log-linear analysis produced a final model retaining only male host species (LOG-LINEAR LIKELIHOOD RATIO,  $\chi^2_{(2)} = 3.55$ ,  $P = 0.169$ ). Female host and female-male host species interaction did not significantly influence mating success with male host explaining the greatest variation ( $\chi^2_{(1)} = 6.908$ ,  $P = 0.009$ ). P-line males won more mating outcomes, 66%, compared to F-line males, 33%, irrespective of female host population (Fig. 6.1). To determine if females were preferentially selecting P-line males, latency of the pre-copulatory struggle was analyzed for each male by host species (ANOVA, male host  $F = 1.53$ ,  $P = 0.23$ ; female host  $F = 0.26$ ,  $P = 0.61$ ; male-female  $F = 1.19$ ,  $P = 0.28$ ). Furthermore, the proportion of failed mounts by males did not differ ( $\chi^2_{(1,26)} = 0.22$ ,  $P = 0.64$ ). These results indicate that female mate choice is not responsible for P-line males winning more mating events.

Significant variation was found in male mating attempts ( $\chi^2_{(1,125)} = 7.734$ ,  $P = 0.0054$ ), with parasitoids attempting to mate assortatively 69% and 88% of the time for F and P-lines respectively. P-line males on average were much larger than F-line males (mean dry weight: F-line  $0.053 \pm 0.015 \mu\text{g}$ ; P-line  $0.11 \pm 0.041 \mu\text{g}$ ). The difference in size between males influenced the probability of a P or F-line male winning a mating contest (LOGISTIC REGRESSION,  $\chi^2_{(1,45)} = 11.09$ ,  $P = 0.0009$ ) (Fig. 6.2A). Female size had no influence on the probability of a P or F-line male successfully mating ( $\chi^2_{(1,39)} = 0.041$ ,  $P = 0.84$ ). The ability to usurp a previously mounted male was influenced by male host species ( $\chi^2_{(1,23)} = 3.89$ ,  $P = 0.048$ ). P-line males usurped F-line males 40% of the time, F-line males usurped P-line males 7.1% of the time.

The second mating experiment with parasitoids reared exclusively in pea aphids revealed that body size played a significant role in a male's ability to win mating events when in competition (LOGISTIC REGRESSION,  $\chi^2_{(1,25)} = 4.81$ ,  $P = 0.028$ ) (Fig. 6.2B).

#### 6.4.2 Insemination

Post-copulated females produced female offspring  $81.43 \pm 8.65\%$  of the time. No difference was found in the number of female offspring produced when comparing the four possible mating pairs ( $\chi^2_{(3,39)} = 1.428$ ,  $P = 0.69$ ). This confirmed there was no post-copulatory mate choice by females.

### 6.5 Discussion

Parasitoid host selection lines differed in the proportion of successful mate pairings (Fig. 6.1). Female mate choices as evaluated by the number of rejected males and latency associated with the pre-copulatory struggle indirectly indicated that females are not actively choosing males. Furthermore, following the pre-copulatory struggle, females remained passive during which time males frequently competed for access to the female by grappling until one male successfully acquired a copulatory lock with the female. This suggests that female mate choice within this system is most likely superseded by male competition. There is the possibility that females may exhibit cryptic male choice in that they have the option to utilize a male's sperm to manipulate sex ratios, however the insemination results indicated that females were equally likely to use at least a portion of sperm to produce female offspring irrespective of the male's natal host population.

Males attempted to mate with females from the same host population more frequently than from the non-natal host population (69% and 88% for F and P-lines, respectively). Male mate choice is widespread in insects and is predicted to be favoured when there is high variance in quality among individual females (Parker 1983). In parasitoid systems where the reproductive success of a male is completely mediated by the female's ability to successfully oviposit daughters, choosing high quality females may have evolved as a means of maximizing male fertilization success (Bonduriansky 2001).

Although males actively attempted to mate assortatively, the asymmetric skew of the mating outcomes suggests there is more to mating success than purely male choice. P-line males mated assortatively 74.2% of the time, compared to F-line males that successfully mated assortatively only 45.1% of the time (Fig. 6.1). When considering the probability of mating successfully based on male body size (Fig. 6.2A), coupled with the average size deviation between the larger P-line compared to the smaller F-line reared males, it appeared that male body size was a factor contributing to P-line males winning a greater number of competitive events. The role of body size was demonstrated using males reared in a single host species (i.e. pea aphids), in that similar sized males have an equal probability of winning a mating event (Fig. 6.2B, left side x-axis), however as the deviation in sizes increased between the two competing males the larger male increased its probability of successfully mating. This demonstrated that body size plays a key role in male competition. Furthermore, larger P-line males had a much greater proportion of usurping events where the male physically removed the other, previously mounted male, or in several cases actually mated overtop the smaller F-line males to win the mating event.

Previous studies have suggested that *Aphidius* parasitoids exhibit some mate choice, in that mating success has been shown to be greatest when individuals are from the same host species population (Powell and Wright 1988). The results from this study demonstrate that it is male *A. ervi* that actively attempt to mate assortatively. In addition, the influence of host-determined body size presents a layer of complexity that has important implications for gene flow between host-associated parasitoid populations. Parasitoids are known to exhibit different degrees of host fidelity, however in many species at least a portion of males disperse to local patches (Hardy et al. 2005). My results suggest that when host-associated conspecific parasitoid populations exist in sympatry, a male's natal host will undoubtedly influence its ability to exploit neighbouring female populations. This presents an interesting scenario wherein the potential for asymmetric gene flow between populations could exist due to the success of migrant males. Parasitoid populations associated with large natal hosts are likely to resist gene flow from parasitoids immigrating from smaller host species due to the advantage large males have in competitive mating on their natal host patch. Furthermore, large

males gain an advantage when dispersing in that they can out-compete smaller males for access to females in a non-natal host patch, making parasitoids utilizing smaller host species more susceptible to having local adaptation slowed or disrupted by the influx of genes from neighbouring populations.

Host-associated genetic divergence has been reported in sympatric aphid parasitoid populations (Vaughn and Antolin 1998). A substantial amount of research has focused on host preferences and host fidelity in females (Godfray 1994), which are mechanisms potentially conserving host-adapted gene pools. Although the aforementioned processes theoretically contribute to local adaptation in host-associated populations, the impact of mating preference coupled with the success of migrant males has virtually been ignored in parasitoids despite its important in mitigating gene flow. Assortative mating in conjunction with phenotypic plasticity in male mating success thus represents a novel mechanism influencing the movement of genes between host-associated parasitoid populations.

Although male-based assortative mating occurred within this study, under the present experimental design it was not possible to determine the signal dictating the male response. Males may have been size-assortatively mating, have evolved mating preferences as a by-product to different selective regimes, or may have conditioned responses based on preimaginal or early emergence learning. Further research is required to determine whether male mating preference is due to genetic changes brought about by adaptation to a host species or preference for a particular phenotype induced by developing in a host for a single generation.

## **6.6 Acknowledgements**

I would like to thank Eva Ravenstein, Bernie Roitberg, David Gillespie and the Roitberg lab group for support and advice as well as NSERC and the BC Greenhouse growers for funding.

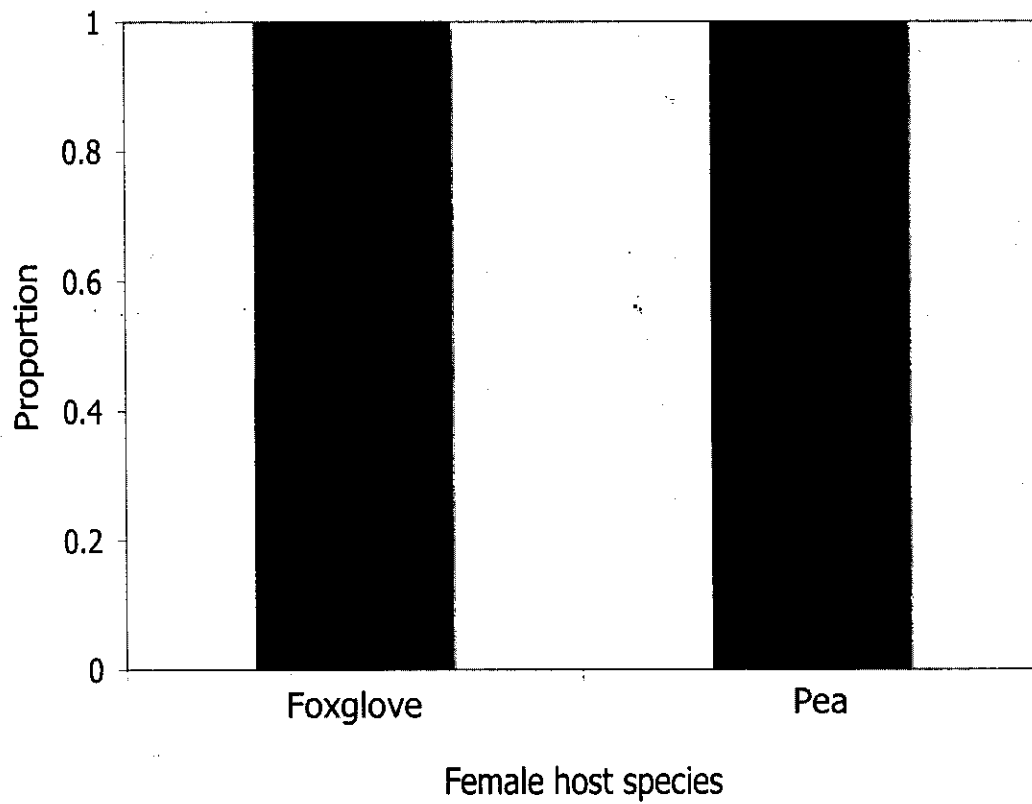
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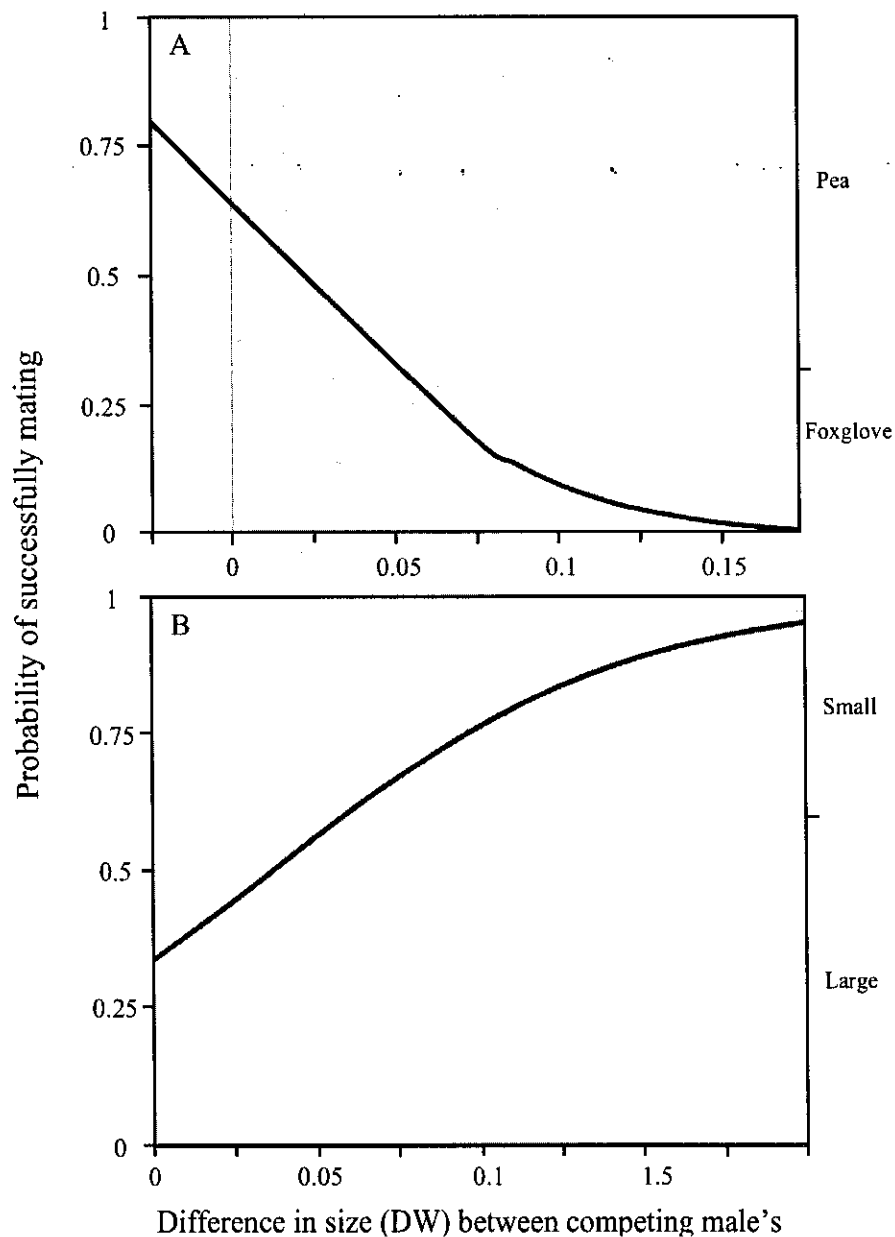
Vaughn, T. & Antolin, M. 1998 Population genetics of an opportunistic parasitoid in an agricultural landscape. *Heredity* 80, 152-162.

**Figure 6.1** Proportion assortative (black) and disassortative (grey) mate pairing outcomes by female host species (x-axis) when 2 males (1 natal host species and 1 non-natal host species) compete for access to a single virgin female.





**Figure 6.2** Logistic regression plot describing the probability of winning a mating contest in relation to the difference in size (DW - dry weight) between the two competing males (x-axis) for competing F and P-line males (a) and males reared only in pea aphids (b). Weight difference = (F-line) - (P-line). The 0 line (a) represents parasitoids of equal size, with values to the right indicating P-line males are larger and to the left F-line males are larger.



## CHAPTER 7: GENERAL CONCLUSIONS

## 7.1 Summary of Main Findings

Although there has been great progress in understanding the ecology and evolution of host use in phytophagous insects, little is known about the processes that mediate host range evolution in insect parasitoids. Despite the lack of knowledge regarding the actual mechanisms that mediate population and species divergence in parasitoids there is evidence that suggests host-affiliation may be responsible for driving differentiation of lineages (Fellowes and Kraaijeveld 1998; Dupas *et al.* 2003; Powell and Wright 1988; Pike *et al.* 1999; Morehead *et al.* 2001; Aldrich and Zhang 2002; Stireman *et al.* 2006). Highly specialized traits designed to cope with host defences and locate concealed hosts combined with the intimate relationship parasitoids share with particular host species suggests that host-parasitoid co-evolution may play an important role in the diversification of parasitoid communities. However, the value of parasitoid systems for the study of host-specialization and speciation is hampered by our lack of a quantitative understanding the sources that generate genetic diversity and the mechanisms that maintain this diversity. The work presented in this dissertation contributes to our basic understanding of the mechanisms that lead to and maintain biological diversity in insect parasitoids.

A fundamental hypothesis surrounding the evolution of specialization in all organisms is that no single genotype is uniformly superior in all environments. The importance of trade-offs in the evolution of specialization is currently in flux, primarily due to the lack of a consensus in the empirical literature. A significant contribution to this fundamental evolutionary process was developed through my doctorate research by investigating the physiological mechanism involved in overcoming host defences to determine this process is under directional selection. Results demonstrate that host species can act as agents of directional selection in insect parasitoids. Furthermore, the results demonstrated a trade-off in the ability of a population of parasitoids to simultaneously maximize fitness on more than one host species, which suggests a genetic basis for host utilization that may limit host range expansion and promote specialization in parasitoids. This work presents the first account of a selection experiment demonstrating an adaptive shift in host use by an insect parasitoid that coincides with a

loss of fitness on the ancestral host species, and one of the few studies for any case of trade-offs in the evolution of host specialization.

The population level outcome of processes driving diversification, such as trade-offs or selection, is dependent on the system-specific mechanisms that impact gene flow. Host fidelity, mate choice, and host-instar selection are important mechanism that can substantially reduce gene flow between populations through processes such as assortative mating or through the success of migrants moving between different host species patches. Behaviours involved in the aforementioned processes were investigated to determine their importance in mediating gene flow between parasitoid populations utilizing different host species. Results demonstrated that mechanisms exist in *Aphidius* parasitoids that act to restrict gene flow between host-associated populations. Host fidelity is an important mechanism that can contribute to the conservation of locally adapted gene pools if mating occurs on the host. Thus, parasitoids exhibiting higher fidelity for the host species in which the parasitoids emerge, as described in chapter 2, can lead to reproductive isolation through on-patch mating prior to dispersal. Parasitoids were also found to assortatively mate, which is another mechanism integrally involved in promoting adaptation through the conservation of locally adapted gene pools.

The success of migrant individuals that are moving between different host species patches was also investigated, as gene flow is not only mediated by dispersal but also by the performance of individuals in their new environment. Mating success in males was significantly influenced by the host species in which the parasitoids were reared, which has important implications for the directionality of gene flow. Research investigating host instar selection in females demonstrated that development in a particular host dramatically influences host-size selection, which can lead to maladaptive host instar selection if parasitoids are switching host species between generations. Typically, parasitoids select host instars optimally when foraging on the same host species patch in which they developed, thus host selection is conditioned for the host species in which the parasitoid developed but can lead to a reduced performance of individuals migrating to alternate host species patches. The foraging model presented in chapter 5 demonstrates that within-generation foraging behaviour, such as adaptive or maladaptive host-instar selection (i.e. the indiscriminate forager), can have important consequences for within

generation species dynamics. Thus the decision made within a parasitoids lifetime, such as the utilization of adaptive or maladaptive host-instar selection strategies, can influence essential components of parasitoid and host life history traits that have important implications for community and evolutionary dynamics.

A primary objective in biology is to understand the adaptive significance of phenotypic plasticity. My doctorate research demonstrated that extensive phenotypic plasticity, induced by development in a particular host species, has a dramatic impact on a parasitoids behaviour and morphology. One major finding was the correlation between behaviours and the highly plastic developmental strategy exhibited by many parasitoid species, which resulted in an adaptive shift in host size selection. This work represents one of the first accounts of an adaptive adjustment in host instar selection behaviour that correlates with a parasitoids adult body size. Additionally, this research introduced a novel theory relating host-determinant growth to behaviours that are critical for the movement of genes between host-associated parasitoid populations. The role of host-determinant growth in mating success demonstrated that although males actively attempted to assortatively mate actual mating outcomes were strongly influenced by the relative size of the adult males, with the males from a population of larger host species winning a greater number of mating events. This demonstrated the importance of assortative mating in combination with the success of migrant males in mitigating gene flow between host-associated insect populations. In combination these studies further contribute to our basic understanding of the mechanisms that generate and maintain genetic diversity in insect parasitoids.

## **7.2 Future Work**

Whether divergent natural selection can promote speciation (i.e. ecological speciation) is no longer in question thanks in part to a number of convincing examples (reviewed in Schluter 2001). However many details of the process remain unknown, and this is especially true for the insect parasitoids. Evidence of host-associated differentiation is mounting and the process of divergence by host-affiliation may be more common than previously thought (Stireman 2005), especially for the insect parasitoids

(Stireman 2006; Smith et al. 2007). The research presented in this dissertation is a necessary first step to isolating the role of specific mechanisms responsible for generating and maintaining diversity in parasitoids. However, the maintenance of genetic differentiation between populations requires specific environmental conditions, such as stable host populations and limited gene flow, which will be specific to each system. Host-parasite systems that are prone to disturbances or those that experience extensive gene flow may have specialization disrupted through the obliteration of locally adapted gene pools. Therefore, in order to gain a better understanding of how these mechanisms function in nature future research should concentrate on linking processes such as those presented in this dissertation to the actual genetic structure of natural populations utilizing different host species through the use of comprehensive population genetics surveys.

Very few studies have investigated host-based population divergence in aphid parasitoids. Vaughn and Antolin (1998) demonstrated the potential for host-based divergence in populations of *Diaeretiella rapae* from two adjacent fields containing Russian wheat aphids and cabbage aphids. However, a follow up study pointed towards incomplete adaptation in *D. rapae* (Antolin et al. 2006). Microsatellite markers have been developed for *A. ervi* (Hufbauer et al. 2001), so the potential to address host-affiliated differentiation through population genetics in *A. ervi* exists. Daza-Bustamante and associates (2002) attempted to study genetic differentiation of *A. ervi* populations utilizing different hosts, unfortunately this study only utilized mitochondrial markers and was conducted on populations of *A. ervi* that had been introduced to the region for less than 50 years. In order to address host-affiliated genetic differentiation researchers should utilize more discriminant genetic markers and concentrate on populations of parasitoids from native regions or at least those that have had a sufficient amount of time that the observed differentiation is at least in part due to the current conditions the populations are under and not a relic of the genetic variation from the initially introduced population. Furthermore, the majority of studies addressing host-associated differentiation in aphid parasitoids have been carried out in agricultural environments. Agricultural settings are not ideal conditions for the study of host-based adaptation, as populations diverge through the accumulation of traits arising from differential selective

pressures exerted over many years. This most commonly occurs with organisms that have occupied an ecosystem that is not prone to yearly disturbances, such as crop rotations.

The parasitoids represent a potentially rich taxa for studying host-affiliated ecological divergence, however the insects and hosts studied to date represent only a fraction of the tremendous diversity of parasitic relationships that exist. In general a greater number of studies concentrating on the mechanisms mediating population divergence and the actual genetic structuring of parasitoid and host populations are required for further development of parasitoids as a model system in speciation biology. For aphid parasitoids in particular, a greater number of population genetics studies that take into account the conditions that will promote or disrupt local adaptation, such as the origin of the parasitoid population and the ephemerality of host or host plant populations, will undoubtedly increase the value of these systems for the study of specialization and host-affiliated diversification.

### 7.3 References

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