COMPARATIVE HOST STAGE UTILIZATION OF TWO PARASITOIDS OF *Liriomyza brassicae* (DIPTERA : AGROMYZIDAE)

BY

ROSS MICHAEL LARDNER

B. Sc. (Hons) Macquarie University

A thesis submitted for the Degree of Doctor of Philosophy in the Faculty of Agricultural and Natural Resource Sciences at The University of Adelaide.

Department of Crop Protection
Waite Agricultural Research Institute
The University of Adelaide

November 1991

To My Family

TABLE OF CONTENTS

CHAPTER FOUR. ATTACK OF HOST STAGES BY NAIVE FEMALES OF *Hemiptarsenus varicornis* AND *Opius cinerariae*

CHAPTER FIVE. ATTACK OF HOST STAGES BY EXPERIENCED FEMALES OF *Hemiptarsenus varicornis* AND *Opius cinerariae*

REFERENCES

ADDENDUM TO THESIS

LIST OF FIGURES

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

CHAPTER SEVEN

CHAPTER EIGHT

APPENDIX ONE

APPENDIX TWO

APPENDIX THREE

APPENDIX FOUR

APPENDIX FIVE

LIST OF TABLES

CHAPTER EIGHT

APPENDIX ONE

APPENDIX TWO

APPENDIX THREE

APPENDIX FOUR

APPENDIX FIVE

SUMMARY

Natural selection theory predicts that parasitoids should evolve a strategy of host utilization that maximizes fitness. This prediction was tested for two parasitoids, a koinobiont *Opius cinerariae* Fischer (Hymenoptera : Braconidae) and an idiobiont *Hemiptarsenus varicornis* (Girault) (Hymenoptera : Eulophidae), attacking the cabbage leafminer, *Liriomyza brassicae* (Riley) (Diptera : Agromyzidae).

Initial studies investigated the biology of *L. brassicae* in the laboratory and its phenology and host plant utilization in the Adelaide region. The complex of parasitoids attacking *L. brassicae* was determined. Field sampling investigated the phenology, host stage utilization and host plant associations of these parasitoids.

Later research focused on *H. varicornis* and *O. cinerariae* and determined the levels of parasitism and parasitoid related mortality, and allocation of sexes in the three host instars. Fitness was estimated by measuring the developmental time, size, longevity, egg load and mating success of individuals developing from each host instar.

H. varicornis emerged from only 2nd and 3rd instar hosts, with greatest parasitism occurring on 3rd instars. Sex ratios were male-biased on 2nd instars and unbiased on 3rd instars. Fitness was greatest for progeny from 3rd instar hosts. Females gained more from developing from 3rd instar hosts than males. *H. varicornis* demonstrated a strategy of host utilization similar to that predicted by the host-size model of Charnov *et al.* (1981).

O. cinerariae emerged from all three host instars, with parasitism increasing significantly in later stage hosts. Sex ratios were generally female-biased from all three host instars. Progeny size increased directly with host stage but no other fitness component showed this relationship. Males developed faster while females lived longer. Host utilization by *O. cinerariae* did not conform to the predictions of the host size model (Charnov *et al.* 1981). The female-biased sex ratios of *O. cinerariae* may indicate a weak local mate competition effect, differential mortality of the sexes or a fixed strategy representing greatest average fitness on a highly variable host population.

This study demonstrated that different selection pressures may influence the host utilization of idiobiont and koinobiont parasitoids. Among these pressures, the relationship between host stage at oviposition and fitness appeared to be particularly important.

DECLARATION

The work presented in this thesis is my own unless otherwise acknowledged, and has not previously been published or submitted to any university for the award of any degree or diploma. This thesis may be made available for loan or photocopying provided that an acknowledgment is made in the instance of any reference to this work.

(Ross M. Lardner)

(November 1991)

ACKNOWLEDGMENTS

I would also like to thank those people who contributed indirectly to my studies. Dinah Hales provoked my interest in insects and encouraged me to undertake a Ph. D. at the Waite. At one time or another throughout my studies, various friends kept me in good spirits by providing a much needed escape from work pressures. These include Huelyn Westby-Hannah, Wayne Peters, Christine Williams, Geraldine Crowe and Adrienne Nieuwenhuis. Finally, I am indebted to my family, particularly my parents, for their love, support (emotional and financial!) and unwavering belief in my abilities.

CHAPTER ONE

INTRODUCTION

Parasitoids play an important role in the regulation of many insect populations (Hassell and Waage 1984). They have been used in numerous biological control programs for insect pests, with varying levels of success (Huffaker and Messenger 1976; Waage and Hassell 1982; Greathead 1986). Why some parasitoids are successful biological control agents while others are not is yet to be fully understood. Numerous factors can influence the efficacy of a biological control agent, including the structure of the host population, environmental conditions and the biology and behaviour of the species of interest (Huffaker and Messenger 1976). To identify characteristics of parasitoids which make them successful biological control agents, further understanding of the biology and behaviour of these insects is needed (Luck 1990).

The importance of evaluating natural enemies prior to their release or acceptance as a biological control agents has been questioned. van Lenteren (1980) suggested that long-term basic research of parasitoid biology was unlikely to lead to the development of more efficient biological control. However, usually some knowledge of a parasitoid's biology is required before it can be used as a control agent. These include its host range, environmental requirements and host utilization strategy, i.e. how progeny and sexes are allocated to different host stages or types (Huffaker and Messenger 1976; van Lenteren 1980; Ehler 1990; Howarth 1991). Knowledge of a parasitoid's host utilization strategy is important for (i) the mass rearing of parasitoids for field releases and experimental purposes, (ii) determining the

optimum time to release the parasitoid in the field in relation to the host population, and (iii) predicting how a particular parasitoid species may perform in the field.

Determining the host utilization strategy of a parasitoid species has proved relatively simple, particularly if it is investigated in relation to some host characteristic. For example, numerous studies have considered parasitism of hosts of different sizes (Opp and Luck 1986; Simbolotti *et al.* 1987; Reeve 1987; Taylor 1988; Heinz and Parrella 1989; Allen 1990), developmental stages (van Alphen 1980; Liu *et al.* 1984; Hopper 1986) and/or ages (Juliano 1982; Pak *et al.* 1986; Wong *et al.* 1990). However, understanding why a particular host utilization strategy is followed has proven more difficult.

Host utilization is a multi-faceted term covering not only what types or stages of hosts are parasitized, but also if hosts are subjected to parasitoid-induced mortality not directly related to parasitism. Parasitoids may cause host mortality through the probing action of their ovipositors (Burnett 1962). This probing may be related to oviposition activity (Sugimoto 1977) or be involved in a process referred to as host feeding (Clausen 1962). Adult females of some parasitoid species require a protein source to mature their eggs which may be gained by injuring the hosts and feeding upon the haemolymph exuding from the wound (Bartlett 1964). Another aspect of host utilization is sex allocation to different host types. Most hymenopteran parasitoids are arrhenotokous, producing haploid male and diploid female progeny. By controlling the release of sperm stored in their spermatheca, mated female parasitoids can potentially manipulate the sex of their offspring (Flanders 1956, 1965).

Therefore, a parasitoid encountering a host has several decisions to make (Charnov and Skinner 1985; Waage and Godfray 1985; Strand 1988). Firstly, it must decide if that host is suitable for oviposition, host feeding or should be rejected. If accepted for oviposition, then the parasitoid must decide which sex to allocate to that

host. Gregarious parasitoids must also decide what clutch size to produce. Charnov and Skinner (1985) suggested four complementary approaches to understanding the oviposition decisions of parasitoids. These were:

- (i) the proximate approach;
- (ii) the development / ontogeny approach;
- (iii) the natural selection or ultimate approach; and
- (iv) the evolutionary / phylogenetic approach.

Most studies investigating host utilization by parasitoids adopt only one of these approaches, namely the proximate approach. This method investigates how different host types come to be parasitized by observing processes such as habitat searching, host location, handling and discrimination, and the physiological events leading to egg deposition (Charnov and Skinner 1985). These processes represent the mechanics leading to oviposition but may not provide a full understanding of the choices made by a parasitoid.

The development / ontogeny approach has been adopted less frequently but may also provide useful insights into host utilization. The oviposition decisions of a parasitoid are likely to be influenced by its age (van den Assem *et al.* 1984; Avilla and Albajes 1984; Wong *et al.* 1990), egg load (Sugimoto and Ishii 1979; Prasad *et al.*, in prep.) and level of previous experience with hosts (van Lenteren 1976: Charnov and Skinner 1985). Furthermore, her physical capabilities resulting from the quality of the resources exploited may influence oviposition activity (Bartlett 1964; Charnov and Skinner 1985).

All oviposition decisions of a parasitoid are made under the ever present force of natural selection. Parasitoids which make decisions increasing their relative fitness will have a selective advantage. Consequently, strategies maximizing fitness should be adaptive and become progressively more frequent within the population.

The natural selection or ultimate approach can be used to interpret if the oviposition decisions of parasitoids tend to maximize fitness (Charnov and Skinner 1985).

The final approach recognizes that parasitoids are bound to host taxa or types of hosts by their evolutionary history. Examples of this phenomenon include parasitoids of spiders' egg sacs (Austin 1985) and the host associations of the Pimplini, Polysphinctini and Rhyssini tribes of the ichneumonid subfamily Pimplinae (Gauld 1986). These constraints mould the host-parasitoid interactions seen today and influence the rate at which these associations can change. This approach has received the least attention, probably as a result of its indirect application to biological control (Charnov and Skinner 1985).

Few studies have attempted to combine two or more of these approaches. In my study, host utilization by two parasitoid species is compared, using a combination of the approaches outlined by Charnov and Skinner (1985). The development / ontogeny and natural selection approaches provide the basis for much of the research, while aspects of the proximate and evolutionary / phylogenetic approaches are considered. Parasitoid utilization of hosts is considered in relation to the 3 larval instars of *Liriomyza brassicae* (Riley). These host-parasitoid associations were chosen on the basis of their local availability, interest to the investigator, usefulness as model systems for understanding behaviour of parasitoids in general, and potential to further the knowledge of the Australian agromyzid fauna and parasitoids associated with this family.

Leafmining flies in the genus *Liriomyza* are of considerable economic importance in many agricultural regions of the world since they are often pests of both ornamental plants and vegetable crops, particularly in temperate regions (Spencer 1973; Parrella 1987). Damage caused by the leafmining larvae of these flies may slow plant growth, reduce the yield or aesthetic value of crops, or result in the death of

plants, particularly seedling stages (Spencer 1973). In addition, at least one species has been shown to act as a vector for plant viruses (Zitter and Tsai 1977). Several species have proven to be serious pests in glasshouses, e.g. *Liriomyza trifolii* (Burgess) and *Liriomyza bryoniae* (Kaltenbach) (Spencer 1973; Minkenberg and van Lenteren 1986).

The economic importance of this genus has increased dramatically over the last 15 years (Parrella 1987), probably due to several factors. Firstly, the use of insecticides on crops suffering *Liriomyza* attack has been shown in several cases to result in an increase in *Liriomyza* populations (Spencer 1973; Minkenberg and van Lenteren 1986; Parrella 1987). Insecticide resistance frequently appears in *Liriomyza* populations (Genung 1957; Wolfenbarger 1958; Parrella and Keil 1984; Keil *et al.* 1985). In addition, the leafmining larvae of *Liriomyza* species are protected from many insecticides by being enveloped in plant tissue (Parrella 1987). Generally, adult parasitoids associated with these leafminers are not similarly protected and may be killed by the application of insecticides, allowing *Liriomyza* populations to increase (Speyer and Parr 1948; Hills and Taylor 1951; Wene 1955; Shorey and Hall 1963; Genung and Janes 1975; Oatman and Kennedy 1976; Johnson *et al.* 1980a,b). However, at least one parasitoid of agromyzids has recently been shown to have some degree of insecticide resistance (Rathman *et al.* 1990).

A second factor increasing the pest status of this genus is the transportation of infested plant material around the world which, in conjuction with inadequate quarantine procedures, has widened the distribution of many *Liriomyza* species in recent years (Parrella 1987; Minkenberg 1988b). Waterhouse and Norris (1987) stated that the three major pest species *Liriomyza sativae* Blanchard, *L. trifolii* and *Liriomyza huidobrensis* (Blanchard), have considerably extended their range. Upon arriving in a new country or region, *Liriomyza* species may achieve pest status because they are attacked by fewer natural enemies, are subjected to pest management

strategies that favour the development of leafminer populations (e.g. the reliance on insecticides), or expand their host plant range.

Several of the major pest species of *Liriomyza* are polyphagous (Spencer 1973; Parrella 1987). For some species, the range of host plants has greatly increased in conjunction with the widening of their geographic range (Parrella 1987), creating further difficulties for the control of these species.

The increase in pest status of several *Liriomyza* species, whether due to the development of insecticide resistance, reduction in control by natural enemies, an increase in geographic distribution or widening of host plant range, indicates the need for further work on the biology of this genus and its natural enemies.

Australia, at present, is fortunate in lacking the major pest species of *Liriomyza* (Spencer 1977). However, Waterhouse and Norris (1987) have shown the expansion of geographic ranges of several pest species westward across the Pacific Ocean. Therefore, it is probable that, despite strict quarantine procedures, one or more of these species will eventually arrive in Australia.

Spencer (1977) concluded that 18 species of *Liriomyza* are currently found in Australia. While none of these species cause serious damage to agricultural or horticultural plants at present, they may prove important as reservoirs for natural enemies of more damaging species. Leafminer parasitoids are frequently polyphagous, being more niche specific than host specific (Waterhouse and Norris 1987). Therefore, it is likely that some of the parasitoid species currently found attacking *Liriomyza* and other agromyzid species in Australia will be capable of transferring activity to pest species of *Liriomyza*, were these to be accidently introduced. For this reason it is desirable to not only chronicle the parasitoid complexes existing on agromyzid species in Australia, but also to investigate the

biology of the component species. My study partially addresses this objective, investigating the biology of *L. brassicae* and the complex of parasitoids attacking it in the Adelaide region of South Australia. To date, this is the first comprehensive investigation of the biology of an agromyzid fly and its associated parasitoids undertaken in Australia.

CHAPTER TWO

THE BIOLOGY AND PHENOLOGY OF
Liriomyza brassicae (DIPTERA : AGROMYZIDAE) IN THE ADELAIDE REGION

2.1. ABSTRACT

The biology of *Liriomyza brassicae* was investigated by a series of laboratory experiments focusing on the development, fecundity and longevity of this species at three constant temperatures (15, 20, 25°C). Lower thresholds for development were calculated (1st instar 12.5°C, 2nd instar 11.5°C, 3rd instar 11.8°C, pupa 8.8°C, egg to adult 8.4°C) and found to be generally within the normal range for *Liriomyza* species, except for the egg stage which had a particularly low threshold (0.1°C). *L. brassicae* proved to be a moderately fecund species ($\bar{x}$ = 126 at 25°C), oviposition being influenced by both temperature and lifespan of the female. The relationship between temperature and longevity varied depending on the experimental conditions. When maintained with plants, the lifespan of *L. brassicae* was reduced and not influenced by temperature. However, when maintained in vials separate from plants, longevity increased and showed a positive relationship with temperature.

Field studies of *L. brassicae* throughout the Adelaide region revealed the phenology, abundance and host plant associations of this species. It was present throughout the year, being multivoltine and having overlapping generations. Numerous host plant species were found, several being new host records for Australia. Considerable variation was found in the frequency, duration and size of *L. brassicae* infestations between host plants and sites.

2.2. INTRODUCTION

Liriomyza brassicae (Riley) (Diptera : Agromyzidae) is a cosmopolitan species (Stegmaier 1968; Spencer 1973; Parrella 1982). It has undoubtedly extended its range by commercial practices (e.g. transportation of infested plant material), making its origins difficult to determine (Spencer 1977). Kleinschmidt (1970) stated there is no doubt that *L. brassicae* was introduced into Australia. It is a polyphagous species, attacking a variety of plant species in the family Brassicaceae, but also some species in the families Capparaceae, Fabiaceae and Tropaeolaceae (Stegmaier 1968; Spencer 1973, 1977). In Australia, Kleinschmidt (1970) provided details of the host plants, life history and parasitoids of *L. brassicae*. However, there has been no comprehensive investigation of the biology of *L. brassicae* in Australia to date.

In some regions of the world it is considered a minor economic pest, occasionally causing considerable losses in cabbage seedlings (Oatman and Platner 1969; Spencer 1973). In Australia, *L. brassicae* populations are relatively common on cruciferous weeds and the ornamental, *Tropaeolum majus* (nasturtium; Kleinschmidt 1970; Spencer 1977). However, it is considered to have no pest status, being found infrequently on cultivated *Brassica* crops in this country.

Dorge and Dalaya (1964) and Beri (1974) described the biology of *L. brassicae* in India in some detail. Eggs are laid within punctures made on the leaf surface, hatching into small maggots which commence to eat the mesophyll layers of the leaf. A serpentine mine is formed, becoming progressively longer as the larva develops. There are three larval stages. Late third instars split the leaf epidermis and

emerge from the mine (Kleinschmidt 1970). A short prepupal period follows before the cuticle hardens to form the puparium. Pupation takes place in the soil (Spencer 1973). Adult flies feed from punctures made on the leaf surface by the female ovipositor (Kleinschmidt 1970).

At present, Australia has no agromyzid pest species. Consequently, little attention has been given to the continent's agromyzid fauna. Waterhouse and Norris (1987) note that several important pest species, including *Liriomyza sativae* Blanchard and *Liriomyza trifolii* (Burgess), have spread westwards across the Pacific region during the last decade. Therefore, it is probable that, despite quarantine procedures, one or more *Liriomyza* pest species will arrive in Australia during the next decade. In other regions of the world, pre-existing populations of *Liriomyza* and other agromyzid species have been shown to serve as potential reservoirs for natural enemies of the more damaging *Liriomyza* species (Spencer 1973; Cock 1985; Waterhouse and Norris 1987). Therefore, the native and introduced agromyzid fauna of Australia may play a similar role, should the need arise.

This chapter deals with the biology of *L. brassicae* in the Adelaide region of South Australia. Investigations were carried out in both the laboratory and the field. Laboratory investigations into the development, fecundity and longevity aimed to provide the background knowledge necessary for the maintenance of a *L. brassicae* culture (Appendix One) and the manipulation of this fly for experimental purposes. Field studies of the phenology and host plant range of *L. brassicae* provided the basis for sampling parasitoid populations (Chapter Three) and also enabled the evaluation of its potential as a refuge for natural enemies.

2.3. MATERIALS AND METHODS

All flies and plants used in the following investigations were taken from a laboratory and shadehouse culture, respectively (Appendix One).

2.3.1. Instar Determination

A quick and reliable method was required to distinguish between instars of *L. brassicae*. Various characters such as mine length and width, larval length and width, and mouth-hook (cephalo-pharyngeal skeleton) length were measured for approximately thirty individuals of each instar. To measure mine length for each instar, ten leaves containing mines were selected. The paths of the mines were traced onto an acetate sheet laid over active mines on each leaf. These traces were then measured three times using a Graphics Tablet™ (Apple) and a mean measurement was calculated. Data for each parameter was analysed using a one-way analysis of variance (ANOVA). Comparisons between life stages for each parameter were made with the Student-Newman-Keuls (SNK) multiple range test (SAS 1985).

2.3.2. Development

Mated females were caged singly within a lantern globe (Maxbilt Trading Co., Lantern Glass 285, approximately 1 litre volume) covering a rape seedling (Figure A1.1). The top of the lantern globe was covered in fine mesh, held in place by a rubber band. Honey was supplied on a circular piece of filter paper (diameter = 30 mm) through which a pin was placed to suspend the paper above the soil. Caged plants were held at $15(\pm 2)$, $20(\pm 2)$ and $25(\pm 2)°C$, 12L:12D photoperiod for approximately 24 hours after which the old plant was exchanged for a fresh one. Care was taken during the exchange not to release the female flies. Following the oviposition period, plants were kept in aerated plastic containers at the same temperature at which oviposition had occurred. These were checked daily with the number of mines and stage of development of each mine being determined. When

larvae were in the late third instar, the infested leaves were excised and transferred to petri dishes containing moistened filter paper. These petri dishes were checked daily for larval emergence. Emerged larvae and pupae were transferred to aerated plastic or glass vials (Appendix One) and stored at the same temperature as previous development had taken place. Vials were maintained until all adults had emerged or several weeks had passed since the last emergence. From the records obtained it was possible to estimate the duration of development of eggs, first, second and third instars and pupae. Developmental rates were determined by taking the inverse of the developmental time. The influence of temperature and sex (where known) on the developmental rate was investigated by a mixed-model ANOVA followed by the SNK test (SAS 1985). Lower temperature thresholds for development were calculated by the temperature-intercept method (Andrewartha and Birch 1954). Thermal constants (day-degrees required to complete development) for each life stage were estimated at each temperature and mean values calculated.

2.3.3. Fecundity and Longevity of Mated Females

Forty-five mated females were caged individually with rape seedlings as described in the development section, split into three groups and maintained at $15(\pm 2)$, $20(\pm 2)$ or $25(\pm 2)°C$ for 24 hours. Each plant was replaced daily by a fresh seedling until the fly was dead. Following the oviposition period, plants were placed in an aerated plastic container and kept at $25(\pm 2)°C$. These plants were checked daily and the number of mines counted to determine the number of viable eggs laid. The influence of temperature and female lifespan on fecundity was investigated by linear regression (SAS 1985).

2.3.4. Longevity of Unmated Flies

Newly-emerged unmated males and females of *L. brassicae* were placed singularly in glass vials (50 x 10 mm) containing a smear of honey on the side. Several small punctures in the caps provided aeration. Thirty flies of each sex were

kept at 15(±2), 20(±2) and 25(±2)°C until death.   The influence of sex and
temperature on longevity was determined by a mixed-model ANOVA, followed by the
SNK test (SAS 1985).

2.3.5.   Field Survey

Field surveys of *L. brassicae* commenced in July 1988.   Several
sites containing weeds in the family Brassicaceae were selected initially but more were
added as other host plants became apparent (Figure 2.1, Table 2.1).   Initially,
sampling occurred at approximately monthly intervals until the thermal constant for *L.
brassicae* development was determined (see development data).   From this time,
sampling proceeded approximately once per generation.   The duration of *L. brassicae*
generations in the field were determined by a degree-day model (Allen 1976) modified
by Allen (1989), using maximum-minimum temperature data collected at the Waite
Agricultural Research Institute.   An arbitrary upper developmental threshold was set at
35°C.

In the first year of surveying (1988-89), up to 100 plants at each site
were searched for *L. brassicae* activity, depending on the size of the plant population
present.   Each plant was examined until a mined leaf was found.   This leaf was
collected in a plastic bag and then placed in an insulated cooler (Esky™) for transport
back to the laboratory.   Following the collection of a leaf, examination of that plant
ceased and another plant was searched for *L. brassicae* activity.   The number of
leaves collected per site indicated the number of plants with *L. brassicae* activity out of
the 100 surveyed.   Once back in the laboratory, these leaves were placed individually
into petri dishes containing moistened filter paper.   These were held at 25(±2) °C and
12L:12D hr photoperiod until all *L. brassicae* and parasitoids had emerged.   Fly
larvae that emerged from leaves were transferred to vials and kept under the same

14

Figure 2.1.   Location of collection sites for field surveys of *L. brassicae* populations within the Adelaide region.   (●) Year 1 (1988-89) and additional sites; (◉)Years 2 and 3 (1989-1991) sites.

N

⊚ Daveyston

⊚ Sheoak Log

Adelaide Plains

GULF
ST
VINCENT

Mt. Lofty Ranges

⊚ Semaphore

■ ADELAIDE

See lower inset

35°S

⊚ Darlington

● Reynella

⊚ O'Sullivan Beach

● Old Noarlunga
Moana Beach
● McLaren Vale

Athelstone ⊚

■ Adelaide C.B.D.

Wheal
⊚ Watkins

W.A.R.I. ▢
Mitcham ⊚

Mt Osmond ●

● Piccadilly

5 km

● Brighton

5 km
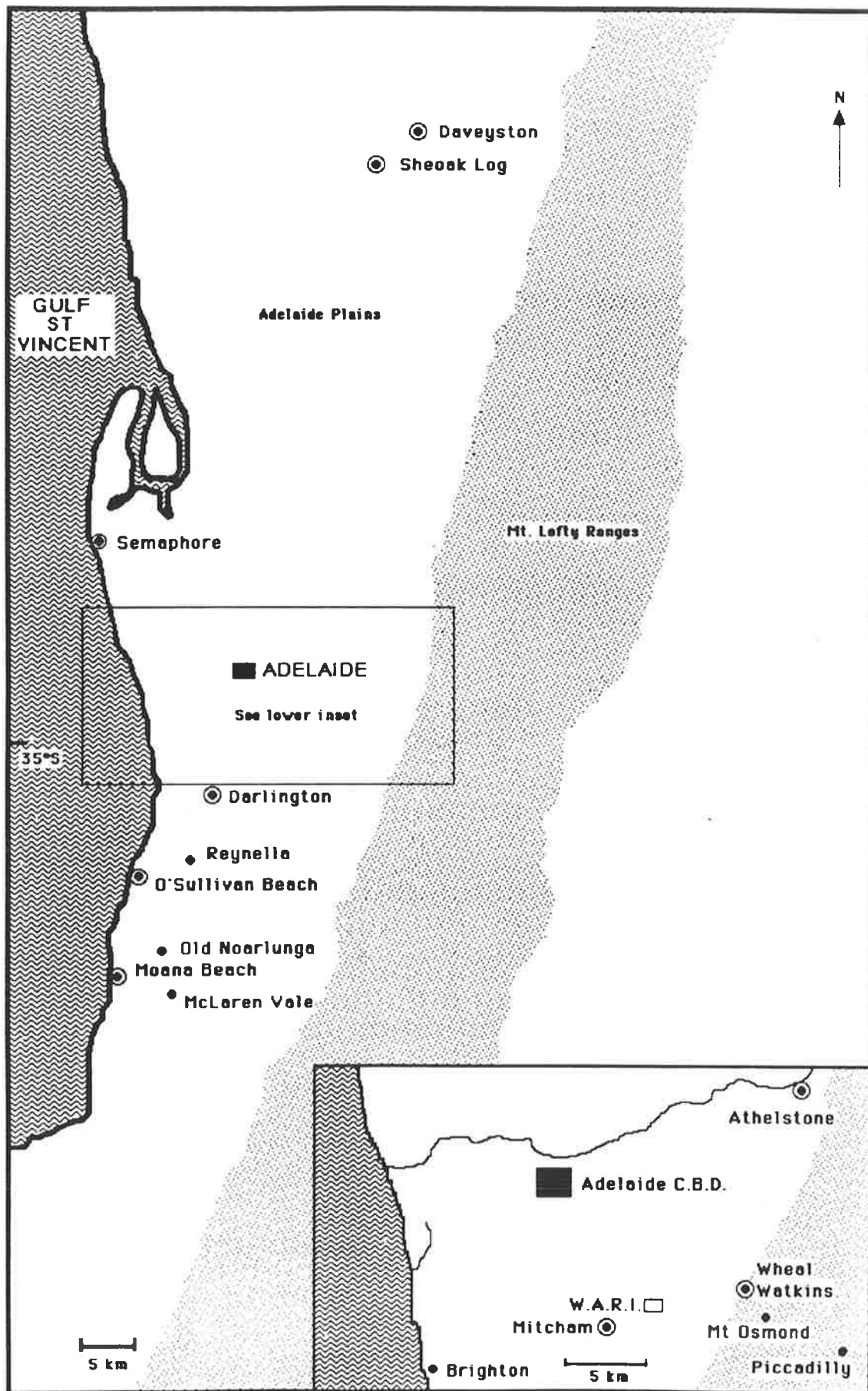
Table 2.1. Plant species and sites included in the first year survey of *L. brassicae* populations (1988-89).

Plant Species	Common Name	Date included in survey	Site	Description of Site
Cakile maritima	Sea Rocket	10th August 1988	Moana Beach	Coastal foredune
Diplotaxis tenuifolia	Lincoln Weed	13th July 1988	Old Noarlunga	Roadside verge in rural area
Lepidium africanum	Peppercress	12th January 1989	Mitcham	Roadside verge in urban area
Raphanus raphanistrum	Wild Radish	9th August 1988	Piccadilly	Roadside verge in rural area
Rapistrum rugosum	Giant Mustard	13th July 1988	Darlington	Vacant urban land
		" " "	McLaren Vale	Roadside verge in rural area
		9th August 1988	Mitcham	Vacant urban land
		13th July 1988	Moana	Roadside verge in urban area
		" " "	Old Noarlunga	Roadside verge in rural area
		10th August 1988	Reynella	Roadside verge in urban area
		9th August 1988	Wheal Watkins	Urban parkland
Sisymbrium officinale	Hedge Mustard	11th January 1989	Darlington	Vacant urban land
Sisymbrium orientale	Indian Hedge Mustard	5th October 1988	Mitcham	Roadside verge in urban area
Tropaeolum majus	Nasturtium	10th November 1988	Wheal Watkins	Along dry creek bed in urban parkland

conditions until adult emergence. Some parasitoids were placed in 70% ethanol for identification at a later stage while others were maintained in glass vials (50 x 18 mm) with honey as a food source.

Following the first year survey, four plant species were chosen as the focus of the second and third year surveys (1989-90 and 1990-91). Each of these plants exhibited at least moderate levels of leafminer and parasitoid activity during the first year of sampling. Three sites were selected for each species (Figure 2.1), as listed below:

Cakile maritima	Moana Beach, O'Sullivan Beach , Semaphore (all coastal dunes);
Rapistrum rugosum	Darlington (vacant urban land), Wheal Watkins (urban parkland), Mitcham (vacant urban land);
Sisymbrium officinale	Darlington (vacant urban land), Daveyston (roadside verge), Sheoak Log (roadside verge);
Tropaeolum majus	Athelstone (urban parkland), Wheal Watkins (along dry creek bed in urban parkland), Mitcham (vacant urban land).

Surveying continued approximately once per *L. brassicae* generation. Sampling methods were similar for *R. rugosum*, *S. officinale* and *C. maritima* but were modified for *T. majus* due to its different growth form. For each species except *T. majus*, up to 100 plants were searched per site and the number with *L. brassicae* activity recorded. From estimating the proportion of the total plant population at the site that had been searched, it was possible to approximate the total plant population present. In order to minimize the effect of sampling on the population dynamics of *L. brassicae*, these estimates were deliberately conservative, tending to under-estimate the actual population size. Using these estimations, the approximate number of plants

with *L. brassicae* activity at the site was calculated. Ten percent of these plants were then collected for further examination in the laboratory. A similar method was used to sample leafminer populations on *T. majus*. Since the growth form of *T. majus* is intertwining and sprawling, it was decided to search up to 500 leaves per site, again recording the number with *L. brassicae* mines. The total number of leaves per site was then estimated and the approximate number of leaves with mines per site determined. Again, ten percent of the calculated number of leaves with mines were then collected for processing in the laboratory.

In the laboratory, mines were classified as being active (i.e. containing a living *L. brassicae* larva or parasitoid), dead (i.e. containing a dead *L. brassicae* larva or parasitoid) or empty (i.e. not containing an insect). The instar of each larva present was determined by measuring the mouth-hook length. Leaves containing active or dead insects were placed in petri dishes and treated as described for the first year survey.

2.4. RESULTS

2.4.1. Instar Determination

The sizes of larvae and their mines differed with instar (P < 0.05; Table 2.2). However, overlap in the range of values recorded was present for every character except mouth-hook length. Therefore, it was decided to use mouth-hook length as the basis for discrimination between instars of *L. brassicae*.

Table 2.2. Length and width of leaf mine, length and width of larvae and mouth-hook length of different life stages of *Liriomyza brassicae*. All measurements in mm.

Instar	N	Mine Length *	Mine Width	Larval Length	Larval Width	Mouth-hook Length
		Mean ± se (Range)	Mean ± se (Range)	Mean ± se (Range)	Mean ± se (Range)	Mean ± se (Range)
1	30	1.33 ± 0.13[a] (0 - 2.99)	0.20 ± 0.01[a] (0.15 - 0.35)	0.46 ± 0.02[a] (0.20 - 0.65)	0.15 ± 0.01[a] (0.08 - 0.20)	0.105 ± 0.002[a] (0.09 - 0.13)
2	30	20.23 ± 1.34[b] (5.57 - 37.28)	0.55 ± 0.04[b] (0.35 - 1.10)	0.95 ± 0.04[b] (0.60 - 1.25)	0.28 ± 0.01[b] (0.20 - 0.50)	0.187 ± 0.002[b] (0.18 - 0.20)
3	30	55.37 ± 2.19[c] (33.28 - 111.38)	1.17 ± 0.04[c] (0.70 - 1.50)	2.18 ± 0.08[c] (1.20 - 2.80)	0.50 ± 0.01[c] (0.40 - 0.65)	0.288 ± 0.002[c] (0.28 - 0.30)
Pre-pupae	11	- -	- -	2.10 ± 0.08[c] (1.50 - 2.40)	0.56 ± 0.04[c] (0.40 - 0.70)	0.286 ± 0.003[c] (0.28 - 0.30)

Means for each parameter followed by the same letter are not significantly different (P > 0.05).
* Sample size of mine length data for 1st (27), 2nd (45) and 3rd (54) instars.

2.4.2. Development

The developmental rate of each life stage showed a significant positive linear relationship with temperature (P < 0.05; Figure 2.2). Lower thresholds for different life stages ranged from 0.1°C for eggs to 12.5°C for 1st instar larvae (Table 2.3). The lower threshold for egg development was much less than for any other life stage, indicating eggs will continue to develop under most field conditions. Highest temperatures were required for larval development (lower thresholds 11-13°C) while development of pupal and total life stages will occur at intermediate temperatures (lower threshold 7-9°C).

Calculation of thermal constants indicates approximately 300 degree days above 8.4°C are required for *L. brassicae* to complete development. The pupal stage occupied the largest portion of the total developmental time (approximately 50%), followed by the egg stage (approx. 30%) and the larval stage (approx. 20%).

No significant difference was found between the developmental rate of males and females.

2.4.3. Fecundity and Longevity

The total number of viable eggs produced by *L. brassicae* increased with temperature (Table 2.4). Longevity of actively reproducing females at the 3 constant temperatures was similar (Table 2.4), indicating greater rates of viable egg production must occur at higher temperatures. Viable egg production was positively correlated with female lifespan (Figure 2.3).

Figure 2.2. Relationship between temperature and developmental rate (mean ± standard error) of various life stages of *L. brassicae*.

(A) egg, developmental rate = -0.000667 + 0.0085temperature, R^2 = 0.885;

(B) 1st instar, developmental rate = -0.971 + 0.0776temperature, R^2 = 1.00;

(C) 2nd instar, developmental rate = -0.671 + 0.0585temperature, R^2 = 0.997;

(D) 3rd instar, developmental rate = -0.605 + 0.0512temperature, R^2 = 0.0972;

(E) egg to 3rd instar, developmental rate = -0.0426 + 0.00587temperature, R^2 = 0.990;

(F) pupa, developmental rate = -0.0581 + 0.00664temperature, R^2 = 0.995;

(G) total development, developmental rate = -0.0277 + 0.0033temperature, R^2 = 0.985.
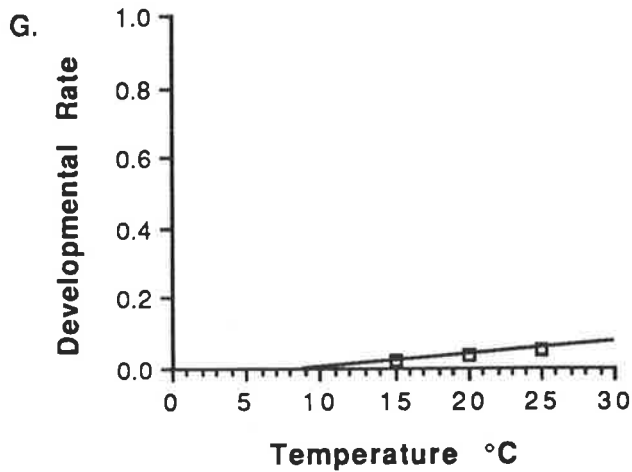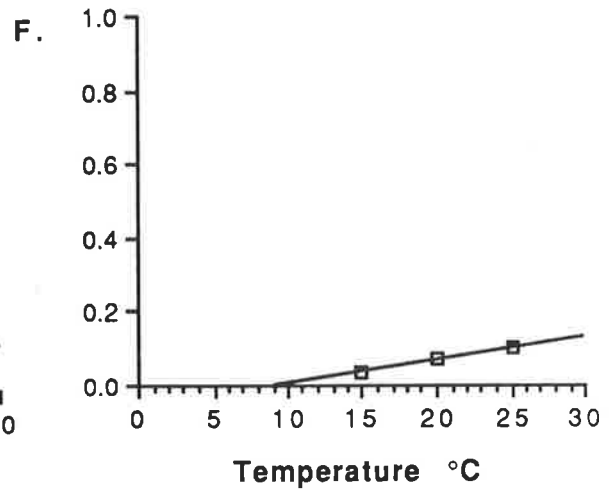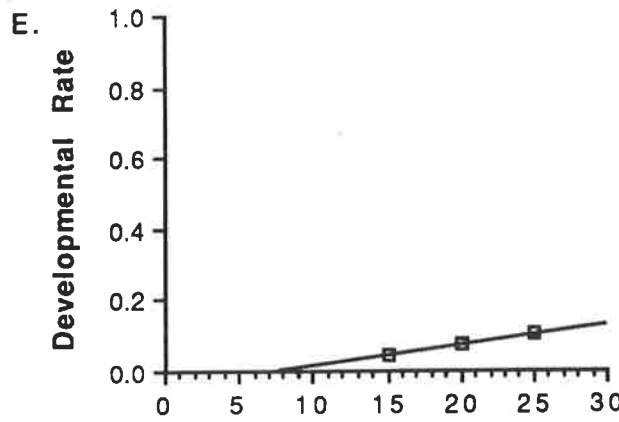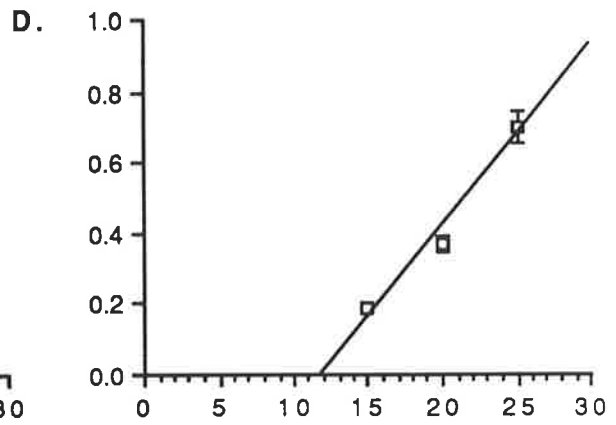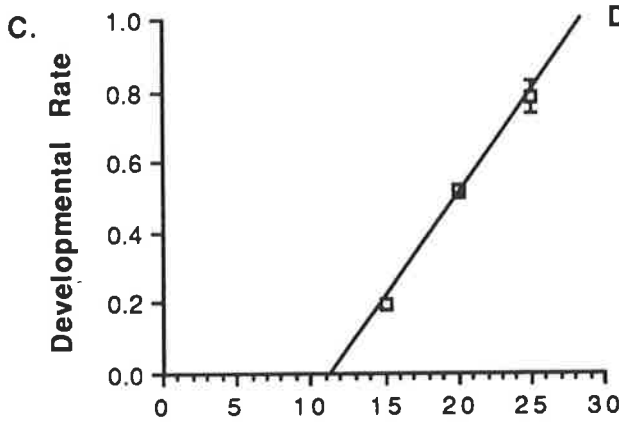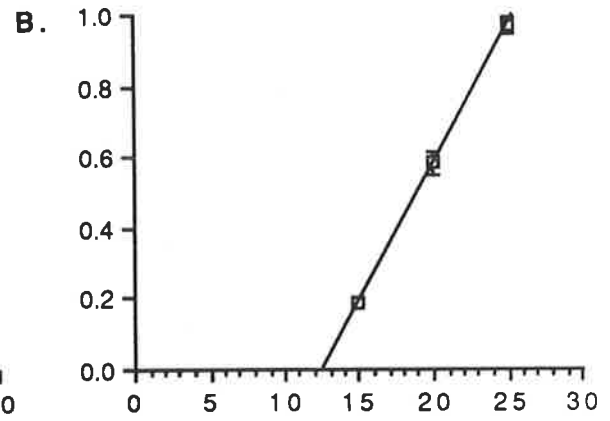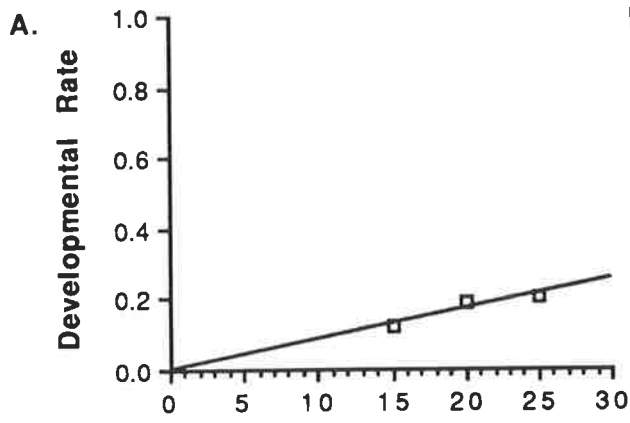
Table 2.3. Lower temperature thresholds and thermal constants calculated for development of various life stages of *L. brassicae* (sexes pooled).

Life stage	N	Lower Temperature Threshold °C	Thermal Constant (day-degrees)
Egg	30	0.1	119
1st instar	30	12.5	13
2nd instar	30	11.5	18
3rd instar	30	11.8	21
Within leaf stage	34	7.3	172
Pupa	52	8.8	164
Total	46	8.4	307

Table 2.4. Fecundity (no. of viable eggs) and lifespan (days) of *L. brassicae* when kept with plants at three constant temperatures. Values are mean ± standard error (and range).

	Temperature °C		
	15	20	25
Fecundity	17.53 ± 6.11 (0 - 88)	60.60 ± 11.68 (4 - 163)	126.07 ± 22.22 (19 - 315)
Lifespan	8.47 ± 1.91 (1 - 27)	8.80 ± 1.44 (3 - 22)	8.80 ± 1.50 (1 - 21)
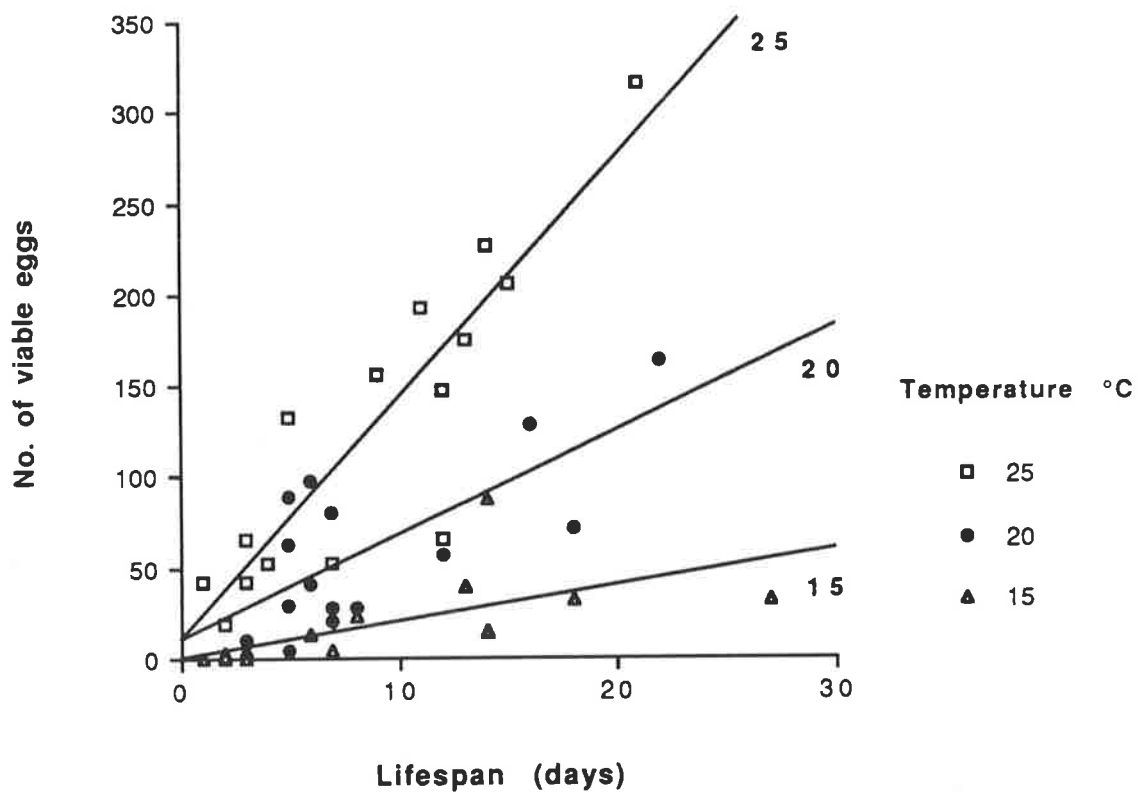
Figure 2.3. Relationship between lifespan and the number of viable eggs laid by female *L. brassicae* at three constant temperatures (15, 20, 25°C).

The lifespan of reproducing females averaged between 8-9 days regardless of temperature (Table 2.4), but the lifespan of non-reproducing individuals varied between the 3 constant temperatures and the sexes (Figure 2.4). At each temperature, females lived longer than males (P < 0.05). For each sex, greatest longevity was observed at 15°C and least at 25°C.

2.4.4. Field Survey

Year One (1988-89)

L. brassicae was active in the field throughout the whole first year of sampling (Figure 2.5). Population levels were variable between plant species, within a plant species and at each particular site. Greatest levels of *L. brassicae* activity, as indicated by the total number of mines present, were observed on *Rapistrum rugosum*, *Cakile maritima*, *Sisymbrium officinale* and *Tropaeolum majus*. Other plant species, such as *Diplotaxis tenuifolia* and *Raphanus raphanistrum* showed consistent but low levels of *L. brassicae* activity. *Lepidium africanuum* and *Sisymbrium orientale* were found to be only occasionally attacked by *L. brassicae*.

Peak activity of *L. brassicae* occurred at different times throughout the year on different plant species and at different sites. Both winter-spring and summer-autumn peaks were observed on *R. rugosum* at Darlington, McLaren Vale, Moana and Mitcham. Only winter-spring peaks in *L. brassicae* activity were observed on *R. rugosum* at Old Noarlunga, Reynella and Wheal Watkins. Summer-autumn peaks were observed on *R. raphanistrum*, *C. maritima*, *S. officinale* and *T. majus*.
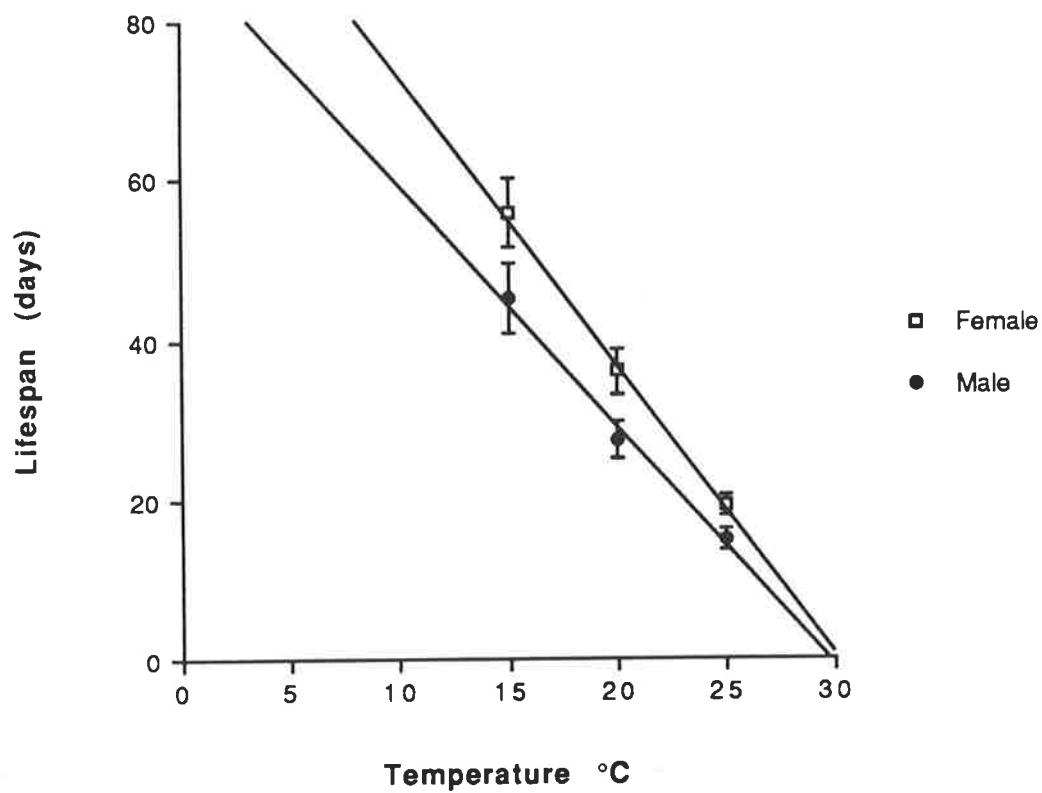
Figure 2.4. Relationship between temperature and lifespan (mean ± standard error)

for male and female adult *L. brassicae*.

Females, Lifespan = 109.62 - 3.63temperature, R^2 = 0.998;

Males, Lifespan = 89.38 - 3.01temperature, R^2 = 0.990.

27

Figure 2.5. Levels of active (black), dead (hatched) and empty (striped) *L. brassicae* mines collected during the first year survey (1988-1989) from various host plants around Adelaide. Solid line above chart indicates host plants present at the site on that date. Broken line indicates no host plants present at the site on that date. No line indicates sampling was not undertaken at the site on that date.

A. *Rapistrum rugosum,* **Darlington**
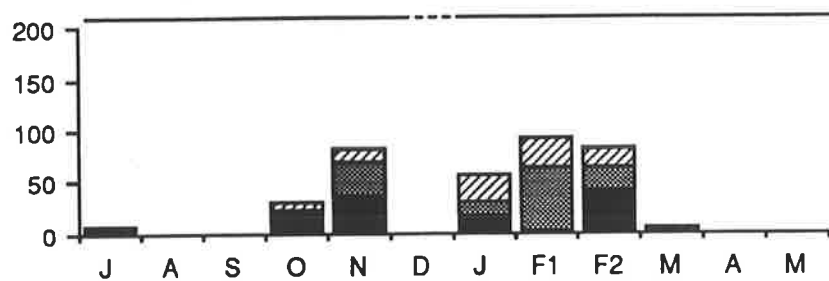
B. *R. rugosum,* **McLaren Vale**
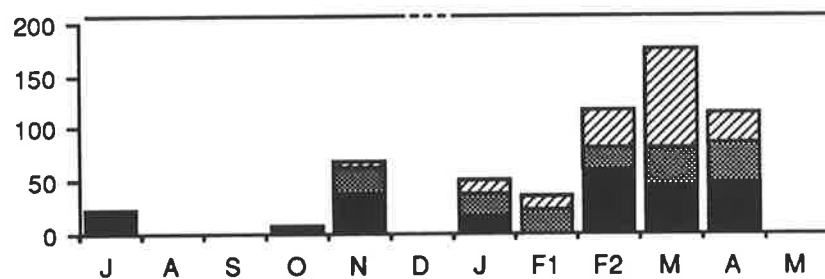
C. *R. rugosum,* **Moana**
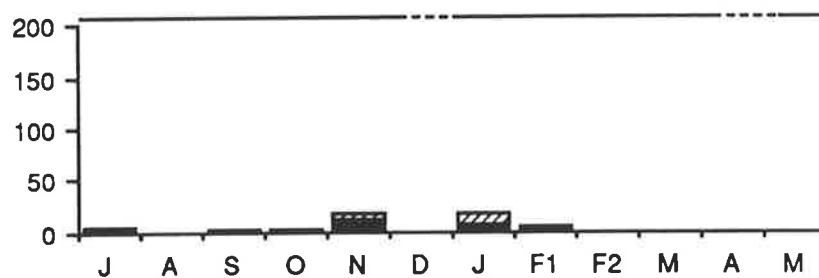
D. *R. rugosum,* **Old Noarlunga**
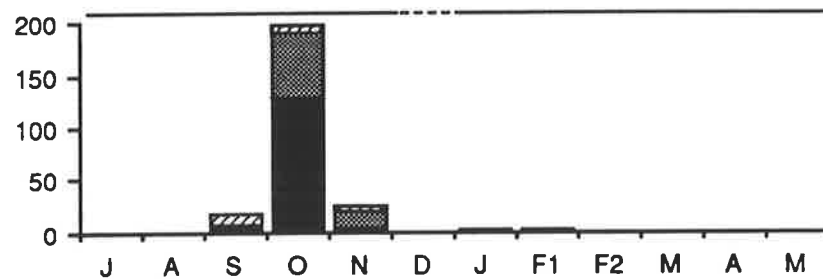
E. *R. rugosum,* **Mitcham**
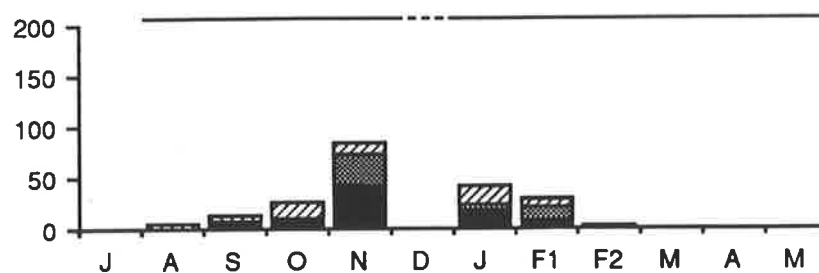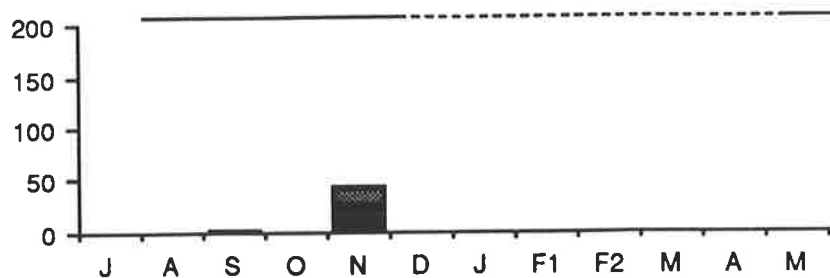
No. of mines

Month of Collection

Month of Collection

K. *Sisymbrium officinale,* Darlington

L. *Sisymbrium orientale,* Mitcham

M. *Tropaeolum majus,* Wheal Watkins

N. *Lepidium africanum,* Mitcham
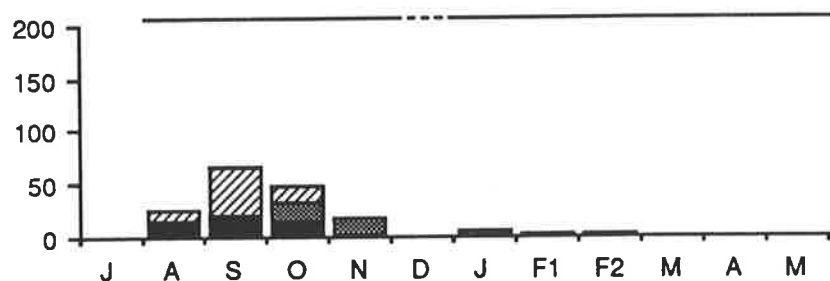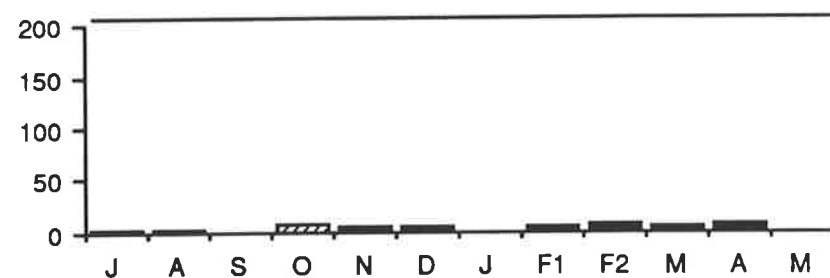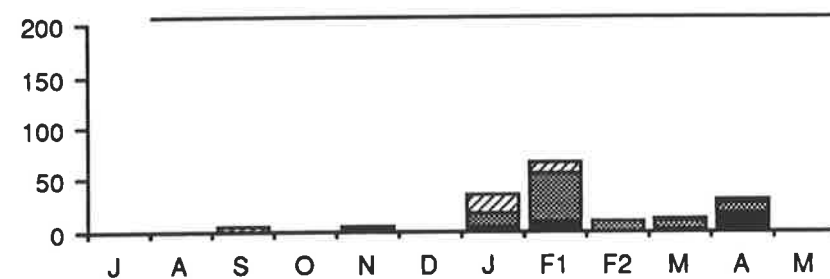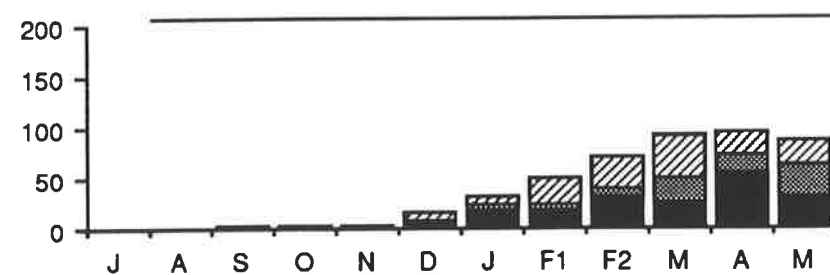
No. of mines
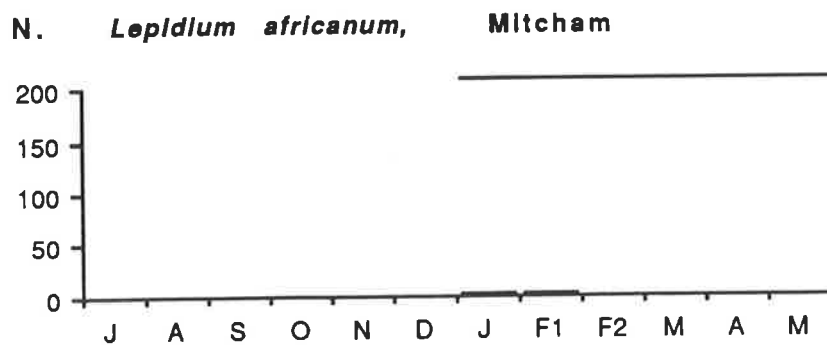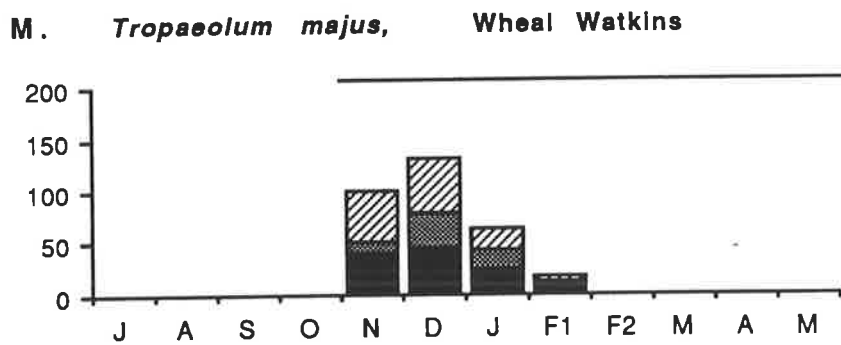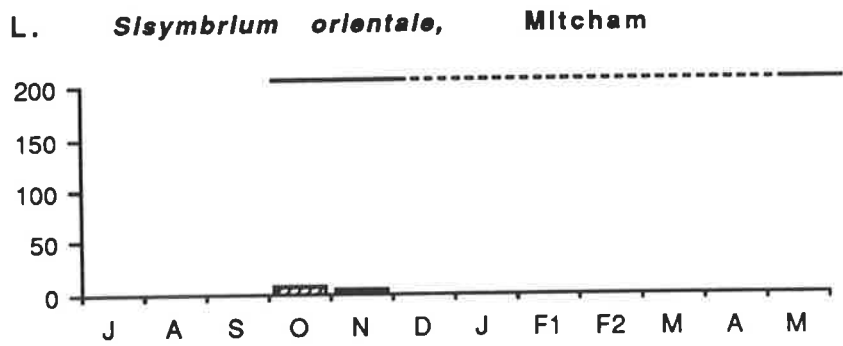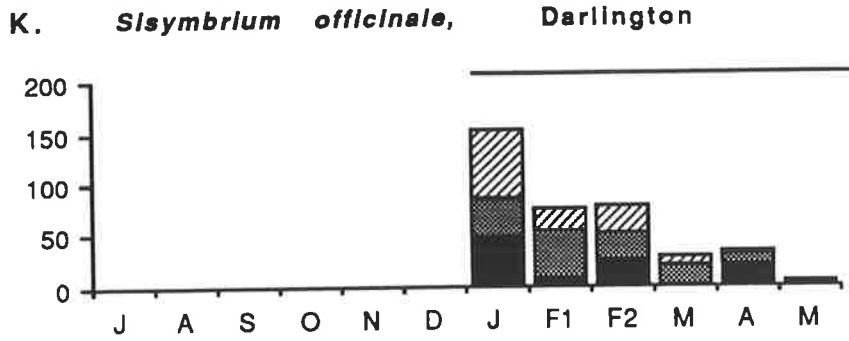
Month of Collection

At the Wheal Watkins site, peak activity was observed on *T. majus* when little activity was occurring on *R. rugosum*. Both *L. africanum* and *S. orientale* showed *L. brassicae* activity at Mitcham at times when mines were also collected on *R. rugosum* from this site, but levels of activity on *R. rugosum* were considerably greater. At Darlington, *R. rugosum* and *S. officinale* displayed similar levels of *L. brassicae* activity during early 1989.

Years 2-3 (1989-91)

L. brassicae activity on *T. majus*, *C. maritima* and *S. officinale* was consistent across all sites where each were sampled (Figure 2.6). Peak activity on *T. majus* occurred during summer. Absence of activity on *T. majus* at Mitcham during the summer of 1990-91 was a result of no *T. majus* plants being present.

On *C. maritima*, *L. brassicae* was active throughout much of the sampling period. During 1989-90, considerable activity was observed on *C. maritima* during summer and autumn. Low levels of *L. brassicae* activity were recorded during the following spring with sporadic infestations occurring during summer and autumn of 1990-91. The Semaphore site had greater activity than either O'Sullivan or Moana Beach. This was also the site which supported the largest *C. maritima* population, being part of a long stretch of minimally disturbed coastal dune.

S. officinale showed greatest *L. brassicae* activity during summer, declining during autumn of both years. Considerably more activity was observed during the 1989-90 than 1990-91 season.

Activity of *L. brassicae* on *R. rugosum* was more variable between sites than on the other 3 plant species (Figure 2.6). Peaks during spring occurred at both Wheal Watkins and Darlington, although these were much greater in 1989 than

Figure 2.6. Levels of active, dead and empty mines of *L. brassicae* on *T. majus*, *C. maritima*, *R. rugosum* and *S. officinale* during the surveys of years 2-3 (1989-91). Solid line above chart indicates host plants present at the site on that date. Broken line indicates no host plants present at the site on that date. No line indicates sampling was not undertaken at the site on that date.

T. majus sites: A, Wheal Watkins; B, Athelstone; C, Mitcham.
C. maritima sites: D, O'Sullivan Beach; E, Moana Beach; F, Semaphore.
R. rugosum sites: G, Wheal Watkins; H, Darlington; I, Mitcham.
S. officinale sites: J, Darlington; K, Sheoak Log; L, Daveyston.
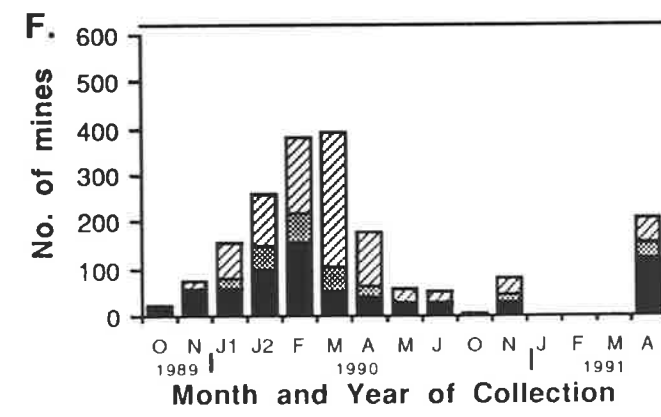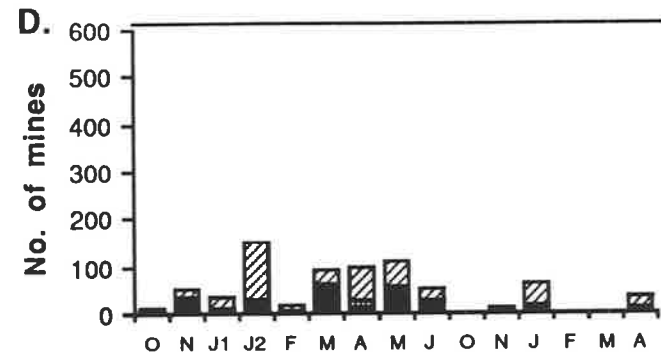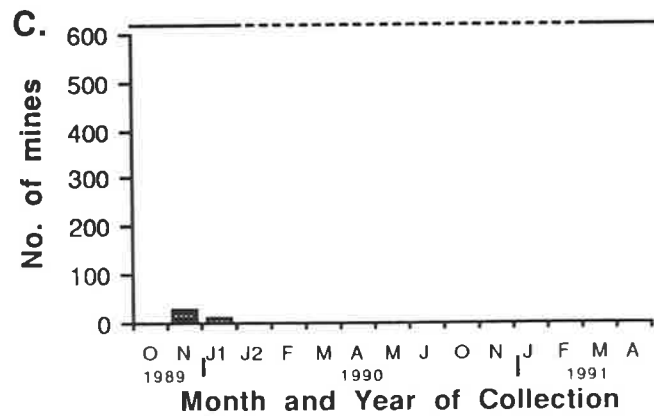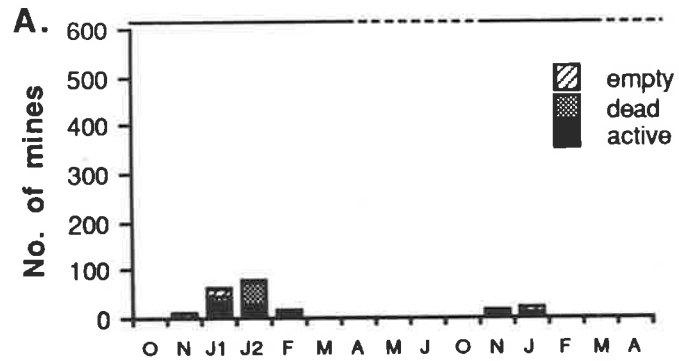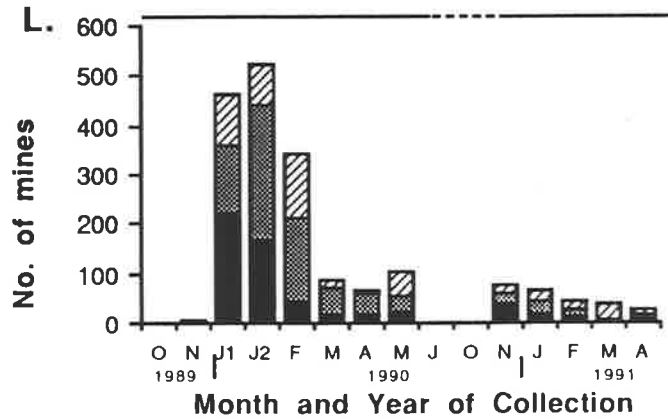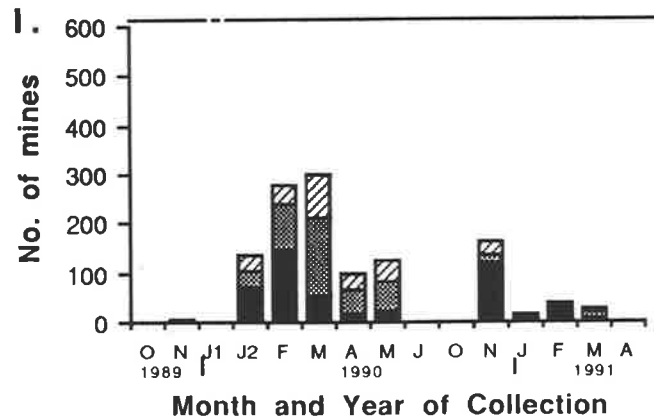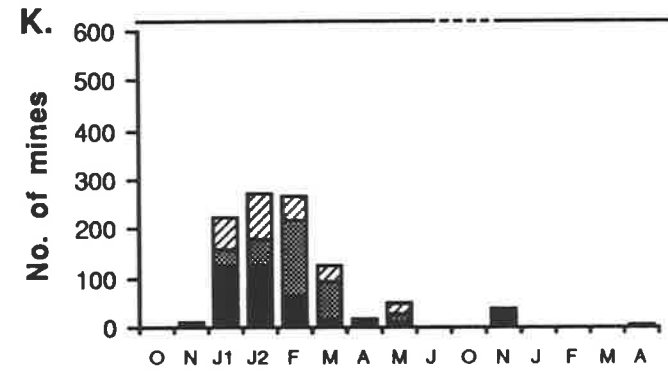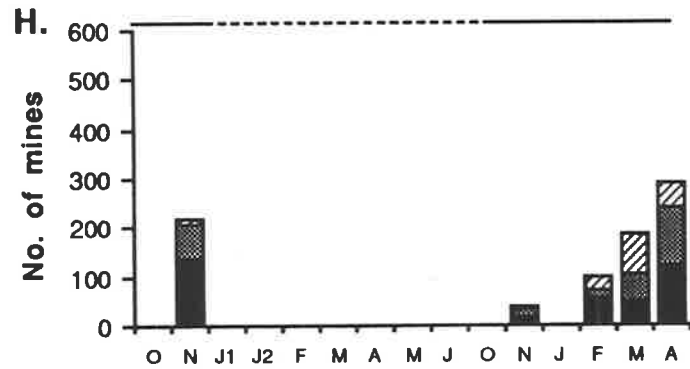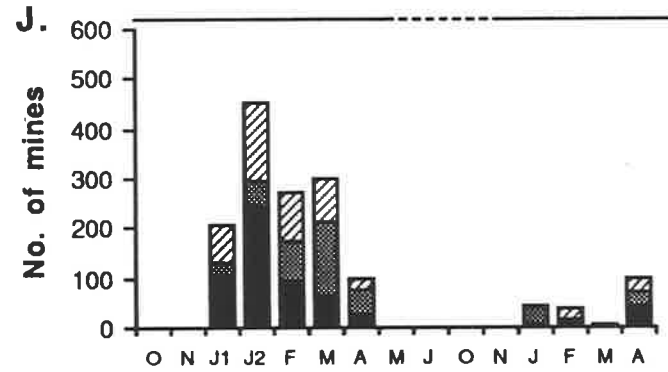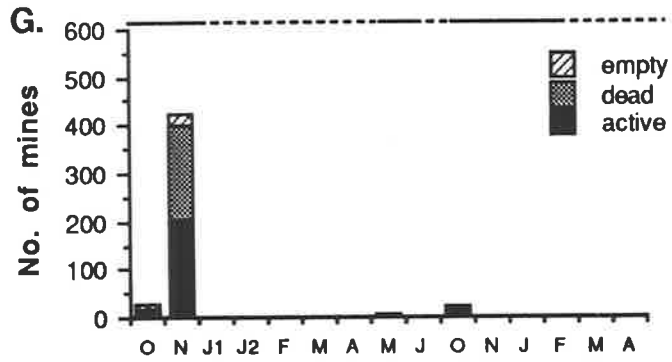
1990. In the 1990-91 season a second, larger peak occurred at Darlington during late summer to autumn. In contrast, peaks in activity at Mitcham occurred during summer into autumn for both years of sampling.

At the Wheal Watkins site, the spring activity on *R. rugosum* preceded the activity peaks on *T. majus* at the same site. In contrast, during the 1989-90 season, activity on *T. majus* at Mitcham preceded the peak observed on *R. rugosum*. Activity on *R. rugosum* preceded that on *S. officinale* at Darlington during both years, but overlap in times of activity occurred during 1990-91.

Early stages of infestation were generally associated with proportionately greater levels of active mines. Correspondingly, later stages had proportionately greater levels of empty or dead mines.

Throughout most infestations all three larval instars were present (Figure 2.7). No discernible trends were evident in life stage frequencies throughout the course of infestation, the proportions of each fluctuating considerably. Collections of eggs were sporadic though it is possible some passed unnoticed, particularly during the field component of the collection process.

Additional Host Plants

During field observations, various host plants not included in sampling programs were noted. These included *Alyssum* sp., *Brassica oleracea*, *Brassica tournefortii*, *Cakile edentula*, *Sisymbrium irio* (Brassicaceae) and *Lathyrus odoratus* (Fabiaceae).

36

Figure 2.7. Population structure of active *L. brassicae* mines collected from *T. majus*, *C. maritima*, *R. rugosum* and *S. officinale* during the surveys of years 2-3 (1989-91).

T. majus sites: A, Wheal Watkins; B, Athelstone; C, Mitcham.
C. maritima sites: D, O'Sullivan Beach; E, Moana Beach; F, Semaphore.
R. rugosum sites: G, Wheal Watkins; H, Darlington; I, Mitcham.
S. officinale sites: J, Darlington; K, Sheoak Log; L, Daveyston.

Figure showing No. of Individuals versus Month and Year of Collection for panels A through F.

Legend:
- 3rd
- 2nd
- 1st
- egg

X-axis labels (panels C and F): O N J1 J2 F M A M J O N J F M A, with years 1989, 1990, 1991

X-axis title: Month and Year of Collection

37

Month and Year of Collection
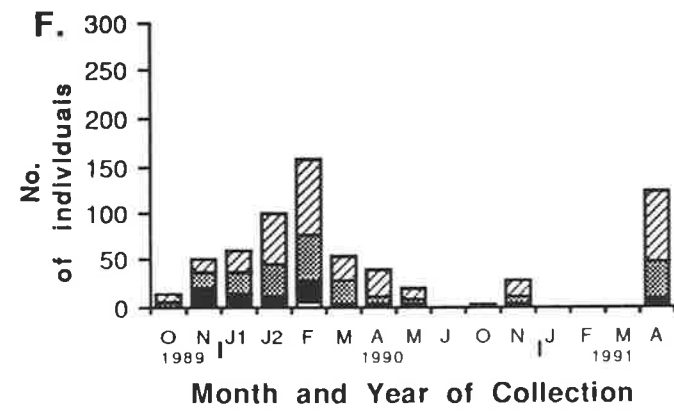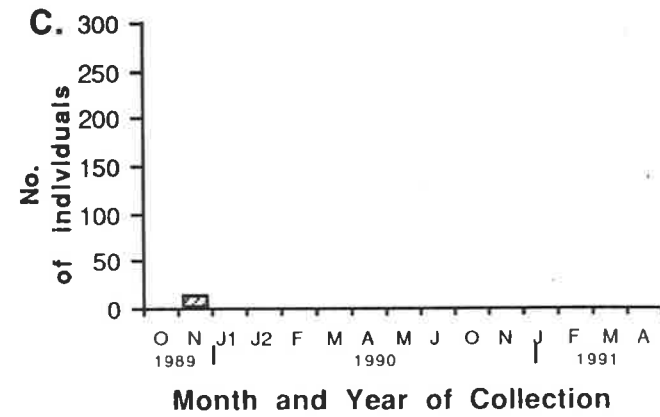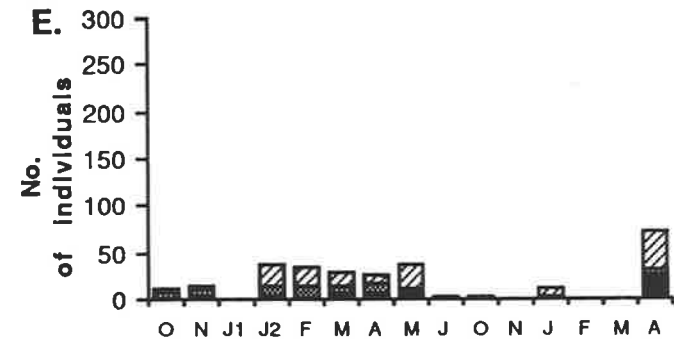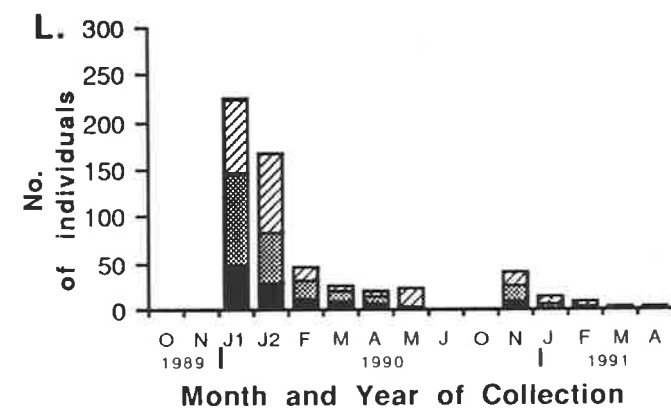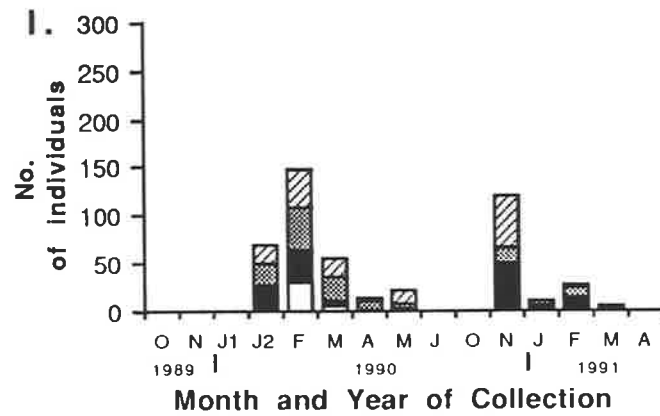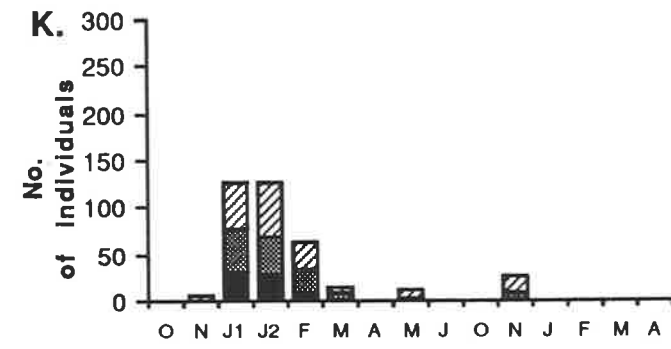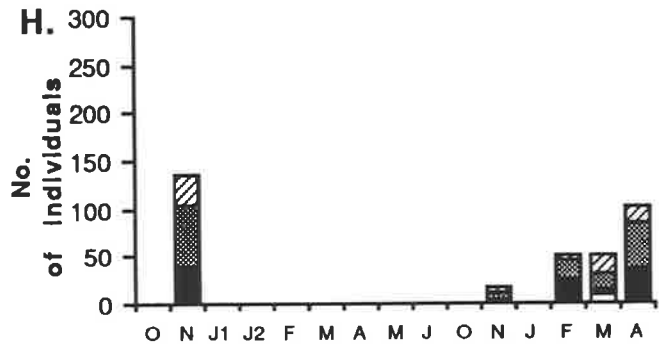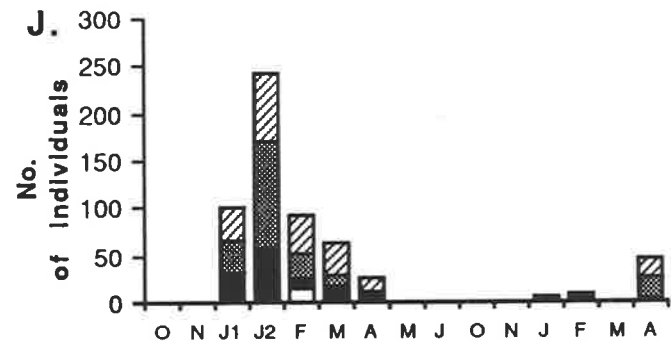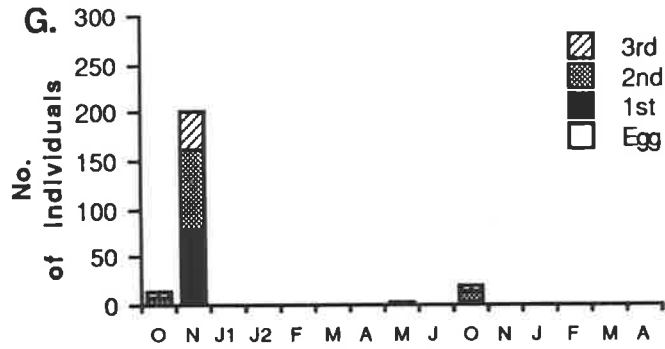
2.5.   DISCUSSION

Laboratory Studies

Larval stages of *L. brassicae* were best separated on the basis of mouth-hook length.   This character has also been used to discriminate between larval stages of *L. trifolii* (Minkenberg 1988a), *L. sativae* (Oatman and Michelbacher 1958), *Liriomyza bryoniae* (Kaltenbach) (Minkenberg and van Lenteren 1986) and *L. brassicae* previously by Beri (1974).   This appears to be a generally useful method for identifying life stages of *Liriomyza* (Oatman and Michelbacher 1958).   Measurements of mouth-hook length reported here differed slightly from those recorded by Beri (1974) for *L. brassicae*, his mean values falling at the lower end of the observed range. This may indicate the different host plants used for this study and that of Beri (1974) have different nutritional values.

Other characters also proved good indicators of larval stage but were not used because of the overlap in the range of values recorded.   Larval length and width, and mine length measurements for each instar reported here varied markedly for some stages from those made by Beri (1974) on *T. majus*.   Lengths of 1st and 2nd instar larvae reared on *T. majus* fell within the range observed here.   However, final instar larvae from *T. majus* were longer then any recorded from *B. napus*.   Similarly, widths of 1st and 2nd instars described by Beri (1974) fell within the range recorded here, but final instars from *T. majus* were wider.   Average mine lengths recorded by Beri (1974) also differed from those observed on *B. napus*.   Lengths of mines formed by both 2nd and 3rd instars were similar in both studies, although mines on *B. napus* were generally shorter.   Mines of 1st instars were considerably shorter on *B. napus* than recorded on *T. majus* by Beri (1974).   Different host plants used by Beri (1974) and this study undoubtedly explains some of the variation observed.   It is also possible that different biotypes of *L. brassicae* exist between localities or different rearing temperatures have influenced mine production and/or larval growth.

Comparison of mine and larval width data indicates that mine width increases disproportionately to larval width. Initially, mines were only 1.3 times wider than the larvae. Mines of 2nd instars were nearly twice as wide as larvae, and by the 3rd instar stage mines were approximately 2.3 times the width of the larvae they contained. This may be important for the movement of larvae in the mine as larger larvae would require more room to manoeuvre. Mine widths greater than larval width would also allow movement back along the mine as an evasive response to parasitoid attack, as observed by Heinz and Parrella (1989).

There was a dramatic increase in mine length with each instar. Second and 3rd instar mines were approximately 15 and 40 times longer, respectively, than those of first instar larvae. When the area of leaf surface covered by mines of each instar was estimated (mine length x 1/2 mine width), mines of 3rd instar were found to cover approximately 250 times more leaf surface than those of 1st instars. While this indicates the elevation of leaf consumption with instar, as observed by Fagoonee and Toory (1984), it also has the potential to influence the behaviour of natural enemies of this leafminer. Parasitoids searching for hosts and using the host mine as a cue would have a much greater chance of encountering older mines (Sugimoto 1977; Hendrikse *et al.* 1980; Minkenberg and van Lenteren 1986).

Lower developmental thresholds calculated for the larval, pupal and total life stages of *L. brassicae* were within the ranges recorded for other *Liriomyza* species (Oatman and Michelbacher 1958; Webb and Smith 1969; Dimetry 1971; Liebee 1984; Bodri and Oetting 1985; Schuster and Patel 1985; Parrella 1987). However, the lower threshold for development of *L. brassicae* eggs was much lower than any previously recorded (Parrella 1987). A lower threshold for egg development approaching 0°C would allow *L. brassicae* to continue development throughout much of the Adelaide winter. Furthermore, the robustness of the egg stage may have

contributed to the cosmopolitan distribution of this species (Spencer 1973; Parrella 1982). Beri and Chandra (1983) presented information on the growth rate and developmental time of *L. brassicae* on *T. majus* at various constant temperatures. Using their information it was possible to calculate lower developmental thresholds for the life stages of *L. brassicae*, enabling comparison with thresholds determined here (Table A2.1). A similar threshold for egg development was calculated from both sets of data. However, other thresholds calculated from the data of Beri and Chandra (1983) were considerably less than those recorded here. The exceedingly low values calculated indicates some of these thresholds are questionable, especially those for larval development. Their sample size and methodology is uncertain and, therefore, their results should be regarded with caution.

In contrast to the observations of Beri (1974), *L. brassicae* readily oviposited in the laboratory. As observed for other *Liriomyza* species (Oatman and Michelbacher 1958; Dimetry 1971; Parrella *et al.* 1983; Parrella 1984; Minkenberg 1988a), temperature influenced the realized fecundity of *L. brassicae*. The mean number of viable eggs laid by *L. brassicae* at 25°C was greater than that observed for *L. trifolii* (Minkenberg 1988a) but less than for *L. sativae* (Oatman and Michelbacher 1958; Parrella 1987), suggesting that *L. brassicae* is a moderately fecund species. Fecundity experiments suggest that the mean reproductive life of *L. brassicae* is relatively short, with flies probably dying before their egg load is depleted.

Unlike other studies, temperature was not found to influence the longevity of *L. brassicae* when maintained in association with host plants (Oatman and Michelbacher 1958; Dimetry 1971; Parrella *et al.* 1983; Parrella 1984; Parrella and Bethke 1984). Mean lifespan of *L. brassicae* when in association with host plant was about half that recorded for other *Liriomyza* (Parrella 1987). However, potential longevity, determined by maintaining flies in small glass vials with constant food supply, indicated that increasing temperature did reduce lifespan. The potential

longevity of both sexes of *L. brassicae* at 25°C are similar to those of other *Liriomyza* (Parrella 1987). Differences in longevity of *L. brassicae* between these two experiments may be due to several factors. Parrella (1987) suggested that increasing the size of the rearing container may have a detrimental influence on longevity. If so, then the larger container used for the fecundity experiments may have contributed to the lower longevity in these experiments. Flies maintained in containers with plants may have been exposed to higher humidities as a result of transpiration. Laboratory studies frequently control for several mortality factors (e.g. limited food supply, predators and pathogens, environmental extremes) and would, therefore, tend to overestimate longevity (Parrella 1987). The fecundity experiments were more representative of the field situation and, hence, the longevity results from these are probably more realistic.

Field Studies

Field studies indicated *L. brassicae* was an ever-present, polyphagous species throughout the Adelaide region. Rarely, though, was it found in association with crop plants. Since of the plant species which *L. brassicae* was found attacking around Adelaide have previously been recorded as host plants for this species (Stegmaier 1967, 1968; Kleinschmidt 1970; Spencer 1973, 1977; Beri and Chandra 1983). Several host plants, including *Alyssum sp.*, *B. tournefortii*, *C. maritima*, *D. tenuifolia*, *L. africanum*, *R. raphanistrum*, *S. officinale* and *L. odoratus*, represent new records for this leafminer in Australia .

This leafminer appears to have distinct plant preferences as some locally abundant species are frequently infested while other species only rarely show *L. brassicae* attack. Phenology of the plant species has a major influence on the frequency and duration of infestations. For instance, decline in infestations on *R. rugosum*, *S. officinale* and *T. majus* was commonly associated with plant senescence. Temperature and rainfall also influence the frequency and size of infestations, having not only a direct relationship with plant abundance and vigour, but

also with *L. brassicae* growth (Beri and Chandra 1983), development (Figure 2.2), fecundity (Table 2.4) and longevity (Figure 2.4). Low levels of *L. brassicae* activity during winter and early spring may be a result of minimal development and fecundity during this period. Site was also seen to influence the size or timing of *L. brassicae* infestations. Proximity to other host plants and, therefore, potential reservoirs of fly activity, or cultural practices may influence the local abundance of *L. brassicae*. The majority of field sites were situated in road verges, vacant urban land or coastal dunes. Road verges and vacant urban land were occasionally subject to weed control measures such as mowing or herbicide applications. Such practices eliminated the *T. majus* population at Mitcham during 1990-91. Clearly, factors affecting the abundance and distribution of *L. brassicae* throughout Adelaide and its environs are numerous and complicated.

L. brassicae is multivoltine, having approximately 8 generations per year based upon degree day requirements. Generation time varied from approximately 3 weeks during summer up to several months over winter. These generations are overlapping so that all larval instars are present in the field at most times of the year. This would give parasitoids of this species a choice of host stages to attack at any one time.

The presence of *L. brassicae* populations throughout the year, its association with common cruciferous weeds and minimal pest status make this leafminer a potentially valuable source of natural enemies for the control of pest *Liriomyza* species, if such a need arises in Australia. Kleinschmidt (1970) recorded 6 species attacking *L. brassicae* in Queensland. Prior to this study, the parasitoid fauna associated with *L. brassicae* in South Australia was unknown. The biology, phenology and abundance of parasitoids attacking *L. brassicae* in the Adelaide region are discussed in Chapter Three.

CHAPTER THREE

THE BIOLOGY OF PARASITOIDS ASSOCIATED WITH *Liriomyza brassicae* IN THE ADELAIDE REGION

3.1. ABSTRACT

Seven species of hymenopteran parasitoids were collected from populations of *Liriomyza brassicae* in the Adelaide region between 1988 and 1991. This parasitoid complex consisted of two braconids and five eulophids. The percentage of the larval host population parasitized varied considerably between plant species and collection dates, with levels greater than 50% sometimes being recorded. *Zagrammosoma* sp. and *Hemiptarsenus varicornis* were the major parasitoid species, being abundant throughout most of the year. *Chrysonotomyia* sp., *Opius cinerariae* and *Opius atricornis* were occasionally abundant species but their activity was restricted to only one or two host plants, mostly during summer. Alternate agromyzid hosts were identified for *Chrysocharis pubicornis*, *Closterocerus* sp. and *H. varicornis*. *O. cinerariae*, *H. varicornis* and *C. pubicornis* were cultured in the laboratory. Culturing of other species was not successful due to insufficient numbers and a reluctance to mate or attack *L. brassicae* in the laboratory.

3.2. INTRODUCTION

Several agromyzid species are serious pests of agricultural and horticultural crops throughout the world (Spencer 1973; Parrella 1987; Minkenberg and van Lenteren 1986). Chemical control methods targeting other pest species have been shown to disrupt the natural control of some agromyzids by reducing or eliminating their parasitoid populations (Oatman and Kennedy 1976; Lange *et al.* 1980; Johnson *et al.* 1980b,c). Species of *Liriomyza* have become serious glasshouse pests in Europe where a combination of favourable environmental conditions and limited parasitoid activity have facilitated their increase (Hendrikse 1980; Wardlow 1985; Minkenberg and van Lenteren 1986). Control of agromyzids using insecticides is often unsuccessful as a result of their mining habit, soil dwelling pupae, high reproductive capacity and ability to rapidly develop insecticide resistance (Parrella and Keil 1984; Wolfenbager 1958; Keil *et al.* 1985; Parrella 1987). Furthermore, chemical control is often not desirable in situations where it may interfere with biological control programs for other pests (Wardlow 1985; Minkenberg and van Lenteren 1986). Consequently, the use of parasitoids for the biological control of agromyzid pests has been increasing during the past two decades (Spencer 1973; Zucchi and van Lenteren 1978; Hendrickson and Plummer 1983; Frijters *et al.* 1986; Westerman and Minkenberg 1986; Harcourt *et al.* 1986; Minkenberg and van Lenteren 1986).

Natural populations of agromyzids commonly support numerous parasitoid species (Minkenberg and van Lenteren 1986). Most belong to the hymenopteran families Braconidae, Eulophidae and Pteromalidae (Spencer 1973; Drea *et al.* 1982; Waterhouse and Norris 1987), and less commonly to the Ceraphronidae, Cynipidae, Encyrtidae, Eucoilidae, Eupelmidae, Eurytomidae, Mymaridae, Scelionidae and Trichogrammatidae (Spencer 1973; Waterhouse and Norris 1987). Cornelius and Godfray (1984) reared 21 species of parasitoids from natural populations of the

chrysanthemum leafminer, *Chromatomyia syngenesiae* Hardy. Similarly, 21 parasitoid species were found to be associated with *Phytomyza lonicerae* Robineau-Desboidy (Kato 1985). In Europe, Drea *et al.* (1982) found 17 and 18 parasitoid species attacking *Agromyza frontella* (Rondoni) and *Agromyza nana* Meigen, respectively. Over 30 species have been identified as parasitoids of *Liriomyza sativae* and *L. trifolii* in North America (Johnson and Hara 1987; Waterhouse and Norris 1987; LaSalle and Parrella 1991).

Large parasitoid complexes and high levels of parasitism have been thought to explain the low abundance of agromyzid leafminers in unsprayed crops (Minkenberg and van Lenteren 1986). For example, between 80-90% of *Liriomyza* larvae on celery were parasitized at harvest for two consecutive years in California (Trumble and Nakakihara 1983). *Opius striativentris* Gahan was found to parasitize approximately 50% of *Phytomyza ilicicola* larvae on native holly in Kentucky (Potter and Gordon 1985). Kleinschmidt (1970) reports about 80% pupal parasitism of *Tropicomyia indigoferae* (Kleinschmidt) by a pteromalid. Miles and Cohen (1936) recorded 98% parasitism of *C. syngenesiae* pupae in England.

Frequently, parasitoids of agromyzids are polyphagous, having more than one host species (Waterhouse and Norris 1987). For example, Drea *et al.* (1982) collected *Dacnusa maculipes* Thomson, *Diglyphus isaea* (Walker) and *Dapsilarthra balteata* (Thomson) from the 3 main agromyzid pests of alfalfa in Europe. Similarly, *Diglyphus begini* (Ashmead), *Diglyphus intermedius* (Girault), *Chrysocharis parksi* Crawford and *Halticoptera circulus* (Walker) were found to parasitize various *Liriomyza* species in North America (Johnson and Hara 1987; Waterhouse and Norris 1987).

Numerous hymenopteran parasitoid species have previously been recorded from *Liriomyza brassicae* (Riley) (Assem 1966; Stegmaier 1967; Oatman and

Platner 1969; Kleinschmidt 1970; Bouček 1988). These include species from the families Braconidae, Cynipidae, Eulophidae, Eupelmidae and Pteromalidae (Spencer 1973). Of particular note, Kleinschmidt (1970) recorded one species of braconid, four genera of eulophids and a eupelmid attacking *L. brassicae* in Queensland. The only species identified, *Opius cinerariae* Fischer (Braconidae), was found to be polyphagous, attacking at least 3 other agromyzids (Kleinschmidt 1970). Two further species known to parasitize *L. brassicae*, *Meruana liriomyzae* Bouček and *Trigonogastrella parasitica* Girault, are present in Australia (Bouček 1988), but so far have not been found in association with this leafminer. Oatman and Platner (1969) provided the only information on parasitism levels in *L. brassicae* populations. They found that up to 84.1% of the larval population was parasitized in California, this rate being reached during October. At present, little information is available on the biology of any of the parasitoids attacking *L. brassicae*.

My study aimed to identify the parasitoids attacking *L. brassicae* in the Adelaide region of South Australia. Abundance, phenology and host plant associations of these parasitoids were investigated by means of surveying host populations. Preliminary investigations of the biology of these species were conducted, with two species being selected for more detailed investigation (see Chapters Four to Eight).

3.3. MATERIALS AND METHODS

Collections of parasitized host larvae were made during the field surveys described in Chapter Two. The stages of development of parasitized larvae were recorded. Parasitoids were reared from hosts by following the same methods used to rear field collected *L. brassicae*. Where possible, the sex of emerging parasitoids was recorded. Those emerging were either (i) placed in 70% ethanol for

identification at a later stage, or (ii) placed into a glass vial (50 x 18mm) with a punctured lid, provided with honey and kept at 15($\pm$ 2) °C.

Attempts were made to establish laboratory cultures of each parasitoid species. Individuals reared from field-collected material were allowed to mate and placed in aerated plastic cages (1.5 litre capacity) containing excised *Brassica napus* leaves infested with 3rd instar *L. brassicae* larvae. Cages were kept at 20($\pm$2)°C and leaves were replaced daily. Leaves removed from cages were placed in large plastic petri dishes and wasps reared using the methods outlined for *L. brassicae* (Appendix One). To obtain mated wasps, several individuals were placed in a gelatine capsule and observed until mating occurred. If unsuccessful, aerated plastic vials (60 x 20 mm) were used. If mating still did not occur, wasps were placed directly in aerated plastic cages with *L. brassicae* infested leaves.

The biology of each species was determined by dissecting additional material collected from field sites at Waite Agricultural Research Insititute (*B. napus*), Mt Osmond (*Tropaeolum majus*) and Brighton (*Cakile maritima*; Figure 2.1) and by culturing parasitoids on *L. brassicae* in the laboratory. The host range of these parasitoids was investigated by collecting plant material infested with other locally available agromyzid species and rearing individuals through to either adult flies or parasitoids. Potential alternative hosts included *C. syngenesiae* on *Sonchus oleraceus* (sow-thistle), *Liriomyza chenopodii* (Watt) on *Chenopodium album* (white goosefoot) and *Calycomyza humeralis* (Roser) on *Aster subulatus*. Voucher specimens of all parasitoid species, *L. brassicae* and *C. syngenesiae* have been lodged in the Waite Agricultural Research Institute's insect collection.

3.4. RESULTS

Seven species of hymenopteran parasitoids were found attacking *L. brassicae* in the Adelaide region (Table 3.1). This complex consisted of 2 braconids and 5 eulophids, all being solitary parasitoids. The percentage of *L. brassicae* parasitized varied considerably, ranging from 0 to 100% (Tables 3.2, 3.3). Rarely were more than 2 parasitoid species present within one collection at a site.

Few individuals of any one species were collected during the first year of surveying (1988-89), probably due to the sampling method used (see Chapter Two). However, percentage parasitism data (Table 3.2) indicates that *L. brassicae* populations were more heavily parasitized on some plant species than others. The highest levels of parasitism were observed on *T. majus*, *S. officinale* and *C. maritima*. *L. brassicae* on *R. rugosum* and *R. raphanistrum* were occasionally parasitized, whereas on *S. orientale*, *L. africanum* and *D. tenuifolia* they were never found to be parasitized. On the basis of this information, and data on the abundance of *L. brassicae* (from Chapter Two), *T. majus*, *C. maritima*, *S. officinale* and *R. rugosum* were chosen as plant species on which to concentrate field studies of parasitism.

Trends in the levels of parasitism results during years 2 and 3 (1989-91) were similar to those recorded during from the first year survey for the 4 plant species (Table 3.3). Parasitism of *L. brassicae* on *T. majus* was greatest during summer; on *C. maritima*, parasitism was most frequent during summer and autumn; while parasitism on *R. rugosum* was sporadic and varied considerably between sites, with least activity being observed during winter and early spring. Peak levels of parasitism occurred during summer and into autumn on *S. officinale*.

Table 3.1. Summary of the biology of the parasitoid species attacking *L. brassicae* in the Adelaide region.

Species	Type of parasitoid	Instars Attacked	Stage from which emergence occurs	Developmental Strategy	Host Plants
Opius atricornis	Primary Endoparasitoid	2nd 3rd	P	K	*Rapistrum rugosum, Tropaeolum majus*
Opius cinerariae	Primary Endoparasitoid	1st 2nd 3rd	P	K	*Brassica napus, Raphanus raphanistrum, R. rugosum, Sisymbrium officinale*
Chrysocharis pubicornis	Primary Endoparasitoid	2nd 3rd	L or P	K	*B. napus, Cakile maritima, R. rugosum, S. officinale*
Chrysonotomyia sp.	Facultative Hyperparasitoid Endoparasitoid	2nd 3rd	L	I	*B. napus, C. maritima, S. officinale, T. majus*
Closterocerus sp.	Primary Ectoparasitoid	3rd	L	I	*B. napis, C. maritima, S. officinale, T. majus*
Hemiptarsenus varicornis	Primary Ectoparasitoid	2nd 3rd	L	I	*B. napus, C. maritima, R. rugosum, S. officinale, T. majus*
Zagrammosoma sp.	Primary Ectoparasitoid	1st 2nd 3rd	L	I	*B. napis, C. maritima, R. rugosum, S. officinale, T. majus*

L, larvae; P, pupae.   K, koinobiont; I, idiobiont.

Table 3.2. Percentage parasitism of *L. brassicae* larval populations on various host plants around Adelaide during the Year 1 survey (1988-89).

Collection no. (date)	Percentage Parasitism													
	RR DT	RR MA	RR MI	RR MV	RR ON	RR RY	RR WW	RA PY	DT ON	CM MB	TM WW	SR MI	SF DT	LA MI
1 (13/7/88)	0	0		0	-				0					
2 (9/8/88)	-	-	0	-	-	-	7	-	0	-				
3 (8/9/88)	-	0	0	-	0	0	5	0	-	0				
4 (5/10/88)	0	0	0	0	5	-	4	-	0	0			0	
5 (9/11/88)	0	0	0	0	0	0	0	33	0	0	83		0	
6 (14/12/88)	np	np	np	np	np	np	np	-	0	0	50	np		
7 (11/1/89)	0	0	6	0	0	np	0	0	-	6	29	np	21	0
8 (2/2/89)	0	0	0	0	0	np	0	0	0	11	100	np	86	0
9 (22/2/89)	5	-	0	0	-	np	0	0	0	19	-	np	19	-
10 (16/3/89)	0	-	-	11	-	np	-	0	0	15	-	np	0	-
11 (13/4/89)	-	np	-	0	-	np	-	6	0	21	-	np	0	-
12 (16/5/89)	-	-	-	-	-	-	-	-	-	-	41	-	0	-

* RR, *Rapistrum rugosum*; RA, *Raphanus raphanistrum*; DT, *Diplotaxis tenuifolia*; CM, *Cakile maritima*; TM, *Tropaeolum majus*; SR, *Sisymbrium orientale*; SF, *Sisymbrium officinale*; LA, *Lepidium africanum*.

** DT, Darlington; MA, Moana; MI, Mitcham; MV, McLaren Vale; ON, Old Noarlunga; RY, Reynella; WW, Wheal Watkins; PY, Piccadilly; MB, Moana Beach (see Figure 2.1).

-, no hosts present; np, no plants present; blank spaces, no collection made.

Table 3.3. Percentage parasitism of *L. brassicae* larval populations on four host plants at three sites each around Adelaide during surveys of years 2 and 3 (1989-91).

Collection no. (date)	Percentage Parasitism											
	T. majus			*C. maritima*			*R. rugosum*			*S. officinale*		
	WW	AT	MI	OS	MA	SM	WW	DT	MI	DT	DY	SL
1 (26/10/89)	-	0	-	17	0	1	13	-	-	-	-	-
2 (28/11/89)	0	100	50	15	0	4	9	2	0	-	0	0
3 (3/1/90)	31	20	100	15	-	40	np	np	np	13	21	13
4 (25/1/90)	50	38	np	13	29	39	np	np	6	7	46	14
5 (22/2/90)	100	0	np	0	49	6	np	np	3	16	18	11
6 (14/3/90)	-	-	np	0	23	28	np	np	11	25	12	7
7 (6/4/90)	np	np	np	7	50	21	np	np	27	42	26	0
8 (2/5/90)	np	np	np	0	18	0	0	np	9	np	38	0
9 (25/6/90)	np	np	np	14	50	0	np	np	-	np	np	np
10 (29/10/90)	np	np	np	-	0	0	0	np	-	np	np	np
11 (30/11/90)	0	12	np	0	0	0	-	0	0	-	0	0
12 (9/1/91)	67	0	np	27	0	-	-	-	9	57	27	-
13 (6/2/91)	0	0	np	-	-	-	-	2	0	13	60	-
14 (4/3/91)	np	-	-	-	0	-	-	6	25	0	0	-
15 (16/4/91)	np	np	-	0	1	7	-	7	-	18	0	100

* WW, Wheal Watkins; AT, Athelstone; MI, Mitcham; OS, O'Sullivan Beach; MA, Moana Beach; SM, Semaphore; DT, Darlington; DY, Daveyston; SL, Sheoak Log. -, no hosts present; np, no plants present.

3.4.1. *Opius atricornis* Fischer

This species is a primary endoparasitoid attacking 2nd and 3rd instar *L. brassicae* but emerging from the anterior end of host pupae (Table 3.1), i.e. it is a koinobiont. It had the most restricted host plant association of any of the parasitoids collected, only being found on 2 species (Table 3.1). No alternative agromyzid hosts were found.

Few *O. atricornis* were collected during the survey of year 1, being found only during January and February 1989 (Table A3.1). More *O. atricornis* activity was detected during January and February 1990, but only one individual was collected the following season, this being in late November 1990 (Table A3.2). This species appears to be active primarily during summer (Figure 3.1) in association with *T. majus* and, therefore, is of restricted importance in the parasitoid complex of *L. brassicae*.

3.4.2. *Opius cinerariae* Fischer

O. cinerariae was associated with *L. brassicae* on 4 plant species (Table 3.1). No alternative agromyzid hosts were found around Adelaide. It is a primary endoparasitoid attacking all 3 larval stages and emerging from the anterior end of the host pupa (Table 3.1), i.e. it is a koinobiont. In the laboratory, *O. cinerariae* fed on host larvae by piercing them with its ovipositor and feeding on haemolymph exuding from the wound.

During the first year survey, *O. cinerariae* was the most frequently collected parasitoid, being found on 8 of the 12 sample dates (Table A3.1). It was present from August through to November, collected once in January, and then

54

Figure 3.1. Phenology of the parasitoids of *L. brassicae* in the Adelaide region. Boxes indicate months when individuals were collected during the field surveys between 1989-1991. Open boxes, collected for one but not all years sampled. Black boxes, collected for all years sampled.
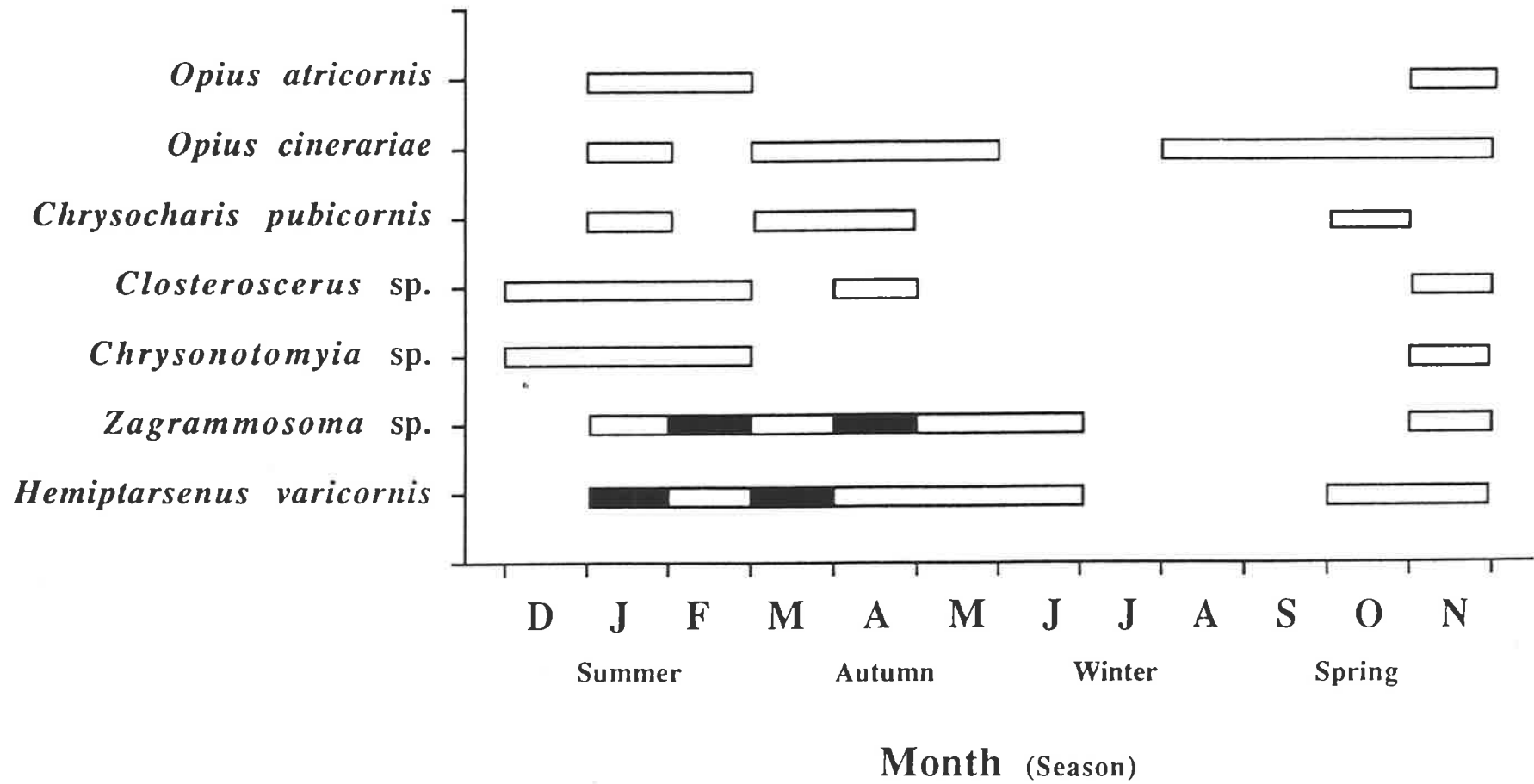
reappeared from March to May. Although few individuals of any parasitoid species were collected during year 1, *O. cinerariae* appeared to be a major component of the parasitoid complex of *L. brassicae*. This was not true in the surveys of years 2 and 3 when few individuals were collected (Table A3.2). The minimal activity detected occurred in summer and autumn. All individuals reared from field collected material during the 2nd and 3rd year surveys were male. Thus, *O. cinerariae* appears to be an occasionally important parasitoid of *L. brassicae* from spring to autumn around Adelaide (Figure 3.1).

3.4.3. *Chrysocharis pubicornis* (Zetterstedt)

L. brassicae was attacked by *C. pubicornis.* on 4 host plants (Table 3.1). *C. syngenesiae* on *S. oleraceus* was an alternate host. It is a primary endoparasitoid found attacking only 2nd and 3rd instar larvae (Table 3.1). Individuals were observed to emerge from both 3rd instar host larvae and pupae , indicating it is a koinobiont (Table 3.1).

During the first year survey, a few individuals were collected on two separate sample dates (Table A3.1). Similarly, *C. pubicornis* was rare throughout the surveys of years 2 and 3 , with no specimens being collected after March 1990 (Table A3.2). The activity observed was in spring, summer and autumn (Figure 3.1). *C. pubicornis* appears to be only a minor member of the parasitoid complex of *L. brassicae* in the Adelaide region.

3.4.4. *Chrysonotomyia* sp.

L. brassicae was attacked by *Chrysonotomyia* sp.on 4 host plants (Table 3.1). This species was normally found as a primary endoparasitoid of 2nd or 3rd instars and pupated within the dead host (Table 3.1). However, occasionally it was found to hyperparasitize other parasitoid larvae. No development of host larvae

took place while *Chrysonotomyia* sp. was developing, indicating it is an idiobiont. No alternate hosts were found.

The largest single collection of any parasitoid species during the first year survey was made for this species (Table A3.1). It was collected in November, December and February, suggesting a summer peak in activity. During the surveys of year 2 and 3, it was again collected mainly in late spring to summer. Sometimes it was the dominant species at a particular site (Table A3.2). *Chrysonotomyia* sp. appears to be an occasionally important component of the parasitoid complex of *L. brassicae*, particularly during summer (Figure 3.1).

3.4.5.   *Closterocerus* sp.

This species is a primary ectoparasitoid of 3rd instars of *L. brassicae* (Table 3.1). Occasionally, two individuals were found on the one host. It is an idiobiont, the host larva being killed shortly after oviposition. Pupation occurs in the host mine adjacent to the dead larva. *L. brassicae* was attacked by *Closterocerus* sp. on 4 host plants (Table 3.1). *C. syngenesiae* on *S. oleraceus* was an alternate host in the Adelaide region.

Several specimens of *Closterocerus* sp. were collected during summer and autumn of the first year survey (Table A3.1). Only one specimen was subsequently collected, this being in January during the 2nd year survey (Table A3.2). *Closterocerus* sp. appears to be only a minor component of the parasitoid complex of *L. brassicae* present only during the warmer parts of the year (Figure 3.1).

3.4.6.   *Hemiptarsenus varicornis* (Girault)

This species is a primary ectoparasitoid attacking 2nd and 3rd instars of *L. brassicae* (Table 3.1). Host larvae are killed at oviposition, indicating *H. varicornis* is an idiobiont. Pupation takes place in the mine adjacent to the dead host.

A semicircular frass deposit is located in the mine, posterior of the pupa. Pupal cases were attached posteriorly to the lower surface of the mine by means of a cuticular thread. Emerging parasitoids made a circular exit hole in the upper surface of the mine. In the laboratory, *H. varicornis* was observed to host feed. *L. brassicae* was parasitized by *H. varicornis* on 5 host plants (Table 3.1). Three alternate hosts were identified, these being *C. humeralis* on aster, *C. syngenesiae* on sowthistle and *L. chenopodii* on goosefoot.

H. varicornis was collected in October, January, February, March and May during the first year survey (Table A3.1). This species was also collected frequently during the surveys of years 2 and 3, only being absent during late spring of 1990 and late summer of 1991 (Table A3.2). It was often the dominant parasitoid species at a particular site. There was a strong bias towards male emergence from 2nd instar hosts while both sexes emerged with approximate equal frequency from 3rd instar larvae (Figure 3.2). *H. varicornis* is a major component of the parasitoid complex of *L. brassicae* throughout the year (Figure 3.1).

3.4.7. *Zagrammosoma* sp.

Zagrammosoma sp. is a primary ectoparasitoid attacking all larval stages of *L. brassicae* (Table 3.1). Host development is halted at oviposition, indicating it is an idiobiont. Pupation takes place in the host mine several millimetres from the dead larva. Emerging parasitoids made a circular exit hole in the upper surface of the mine. *Zagrammosoma* sp. was collected in association with *L. brassicae* on 5 host plants (Table 3.1). No alternate hosts were found.

During the first year survey, *Zagrammosoma* sp. was collected from February through to May (Table A3.1). Regular collections of this species were also made during the 2nd and 3rd year surveys (Table A3.2), it being absent only from collections made in October 1990 and March 1991. *Zagrammosoma* sp. was

59
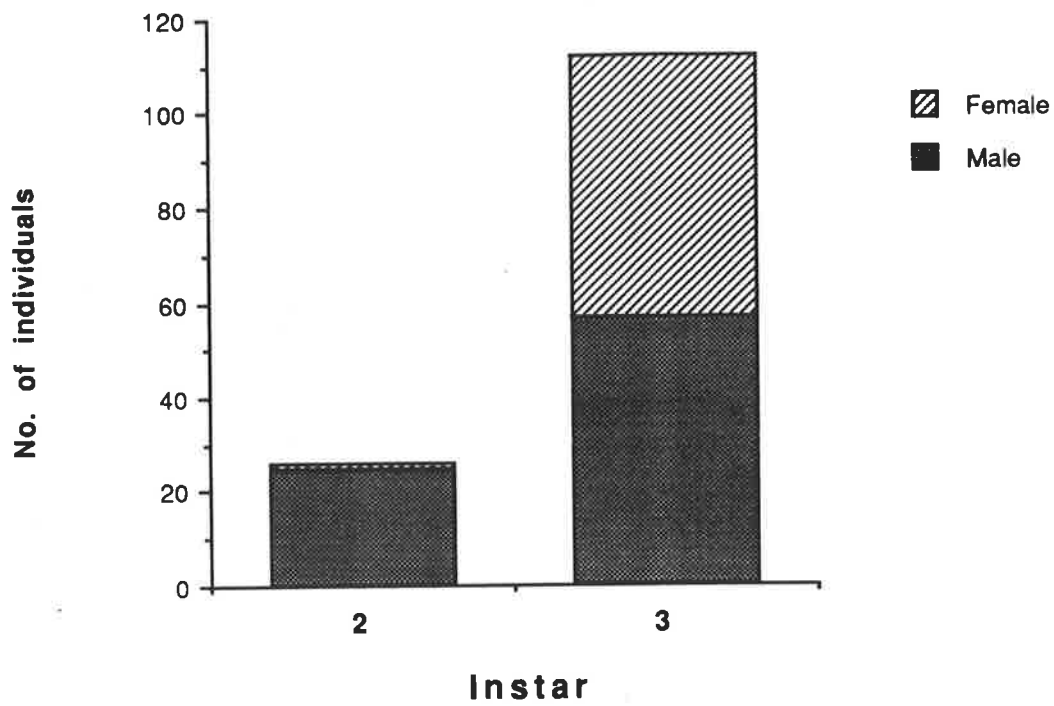
Figure 3.2. Number of emergences of each sex of *H. varicornis* from 2nd and 3rd

instar *L. brassicae* larvae pooled for all sites during the 2nd and 3rd year surveys

(1989-91).

frequently the dominant parasitoid at a particular site and is probably a major component of the parasitoid complex of *L. brassicae* within the Adelaide region thorught the year (Figure 3.1).

3.4.8. Culturing Parasitoids

Attempts were made to establish laboratory cultures of parasitoid species attacking *L. brassicae*. For *Closterocerus* sp., *Chrysonotomyia* sp. and *O. atricornis* these attempts were unsuccessful, probably because of the few individuals available at any one time.   Problems with getting individuals to mate (e.g. *C. pubicornis* and *Zagrammosoma* sp.) or to attack *L. brassicae* in the laboratory (e.g. *Zagrammosoma* sp.) also hindered culturing efforts.   Young females of both *O. cinerariae* and *H. varicornis* were found to mate readily at room temperature (18-25°C) in gelatine capsules.   Older females were less inclined to mate, while those previously mated never remated.   These two species, plus *C. pubicornis*, also attacked *L. brassicae* on excised *B. napus* leaves in laboratory cages.   Each of these 3 species was able to develop successfully under laboratory conditions.

3.5. DISCUSSION

The parasitoid complex of *L. brassicae* in the Adelaide region is relatively small compared to those of other agromyzid species (Takada and Kamijo 1979; Drea *et al.* 1982; Cornelius and Godfray 1984; Kato 1985).   Commonly, only a few species were active on *L. brassicae* populations at a site on a particular date (Table A2.2).   The small number of parasitoids attacking *L. brassicae* in the Adelaide region may be a consequence of it being an introduced species (Kleinschmidt 1970; Spencer 1973).   Native species are likely to have larger parasitoid complexes as a consequence of their longer exposure to native parasitoid speciation (e.g. Austin and Allen 1989). Conversely, species are frequently attacked by few parasitoids when introduced into

areas out of their normal geographic range (Pollard 1979; Wilson and Huffaker 1976; van den Bosch *et al.* 1982). Parasitoid complexes of introduced species are usually comprised of parasitoids introduced along with the host, specifically introduced biological control agents or polyphagous species which attack an alternative host (Zwölfer 1971; Waterhouse and Norris 1987; Ehler 1990). Few comparative studies have been undertaken to directly test these hypotheses, although the numerous examples where introduced species have become agricultural or horticultural pests support these claims (Turnbull and Chant 1961; Wilson and Huffaker 1976; Drea *et al.* 1982; van den Bosch *et al.* 1982; Minkenberg 1988b).

The activity of parasitoids of *L. brassicae* was influenced by the host plant and its phenology. For example, in the Adelaide region *T. majus* undergoes most growth during late spring, senescing during the dry summer. Few viable plants persist during autumn and winter. Correspondingly, peak parasitism was observed during summer when *L. brassicae* was most active on this plant (Chapter Two, Tables 3.2, 3.3). *C. maritima* is a perennial plant found along coastal dunes. Numerous seedlings appear during autumn and spring, while new foliage is added primarily in spring. *L. brassicae* and its parasitoids were active on *C. maritima* for most of the year (Chapter Two, Tables 3.2, 3.3). *R. rugosum* populations were short lived, usually occurring after spring and summer rains. Periods of growth were followed by rapid senescence. Several generations could occur at the one site per year. Presumably as a result of this plant's short lifespan, *L. brassicae* was only sporadically encountered on *R. rugosum* (Chapter Two) and parasitoid activity was similarly sporadic (Tables 3.2, 3.3). *S. officinale* populations increased during late spring and summer, followed by a long period of senescence during autumn. *L. brassicae* was most active on this plant species during its summer growth phase (Chapter Two). Parasitoid populations also showed peak activity during this period (Tables 3.2, 3.3).

Collections of parasitoids, and knowledge of *L. brassicae* and host plant phenology, indicate that considerable movement of parasitoids must occur in the field. Long periods of host plant and *L. brassicae* quiescence followed by bursts in activity may stimulate the immigration of parasitoids from other host plant or host insect patches. The host range of parasitoids is a factor frequently overlooked when considering parasitoid population dynamics (Askew and Shaw 1986). The potential use of alternate hosts for many of the parasitoids of *L. brassicae* undoubtedly influences their abundance and phenology. Most of the parasitoids found attacking *L. brassicae* in the Adelaide region are known to be polyphagous. While alternative hosts were found for only a few species (Table 3.1), this search was not exhaustive. Many of the alternate hosts recorded for some of these parasitoids are known to be present in Australia and could, therefore, act as reservoirs or refuges for these parasitoids.

Parasitoids belonging to the genus *Opius* are only associated with larvae of Diptera (Wharton 1988) and have been used for the biological control of fruit flies (Wharton and Gilstrap 1983). *O. atricornis* has previously been collected from *Ophiomyia lantanae* (Froggatt), *Tropicomyia polyphyta* (Kleinschmidt), *Tropicomyia wilkstroemiae* (Kleinschmidt), *C. humeralis* and *C. syngenesiae* in Australia (Kleinschmidt 1970; Fischer 1971). These last two species were among the potential alternative hosts collected in Adelaide, yet no *O. atricornis* were found in association with them. This is the first record of *O. atricornis* attacking *L. brassicae*.

O. cinerariae was previously collected from *L. brassicae* by Kleinschmidt (1970). Alternative hosts recognized for this species include *L. chenopodii*, *Tropicomyia pisi* (Kleinschmidt) and *C. syngenesiae* (Kleinschmidt 1970; Fischer 1971). However, no alternative hosts were found around Adelaide even though two of these suitable species were known to be present.

C. pubicornis has not previously been collected from *L. brassicae*. However, Stegmaier (1967) and Oatman and Platner (1969) in North America and Kleinschmidt (1970) in Australia reared unidentified specimens of *Chrysocharis* from *L. brassicae*. Species of *Chrysocharis* originate primarily in the northern hemisphere. *C. pubicornis* is the only species known from Australia and probably was introduced from Europe (Bouček 1988; La Salle pers. comm.). Therefore, the species collected by Kleinschmidt (1970) is probably *C. pubicornis*. It is known to parasitize several species of agromyzid including *Liriomyza congesta* (Becker), *Liriomyza strigata* (Meigen), *Phytomyza atricornis* Meigen (now a junior synonym of *Chromatomyia horticola* Goureau and *C. syngenesiae*) (Bouček and Askew 1968), *L. bryoniae*, *Phytomyza petoei* Hering, *Phytomyza fuscula* Zetterstedt and *Phytomyza heringiana* Hendel (Spencer 1973). Kleinschmidt (1970) collected unidentified specimens of *Chrysocharis* from *L. chenopodii* in Queensland which may be *C. pubicornis*. *C. syngenesiae* was an alternate host in the Adelaide region, but *L. chenopodii* was not, even though it is known to be a suitable host (Kleinschmidt 1970).

Closterocerus species are parasitoids of small leafmining larvae of Lepidoptera, Coleoptera and Diptera (Bouček 1988; Spencer 1973). This is the first record of a *Closterocerus* species attacking *L. brassicae* or any other agromyzid in Australia. In the Adelaide region it was also found attacking *C. syngenesiae*.

This is the first record of a *Chrysonotomyia* species attacking *L. brassicae* in Australia. However, specimens of several genera now synonymized with *Chrysonotomyia* have previously been collected. Kleinschmidt (1970) collected specimens of *Achrysocharis* attacking *L. brassicae* in Queensland. Assem (1966) found specimens of *Achrysocharella* associated with this leafminer in Egypt. Johnson *et al.* (1980a) and Johnson (1987) found a *Chrysonotomyia* sp. attacking *L. brassicae* in North America. Specific host records are difficult to determine but other

agromyzids known to be attacked by this genus in Australia include *C. humeralis*, *T. wilkstroemiae*, *T. polyphyta* and *L. chenopodii* (Bouček 1988).

H. varicornis is a widely distributed species found in association with dipterous leafminers (Bouček 1988). It is the only *Hemiptarsenus* species known from Australia and while not positively identified as attacking *L. brassicae*, the record of a *Hemiptarsenus* sp. by Kleinschmidt (1970) may be this species. Alternate hosts previously recorded from Australia include *Ophiomyia phaseoli* (Tryon), *C. humeralis*, *L. chenopodii*, *T. pisi* and *C. syngenesiae* (Kleinschmidt 1970). It has been found in association with many other agromyzid species throughout the world (Spencer 1973; Johnson and Hara 1987; Lynch and Johnson 1987; Waterhouse and Norris 1987). In the Adelaide region, *H. varicornis* was the species most commonly found in association with alternate agromyzid hosts.

Zagrammosoma species are known to attack dipterous leafminers, including *Liriomyza*, in North America and the Pacific region (Johnson and Hara 1987; Waterhouse and Norris 1987). However, *L. brassicae* is the first host known for this genus in Australia. No alternative hosts were identified in the Adelaide region.

Zagrammosoma sp. and *H. varicornis* were the most abundant parasitoid species attacking *L. brassicae* around Adelaide. These species share several features. They are both idiophytic ectoparasitoids and, therefore, are likely to have a competitive advantage over the koinophytic or endoparasitic species (Askew 1975). In addition, they are both active throughout most of the year and are found in association with all the major host plants of *L. brassicae* (Figure 3.1, Table 3.1) Other species, such as *Chrysonotomyia* sp., *O. atricornis* and *O. cinerariae*, have more restricted periods of activity on this host and fewer host plant associations (Figure 3.1, Table 3.1). Shorter windows of activity could place these species at a competitive disadvantage compared to those which are active for most of the year.

The collection methods used in this study provided information on the parasitism of larval populations. No attempt was made to sample pupae of the host in the soil. Therefore, parasitoids which attack larval stages but complete development within the host pupa may be under-represented in these samples. This would include *O. atricornis*, *O. cinerariae* and *C. pubicornis*. Harding (1965) indicates that the percentage of total parasitism recorded for larval-pupal species may be quite different between pupal and larval samples.

Attempts to rear many of the parasitoid species of *L. brassicae* were confounded by insufficient specimens, as well as problems with mating and/or attacking *L. brassicae* in the laboratory. Recent work by Field (1990) indicates the need for suitable mating chambers for parasitoids. It is possible that the chambers used here (i.e. gelatine capsules, aerated vials or cages) did not provide suitable surfaces upon which courtship signals could be transmitted, or provided inadequate circulation of pheromones involved in courtship. The reluctance of some species to attack caged *L. brassicae* may also indicate impairment of normal search behaviour due to insufficient air circulation or changes in the type or quantity of synomones present.

On the basis of field abundance (year 1 survey, Table A3.1) and ease of rearing in the laboratory, *H. varicornis* and *O. cinerariae* were chosen as species on which to concentrate further investigations. Information from the 2nd and 3rd years surveys (Table A3.2) suggested considerable differences exist in the way these two parasitoids utilized the host population. In addition to the obvious differences in their basic biology (Table 3.1), *O. cinerariae* was found to attack all larval stages of *L. brassicae* while *H. varicornis* only attacked 2nd and 3rd instar hosts. Furthermore, field sex ratios of *H. varicornis* were male biased from 2nd instar and unbiased from 3rd instar hosts. In contrast, all *O. cinerariae* individuals collected during the 2nd and 3rd year surveys were male, however, the small sample size makes it difficult to draw

conclusions from this result. These two species appear to be interesting subjects with which to test predictions of progeny and sex ratio theory (Fisher 1930; Charnov *et al.* 1981; Bull 1981; Werren 1984; Waage and Godfray 1985; Charnov and Stephens 1988; Strand 1988; Mangel 1989), particularly in relation to host development (size/age). A series of laboratory experiments were conducted comparing the host stage utilization strategies of these two species, and these are discussed in the following chapters.

CHAPTER FOUR

ATTACK OF HOST STAGES BY NAIVE FEMALES OF *Hemiptarsenus varicornis* AND *Opius cinerariae*

4.1. ABSTRACT

Progeny production and host-feeding were investigated in relation to the 3 instars of *Liriomyza brassicae* for mated or unmated naive females of the idiobiont, *Hemiptarsenus varicornis,* and the koinobiont, *Opius cinerariae.* Responses of females were determined in situations with no choice between host instars and with all 3 host instars available. Low levels of reproduction and host feeding were observed for both species. *O. cinerariae* showed no differential oviposition on host stages. However, *H. varicornis* only utilized 2nd and 3rd instar hosts, with greater reproduction on 3rd instars. Mated females of both species produced more progeny than their unmated counterparts. Higher than expected levels of mortality occurred in 1st and 3rd instar hosts for both species, suggesting that host feeding and injury through ovipositor probing were important mortality factors. The range of host stages available had little influence on host stage utilization.

4.2. INTRODUCTION

Parasitoids attacking hosts with overlapping generations have a range of host stages to attack at any one time. It is common for host stages to be attacked differentially, with particular stages suffering higher rates of parasitism than others. For example, *Diglyphus isaea* (Walker) parasitizes only the 3rd instars of *Liriomyza trifoliearum* Spencer and 2nd and 3rd instars of *Chromatomyia syngenesiae* Hardy (Hendrickson 1975; Ibrahim and Madge 1979). Sugimoto and Ishii (1979) found that *Chrysocharis pentheus* Walker preferentially attacks 3rd instars of *Phytomyza ranunculi* Schrank. *Ganaspidium hunteri* (Crawford) attacks only 3rd instars of *Liriomyza trifolii* (Burgess) (Chandler *et al.* 1988). Heinz and Parrella (1989) observed parasitism by *Diglyphus begini* (Ashmead) increasing directly with host stage of *L. trifolii* .

Attack by parasitoids may involve oviposition, host feeding or mechanical injury by ovipositor probing. Parasitoids which host feed will frequently choose different host stages to parasitize and feed on (De Bach 1943; Bartlett 1964; Quednau 1964; Sugimoto 1977; Sugimoto and Ishii 1979; Jervis and Kidd 1986; Heinz and Parrella 1989). Mortality resulting from ovipositor probing is most often associated with early stages of host development (Burnett 1962; Sugimoto 1977).

Differential rates of attack of host stages by parasitoids may arise by several processes. The range of host stages encountered has been suggested to influence patterns of parasitoid oviposition (Charnov *et al.* 1981; Jones 1982; Charnov and Skinner 1985; Strand 1988). The chance of encountering different host stages may lead to differential attack (Price 1972; Sugimoto 1977; van Alphen 1980; van Alphen and Drijver 1982; Liu *et al.* 1984). Variation in the acceptance of host stages by parasitoids may also result in different levels of parasitism. Acceptability has been shown to be influenced by host size (Opp and Luck 1986; Reeve 1987; Simbolotti *et al.* 1987; de Jong and van Alphen 1989), age (van Alphen 1980; Juliano 1982), morph

(Liu *et al*. 1984; Lardner and Hales 1990), and stage of development (van Alphen *et al*. 1976; Hopper 1986). Physical defences of the host may reduce the oviposition ability of parasitoids (van Alphen 1980; Taylor 1988; Allen 1990). In addition, oviposition is influenced by a parasitoid's egg supply (Bartlett 1964; Sugimoto and Ishii 1979; Morrison and Strong 1981; Prasad *et al*., in prep.). These factors may all be influenced by the age or level of experience of the female parasitoid (van Lenteren 1976; van den Assem *et al*. 1984; Avilla and Albajes 1984; Charnov and Skinner 1985; Wong *et al*. 1990).

Naive parasitoids may utilize host stages differently than experienced females. Having no prior experience with any host stage, they lack information about the relative size or suitability of a host encountered (Charnov and Skinner 1985). Therefore, oviposition decisions are likely to be made on an absolute basis. If host stages differ in their acceptability or suitability (Vinson and Iwantsch 1980a, b), then limiting oviposition until the range of host stages available has been assessed would conserve reproductive potential. This would enable the parasitoid to allocate the majority of eggs in a manner which maximized fitness.

Similarly, whether a parasitoid is mated or unmated may influence her oviposition activity. It is generally assumed that female offspring represent a better investment than male progeny (Charnov *et al*. 1981; Charnov and Skinner 1985). A strategy conserving reproductive potential until mated may serve to increase overall fitness. If this is the case, then unmated females would be expected to produce fewer progeny than their mated counterparts. Furthermore, older unmated females may increase their oviposition activity as a consequence of the diminishing time in which to realise their reproductive potential. Age has been shown to influence the oviposition activity of mated parasitoids (Avilla and Albajes 1984; Wong *et al*. 1990) but has not previously been investigated for unmated females.

These predictions were tested for *Opius cinerariae* Fischer and *Hemiptarsenus varicornis* (Girault), two parasitoids of *Liriomyza brassicae* (Riley). The biology of these two species was discussed in Chapter Three. Levels of reproduction on each host instar were investigated for naive parasitoids in choice and no-choice situations. In addition, mortality as a result of naive parasitoid activity was determined. The influence of a female's age and mating status upon host-stage utilization was determined for each species.

4.3. MATERIALS AND METHODS

Adult female parasitoids were reared from 3rd instar hosts and kept separated from males at 15($\pm$2)°C. They were less than 6 days old and fed with honey prior to use. A single male and female wasp were placed together in a gelatine capsule and observed until mating occurred.

4.3.1. No-choice Experiments

Levels of parasitism on the three larval instars of *L. brassicae* were determined by caging a single naive female parasitoid with 3 infested *B. napus* leaves. Each leaf contained at least 10 host larvae, all at the same stage of development (Appendix One). The petioles of these leaves were placed in a vial of water (55 x 25 mm), which was then plugged with cotton wool and placed on the inverted lid of a plastic petri dish (150 mm). A 1.5 litre capacity plastic container (British Plastics Pty Ltd, Medium, No. 532) was inverted over the vial and leaves. Two holes (60 mm diameter) were cut into opposite sides and covered with fine gauze to provide aeration. A single female, either mated or unmated, was introduced into the cage and left undisturbed at 20($\pm$2)°C, photoperiod 12L:12D for 24 hours. Following this, the parasitoids were removed. Leaves from cages with *O. cinerariae* were transferred to a large plastic petri dish containing filter paper (Whatman No. 1, 12.5 cm) and held at

$25(\pm 2)°C$. As *H. varicornis* pupates within the mine, leaves from these cages were maintained in the experimental set up (minus the parasitoid) for 2-3 days to preserve leaf turgor. They were then transferred to plastic petri dishes and treated as for leaves from *O. cinerariae* cages. Petri dishes were checked daily and emerged larvae were transferred to vials (glass, 50 x 18 mm or plastic, 40 x 15 mm; see Appendix One) to pupate. Pupae were held at 25°C until all adult flies and parasitoids emerged or a period of 3 weeks had elapsed. Leaves from cages with *H. varicornis* were kept in storage for one week after the last adult parasitoid emerged or a period of 3 weeks had elapsed, whichever occurred first.

Cages containing each host instar were replicated 40 times for mated and unmated parasitoids of each species. In addition, 20 control cages using the same experimental set up but with no parasitoid, were established for each instar and species. The numbers of parasitoids and hosts emerging, and the incidence of mortality were recorded for each replicate. Parasitoid emergence was used as a measure of reproduction. Mortality resulting from parasitoid activity other than parasitism will be referred to throughout this chapter as "parasitoid-related mortality".

4.3.2. Choice Experiments

In choice experiments, the experimental set up was identical to that described for no-choice experiments except that each leaf within a cage contained hosts of a different developmental stage. Following parasitoid removal from *H. varicornis* cages, each leaf was transferred to a separate cage to isolate emergences of each cohort but maintained in water to retain leaf turgor. Otherwise, experimental procedures did not differ from those described for no-choice experiments.

Forty experimental cages for mated and unmated females and 20 control cages were established for each parasitoid species.

4.3.3. Statistical Analysis

The number of cages in which parasitism occurred were compared between host instars and mated and unmated females by the log-likelihood (G) test (SAS 1985). Cages were classified into mated or unmated, active (where parasitism was observed) and inactive (where no parasitoid emergences occurred). As many more hosts of each instar were offered than could be parasitized within the experimental period, the importance of different host densities between replicates was considered minimal. Therefore, parasitoid reproduction was analysed by non-parametric analysis of variance (Kruskal-Wallis; SAS 1985) using host instar, age and mating status of females, and activity status of cage as the independent variables. Multiple comparisons followed the methods of Conover (1980). Parasitoid-related mortality levels were determined by

$$M_{parasitoid-related} = M_{observed} - M_{expected} \qquad \text{(Equation 4.1.)}$$

where $M_{expected}$ was calculated by multiplying the rate of mortality per host instar for each species from the results of the control cages by the total number of hosts per cage. If expected mortality was greater than that observed, a negative result was recorded. Levels of mortality estimated to be parasitoid-related were compared between host instars and cage types (mated vs unmated, active vs inactive) by Kruskal-Wallis (SAS 1985). Multiple comparisons followed the methods of Conover (1980).

4.4. RESULTS

4.4.1. No-choice Experiments

4.4.1.1. Parasitism

H. varicornis

Reproduction was recorded only from cages with 2nd and 3rd instar hosts for both mated and unmated females (Table 4.1; Figure 4.1). More mated

Table 4.1. Numbers of cages in which reproduction by mated and unmated parasitoids occurred for each host instar from the no-choice experiments with naive parasitoids.

Instar	Wasp Status	*Hemiptarsenus varicornis*		*Opius cinerariae*	
		Parasitoid emergences recorded	No parasitoid emergences recorded	Parasitoid emergences recorded	No parasitoid emergences recorded
1	Mated	0	40	19	21
	Unmated	0	40	8	32
2	Mated	3	37	21	19
	Unmated	4	36	5	35
3	Mated	22	18	26	14
	Unmated	6	34	9	31

74

Figure 4.1. Fate of *L. brassicae* exposed to naive, (A) mated and (B) unmated female *H. varicornis* and those in (C) control cages for the no-choice experiment (data pooled for each type of cage).

A. Mated

B. Unmated

C. Control
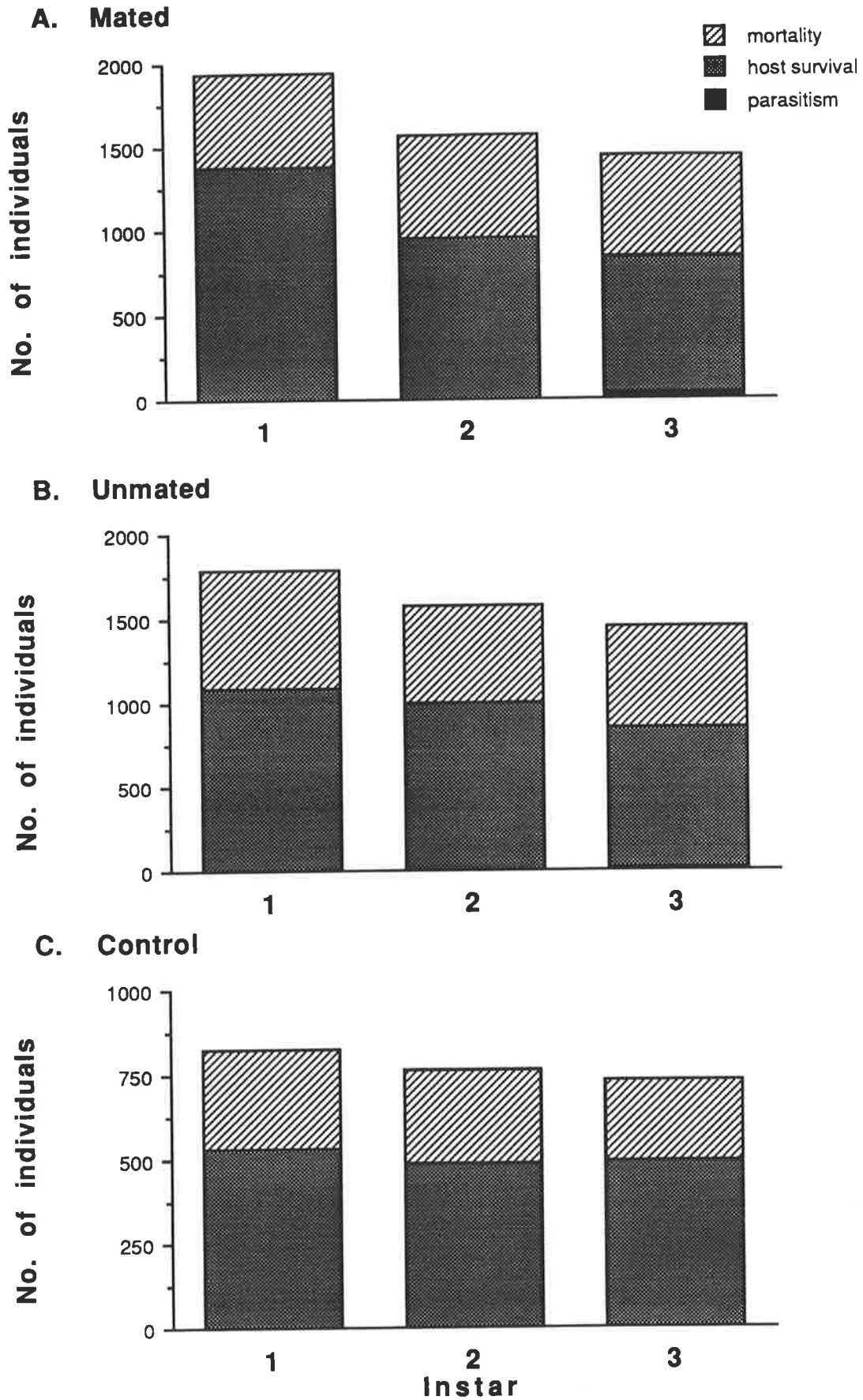
females reproduced in cages with 3rd instars than unmated females. The number of cages in which mated parasitoids reproduced varied between host instars. Considerably more cages with 3rd instar hosts had emergences than those with 1st or 2nd instars.

For *H. varicornis*, only one parasitoid emerged in the majority of cages and, consequently, these contained only one leaf with parasitized hosts (Table 4.2). Reproduction on 2nd and 3rd instar hosts was low for both mated and unmated females (Tables 4.3 and 4.4), varying with both instar of host at oviposition and mating status of the female. Multiple comparisons revealed greater reproduction occurred in cages with 3rd instar hosts and mated females.

O. cinerariae

Reproduction was recorded from all 3 instars of *L. brassicae* (Table 4.1; Figure 4.2). Mated parasitoids reproduced in more cages than unmated females for all three instars. The number of cages from which *O. cinerariae* progeny emerged was independent of host instar for both mated and unmated females.

Emergences of *O. cinerariae* were frequently spread over 2 or 3 leaves (Table 4.2). Greater levels of reproduction were observed for *O. cinerariae* than *H. varicornis* (Tables 4.3. and 4.4.) but this was found not vary with host instar for either mated or unmated parasitoids. However, *O. cinerariae* produced more progeny in cages with mated than unmated females.

Table 4.2. Distribution of parasitoid emergences among leaves in cages containing each host instar separately or all together (choice) for mated and unmated females of *H. varicornis* and *O. cinerariae*.

		Hemiptarsenus varicornis				*Opius cinerariae*			
Number of leaves with parasitism (maximum = 3)		0	1	2	3	0	1	2	3
Instar	Wasp status								
1st	Mated	40	0	0	0	21	8	6	5
	Unmated	40	0	0	0	32	3	3	2
2nd	Mated	37	3	0	0	19	6	5	10
	Unmated	36	3	1	0	35	2	0	3
3rd	Mated	18	16	6	0	15	7	9	9
	Unmated	33	4	3	0	31	3	2	4
Choice	Mated	24	13	3	0	19	9	11	1
	Unmated	31	8	1	0	32	1	6	1

Table 4.3. Mean numbers of offspring produced on each host instar by mated and unmated females for the no-choice experiment with naive parasitoids.

		Hemiptarsenus varicornis *		Opius cinerariae	
Instar	Wasp status	Total	Mean ± standard error	Total	Mean ± standard error
1	Mated	40	0	40	2.44 ± 0.58 [a]
	Unmated	40	0	40	1.10 ± 0.42 [b]
2	Mated	40	0.10 ± 0.06 [b]	40	3.63 ± 0.87 [a]
	Unmated	40	0.15 ± 0.08 [b]	40	1.68 ± 0.86 [b]
3	Mated	40	0.95 ± 0.18 [a]	40	3.80 ± 0.73 [a]
	Unmated	40	0.29 ± 0.12 [b]	40	1.38 ± 0.51 [b]

* 1st instars not included in analysis for *H. varicornis*.
For each species, means followed by the same letter are not significantly different (P > 0.05).

Table 4.4. Mean numbers of offspring produced on each host instar by mated and unmated females from cages where parasitism was present (active) for the no-choice experiments with naive parasitoids.

		Hemiptarsenus varicornis *NS		Opius cinerariae NS	
Instar	Wasp status	Total	Mean ± standard error	Total	Mean ± standard error
1	Mated	0	0	19	5.00 ± 0.85
	Unmated	0	0	8	5.50 ± 1.25
2	Mated	3	1.33 ± 0.33	21	6.90 ± 1.29
	Unmated	4	1.50 ± 0.29	5	13.4 ± 4.37
3	Mated	22	1.73 ± 0.20	26	5.85 ± 0.90
	Unmated	7	1.71 ± 0.36	9	6.11 ± 1.46

* 1st instars not used in analysis for *H. varicornis*.
NS No significant difference among means.

Figure 4.2. Fate of *L. brassicae* exposed to naive, (A) mated and (B) unmated female *O. cinerariae* and those in (C) control cages for the no-choice experiment (data pooled for each type of cage).

A. Mated

B. Unmated

C. Control
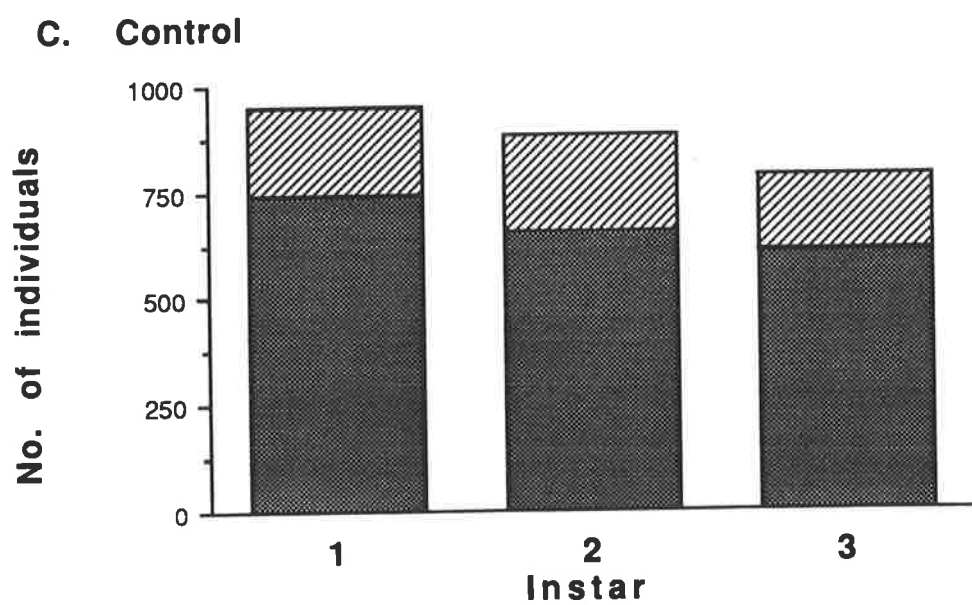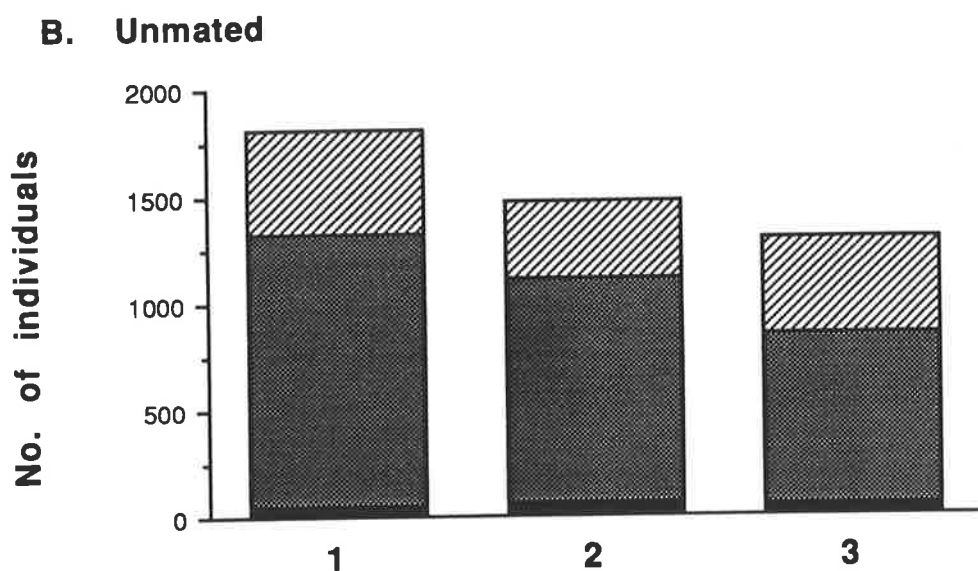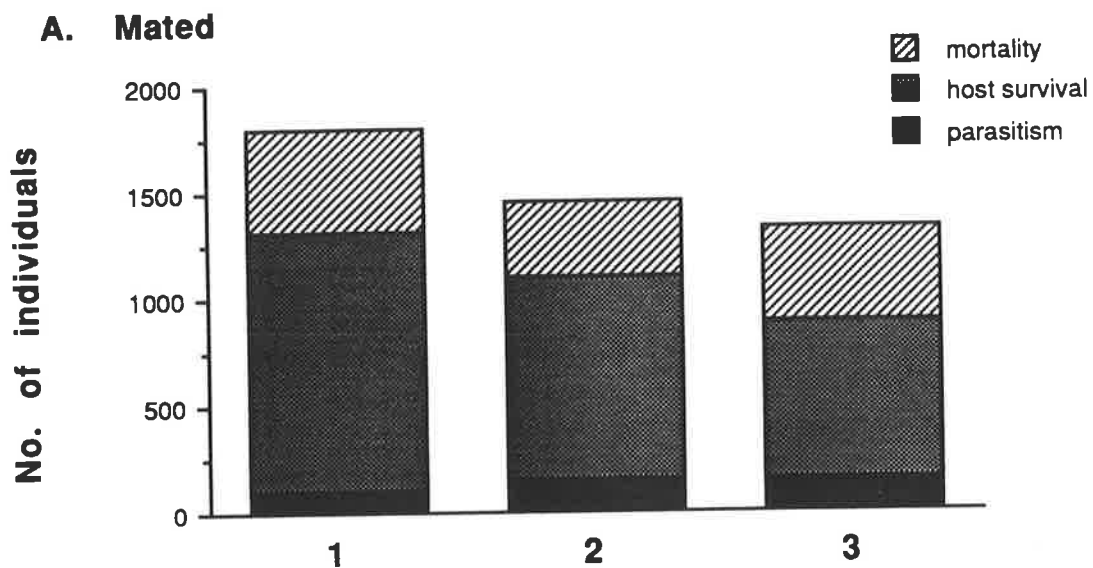
The number of *H. varicornis* or *O. cinerariae* progeny emerging from no-choice cages where parasitism occurred were not influenced by host instar or the mating status of the female (Table 4.4).

Female age did not influence the number of progeny produced for either parasitoid species (Tables 4.5 and 4.6).

4.4.1.2. Host Mortality

H. varicornis

Levels of *H. varicornis* -related host mortality were significantly influenced by host instar and the mating status of the female (Table 4.7). Multiple comparisons revealed more 3rd instar mortality could be attributed to parasitoid activity than either 1st or 2nd instar mortality in cages with mated females. In addition, unmated females caused greater mortality of 1st instar hosts than mated females. Results indicate that there is actually greater host survival than expected for 1st instars exposed to mated females.

Host mortality levels resulting from *H. varicornis* which reproduced and those that did not were not significantly different (Table 4.8).

O. cinerariae

Levels of host mortality attributed to *O. cinerariae* were influenced by the host instar but not by the mating status of the female (Table 4.7). More parasitoid-related mortality occurred in 3rd instar hosts than either 1st or 2nd instars. First instar hosts suffered greater mortality as a result of the parasitoid than 2nd instars.

Table 4.5. Mean numbers of offspring produced on each host instar by mated or unmated *H. varicornis* of different ages from the no-choice experiment with naive parasitoids.

	Mated		Unmated	
Age (days)	N	Mean ± standard error	N	Mean ± standard error
2nd instar				
1	17	0.06 ± 0.06	10	0
2	7	0	4	0
3	6	0	8	0.25 ± 0.16
4	0	-	5	0
5	4	0.25 ± 0.25	12	0.33 ± 0.22
6	6	0.50 ± 0.34	2	0
3rd instar				
1	11	1.27 ± 0.47	16	0.56 ± 0.20
2	7	0.57 ± 0.30	3	0.67 ± 0.67
3	6	1.17 ± 0.31	14	0.21 ± 0.15
4	12	1.08 ± 0.26	9	0.67 ± 0.44
5	4	1.00 ± 0.58	3	0.33 ± 0.33
6	0	-	0	-

Table 4.6. Mean numbers of offspring produced on each host instar by mated or unmated *O. cinerariae* of different ages from the no-choice experiment with naive parasitoids.

Age (days)	Mated		Unmated	
	N	Mean ± standard error	N	Mean ± standard error
1st instar				
1	2	0	8	0
2	10	2.10 ± 0.96	18	1.11 ± 0.50
3	10	3.70 ± 1.51	6	0
4	11	1.73 ± 1.04	12	2.08 ± 1.16
5	4	3.25 ± 1.97	0	-
6	2	2.00 ± 1.41	0	-
2nd instar				
1	2	1.00 ± 0.71	2	0
2	16	2.13 ± 0.85	6	0
3	17	3.59 ± 1.31	30	1.93 ± 0.98
4	8	6.50 ± 2.90	5	4.60 ± 4.60
5	0	-	0	-
6	0	-	0	-
3rd instar				
1	12	2.83 ± 1.17	7	0.29 ± 0.29
2	7	1.86 ± 1.37	4	1.00 ± 0.87
3	8	6.13 ± 2.36	13	0.46 ± 0.31
4	3	1.67 ± 1.36	7	0.86 ± 0.86
5	8	5.63 ± 1.40	8	2.75 ± 1.52
6	1	2.00	1	15.00

Table 4.7. Mean numbers of parasitoid-related mortalities (estimated by Equation 4.1) of each host instar when exposed to mated and unmated females during the no-choice experiment with naive parasitoids.

Instar	Cage type	*Hemiptarsenus varicornis*		*Opius cinerariae*	
		Total	Mean ± standard error	Total	Mean ± standard error
1	Mated	40	-3.16 ± 1.35 ^{c}	40	2.44 ± 0.99 ^{b}
	Unmated	40	1.50 ± 1.31 ^{ab}	40	2.41 ± 0.92 ^{b}
2	Mated	40	0.96 ± 0.86 ^{bc}	40	-0.25 ± 0.69 ^{c}
	Unmated	40	-0.21 ± 0.72 ^{bc}	40	-0.08 ± 0.62 ^{c}
3	Mated	40	3.17 ± 0.58 ^{a}	40	3.37 ± 0.65 ^{a}
	Unmated	40	3.37 ± 0.66 ^{a}	40	3.72 ± 0.70 ^{a}

For each species, means followed by the same letter are not significantly different (P > 0.05).

Table 4.8. Mean numbers of *H. varicornis* -related mortalities (estimated by Equation 4.1) of each host instar in cages where parasitoid reproduction occurred (active) and where it did not (inactive) from the no-choice experiment with naive females.

Instar	Wasp Status	Active		Inactive	
		Total	Mean ± standard error	Total	Mean ± standard error
1 *	Mated	0	-	40	-3.16 ± 1.35
	Unmated	0	-	40	1.50 ± 1.31
2	Mated	3	1.46 ± 1.14^b	37	0.92 ± 0.93^b
	Unmated	4	-0.50 ± 1.29^b	36	-0.17 ± 0.79^b
3	Mated	22	3.40 ± 0.67^a	18	2.90 ± 1.00^a
	Unmated	7	3.90 ± 1.51^a	33	3.25 ± 0.74^a

* Not used in the analysis.
Instars followed by the same letter are not significantly different (P > 0.05; data pooled for mating status and activity).

Table 4.9. Mean numbers of *O. cinerariae* -related mortalities (estimated by Equation 4.1) of each host instar in cages where parasitoid reproduction occurred (active) and where it did not (inactive) from the no-choice experiment with naive females.

Instar	Wasp Status	Active		Inactive	
		Total	Mean ± standard error	Total	Mean ± standard error
1	Mated	19	2.94 ± 1.44 [ab]	21	1.98 ± 1.38 [ab]
	Unmated	8	1.33 ± 1.82 [abc]	32	2.68 ± 1.06 [ab]
2	Mated	21	0.65 ± 1.13 [bc]	19	-1.25 ± 0.69 [bc]
	Unmated	5	-3.31 ± 1.66 [c]	35	0.38 ± 0.63 [bc]
3	Mated	25	2.98 ± 0.76 [ab]	15	4.02 ± 1.19 [ab]
	Unmated	9	6.53 ± 1.42 [a]	31	2.90 ± 0.75 [ab]

Means followed by the same letter are not significantly different (P > 0.05).

Third instar hosts suffered greater mortality when exposed to unmated but reproducing *O. cinerariae* than 2nd instars when exposed to any parasitoid group (Table 4.9). Second instar hosts exposed to active, unmated females exhibited greater survival than all 3rd instars and those 1st instars exposed to either mated, or inactive and unmated parasitoids.

4.4.2. Choice Experiments

4.4.2.1. Parasitism

H. varicornis

Mated and unmated *H. varicornis* reproduced on only 2nd and 3rd instar hosts (Table 4.10). More mated than unmated females reproduced on 3rd instar hosts. Mated females reproduced more often on 3rd than 2nd instars.

In choice experiments, females produced none or only one offspring in the majority of cages (Table 4.2). All emergences were from leaves with 3rd instar hosts from cages with mated females. For cages containing unmated females, three quarters of emergences were from 3rd instar hosts.

Low levels of reproduction by females of either mating status occurred on both 2nd and 3rd instar hosts (Tables 4.11 and 4.12; Figure 4.3). Reproduction varied with both the host instar at oviposition and the mating status of the female but there was no significant interaction between these factors. More 3rd instar hosts were parasitized by mated than unmated females. Mated females parasitized more 3rd than 2nd instar hosts.

Table 4.10. Numbers of cages in which reproduction by mated and unmated parasitoids occurred for each host instars from the choice experiments with naive parasitoids.

Instar	Wasp Status	Hemiptarsenus varicornis		Opius cinerariae	
		Parasitoid emergences recorded	No parasitoid emergences recorded	Parasitoid emergences recorded	No parasitoid emergences recorded
1	Mated	0	40	10	30
	Unmated	0	40	2	38
2	Mated	3	37	8	32
	Unmated	2	38	7	33
3	Mated	16	24	15	25
	Unmated	7	33	7	33
Overall	Mated	16	24	18	22
	Unmated	9	31	8	32

Figure 4.3. Fate of *L. brassicae* exposed to naive, (A) mated and (B) unmated female *H. varicornis* and those in (C) control cages for the choice experiment (data pooled for each type of cage).
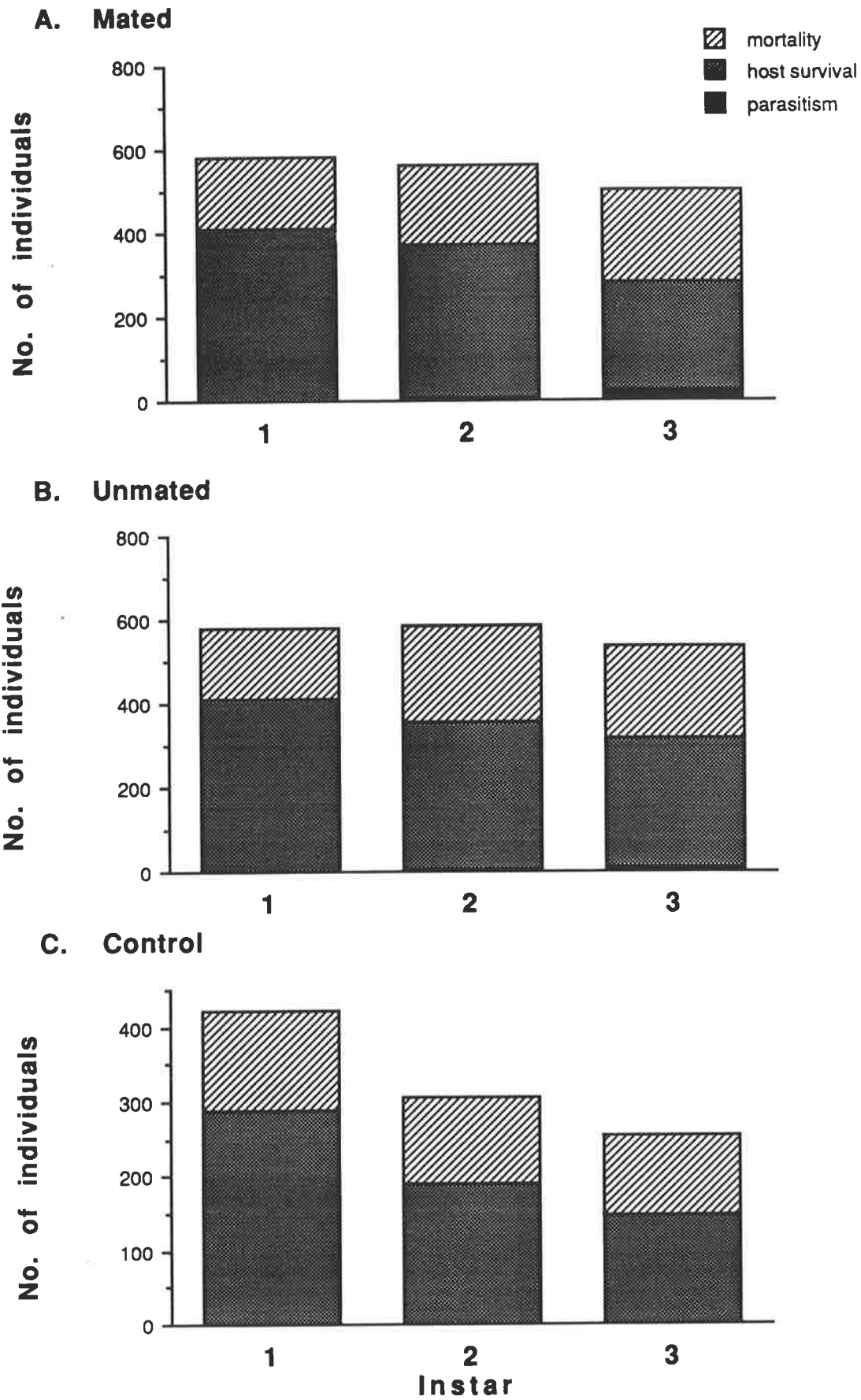
Table 4.11. Mean numbers of offspring produced on each host instar by mated and unmated females for choice experiments with naive parasitoids.

Instar	Wasp Status	*Hemiptarsenus varicornis* [*]		*Opius cinerariae* [NS]	
		Total	Mean ± standard error	Total	Mean ± standard error
1	Mated	40	0	40	0.60 ± 0.22
	Unmated	40	0	40	0.08 ± 0.06
2	Mated	40	0.10 ± 0.06 [b]	40	0.63 ± 0.23
	Unmated	40	0.08 ± 0.06 [b]	40	0.60 ± 0.23
3	Mated	40	0.63 ± 0.14 [a]	40	1.15 ± 0.33
	Unmated	40	0.20 ± 0.07 [b]	40	0.48 ± 0.21

[*] 1st instars not included in analysis for *H. varicornis*.
[NS] No significant difference between means.
For *H. varicornis*, means followed by the same letter are not significantly different (P > 0.05).

Table 4.12. Mean numbers of offspring produced on each host instar by mated and unmated females for cages where parasitism was present (active) for the choice experiments with naive parasitoids.

Instar	Wasp Status	*Hemiptarsenus varicornis* [*NS]		*Opius cinerariae* [NS]	
		Total	Mean ± standard error	Total	Mean ± standard error
1	Mated	0	0	10	4.30 ± 0.86
	Unmated	0	0	2	4.00 ± 2.00
2	Mated	3	1.33 ± 0.33	8	3.25 ± 0.86
	Unmated	2	1.50 ± 0.50	7	4.71 ± 1.17
3	Mated	16	1.56 ± 0.16	14	4.79 ± 0.43
	Unmated	7	1.14 ± 0.14	7	4.57 ± 1.23

[*] 1st instar not used in analysis for *H. varicornis*.
[NS] No significant difference between means.

O. cinerariae

Both mated and unmated *O. cinerariae* reproduced on all three host instars (Table 4.10). More mated females reproduced than unmated females on both 1st instar and 3rd instar hosts. Host instar did not influence the number of females reproducing, regardless of mating status.

For cages where reproduction occurred, progeny frequently emerged from more than one leaf (Table 4.2). Overall levels of reproduction were low on all three host instars (Tables 4.11; Figure 4.4) and did not vary with mating status of the parasitoids or host instar. A similar number of progeny were produced on each host instar by females which reproduced, regardless of whether they were mated (Table 4.12).

4.4.2.2. Host Mortality

H. varicornis

Host mortality attributed to *H. varicornis* was low during the choice experiment (Table 4.13). Levels of *H. varicornis* -related host mortality were not influenced by host instar or the mating status of the female. Host mortality attributed to *H. varicornis* which did or did not reproduce was not significantly different (Table 4.14).

O. cinerariae

Host mortality attributed to *O. cinerariae* was influenced by host instar but not the mating status of the female (Table 4.13). Multiple comparisons revealed most parasitoid-related host mortality occurred on 3rd instars, less on 2nd instars and least on 1st instar hosts. Slightly greater host survival than expected was observed for 1st instar hosts when exposed to either mated or unmated *O. cinerariae*. Whether females were actively reproducing or not had no influence on the level of host mortality that could be attributed to them (Table 4.15).

Figure 4.4. Fate of *L. brassicae* exposed to naive, (A) mated and (B) unmated

female *O. cinerariae* and those in (C) control cages for the choice experiment (data

pooled for each type of cage).

A. Mated

B. Unmated

C. Control
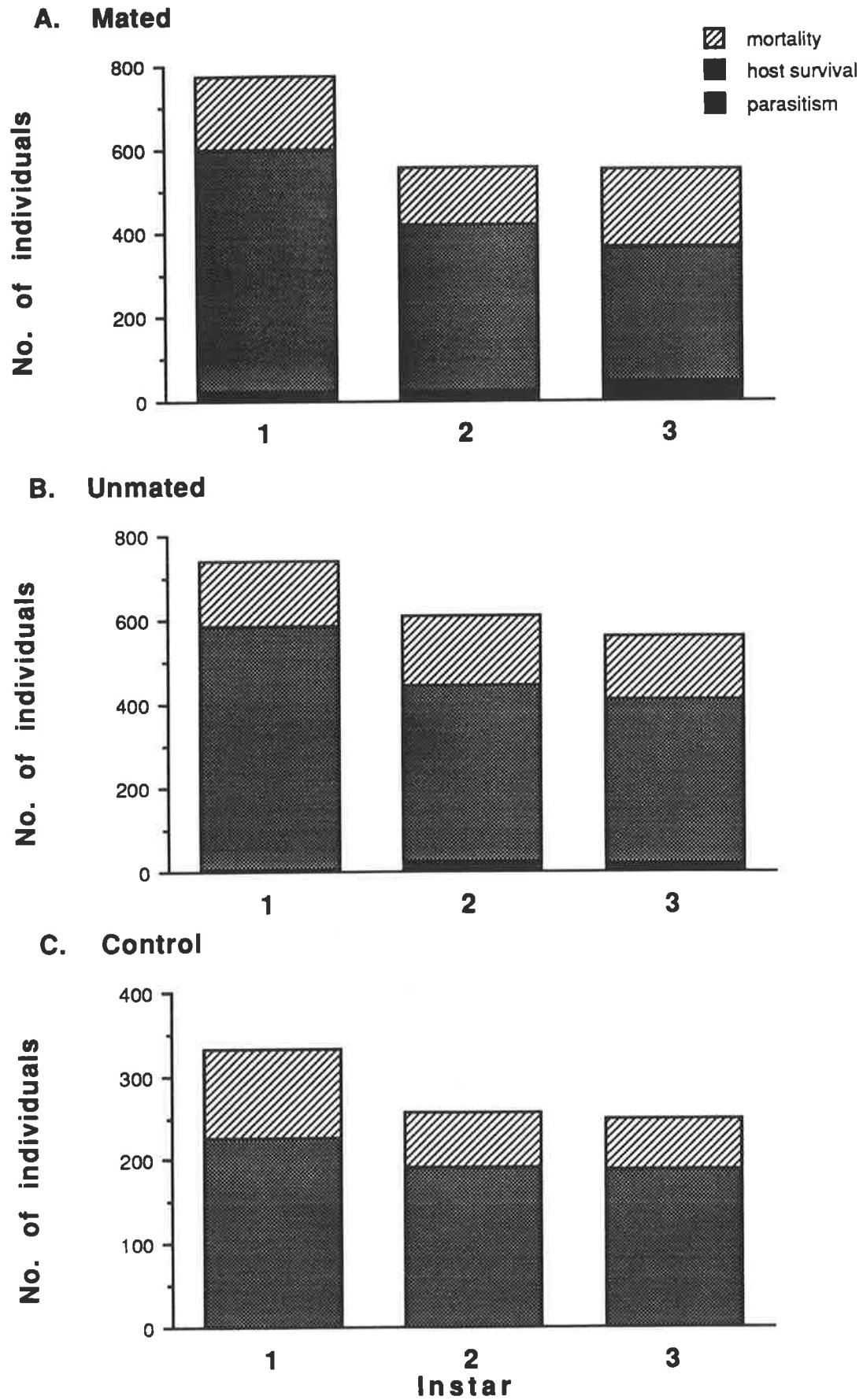
Table 4.13. Mean numbers of parasitoid-related mortalities (estimated by Equation 4.1) of each host instar when exposed to mated and unmated females from the choice experiments with naive parasitoids.

Instar	Wasp Status	*Hemiptarsenus varicornis* NS		*Opius cinerariae*	
		Total	Mean ± standard error	Total	Mean ± standard error
1	Mated	40	-0.54 ± 0.51	40	-1.63 ± 0.67 [c]
	Unmated	40	-0.84 ± 0.38	40	-1.68 ± 0.42 [c]
2	Mated	40	-0.56 ± 0.47	40	-0.17 ± 0.37 [b]
	Unmated	40	0.47 ± 0.48	40	0.22 ± 0.42 [b]
3	Mated	40	-0.23 ± 0.41	40	1.25 ± 0.30 [a]
	Unmated	40	-0.48 ± 0.46	40	0.34 ± 0.34 [a]

NS No significant difference between means.
Means followed by the same letter are not significantly different ($P > 0.05$).

Table 4.14. Mean numbers of *H. varicornis* -related mortalities (estimated by Equation 4.1) of each host instar in cages where parasitoid reproduction occurred (active) and where it did not (inactive) from the choice experiment with naive females.

Instar	Wasp Status	Active		Inactive	
		Total	Mean ± standard error	Total	Mean ± standard error
1 [*]	Mated	0	0	40	-0.54 ± 0.51
	Unmated	0	0	40	-0.84 ± 0.38
2	Mated	3	-1.12 ± 0.88	37	-0.52 ± 0.51
	Unmated	2	0.82 ± 2.40	38	0.46 ± 0.49
3	Mated	16	0.53 ± 0.56	24	-0.74 ± 0.55
	Unmated	7	-1.06 ± 0.99	33	-0.36 ± 0.52

No significant difference between means.
[*] Not used in the analysis.

Table 4.15. Mean numbers of *O. cinerariae* -related mortalities (estimated by Equation 4.1) of each host instar in cages where parasitoid reproduction occurred (active) and where it did not (inactive) from the choice experiment with naive females.

		Active		Inactive	
Instar	Wasp Status	Total	Mean ± standard error	Total	Mean ± standard error
1 [c]	Mated	10	-1.39 ± 0.99	30	-1.71 ± 0.84
	Unmated	2	-1.06 ± 0.42	38	-1.71 ± 0.44
2 [b]	Mated	8	-0.59 ± 0.79	32	-0.07 ± 0.42
	Unmated	7	0.32 ± 0.96	33	0.20 ± 0.48
3 [a]	Mated	14	0.77 ± 0.46	26	1.51 ± 0.39
	Unmated	7	0.62 ± 1.16	33	0.27 ± 0.34

Instars followed by the same letter are not significantly different ($P > 0.05$; data pooled for mating status and activity).

4.5. DISCUSSION

Utilization of host stages differed between *H. varicornis* and *O. cinerariae*. Whether the female was mated and the range of host stages encountered influenced the amount and type of parasitoid attack of host stages of *L. brassicae*.

There was no differential reproduction on host stages by naive *O. cinerariae* in both choice and no-choice experiments. Similar levels of parasitism occurred on all host stages. However, greater parasitism of 3rd instar hosts than 2nd instars was observed for *H. varicornis* during both the choice and no-choice experiments. The number of actively reproducing parasitoids varied in relation to host instar and the mating status of the female, particularly for *H. varicornis*. However, the mean number of progeny produced by active females did not vary for either species. This indicates that once active, parasitoids did not respond differently to each host stage and were not influenced by their mating status. Both species produced few progeny, although considerably more *O. cinerariae* emerged than *H. varicornis*. Low levels of reproduction may indicate egg limitation. Alternatively, this may reflect a strategy conserving reproductive potential until the range of hosts present has been assessed. The egg limitation hypothesis is addressed in Chapter Six.

Differential reproduction of parasitoids on different host stages may result from:

(i) variation in rates of encountering each host stage;

(ii) discrimination between host stages by the female parasitoid on the basis of some host characteristics;

(iii) defense reactions of hosts affecting the success rate of attack or development; and/or

(iv) differential mortality of parasitoids developing in different host stages.

These are proximate explanations of host utilization (Charnov and Skinner 1985).

Physical defense by *L. brassicae* would be limited by its leafmining habit. Injury to the attacking parasitoids, as observed in other host-parasitoid systems (e.g. Allen 1990) would not be possible for *L. brassicae*. Thrashing or wriggling of host larvae within the mine could reduce the efficacy of parasitoid attack. Such a response has been described for *L. trifoliearum* when attacked by *Diglyphus intermedius* (Girault) (Hendrickson and Barth 1978). *L. brassicae* was not observed to behave in this way, although 3rd instars were seen to move backwards along the mine following the initiation of probing by *O. cinerariae* or *H. varicornis*. It is uncertain if other host instars behave in this way. Similar escape tactics have been recorded for *L. trifolii* (Heinz and Parrella 1989). Immunological defenses of *L. brassicae* were not investigated. However, both physical and immunological defense reactions are more common in older host stages (Salt 1970; Lewis and Vinson 1971; Taylor 1988; Allen 1990). Therefore, it is unlikely that either physical or immunological defense by *L. brassicae* could be responsible for the observed differential parasitism of host stages by *O. cinerariae* and *H. varicornis*.

Parasitoids of agromyzids have been shown to use the host mine as a means of locating hosts (Sugimoto 1977; Zucchi and van Lenteren 1978; Sugimoto *et al.* 1986; Kato 1984; Minkenberg and van Lenteren 1986). Mines of *L. brassicae* increase considerably in length and width with host stage (Chapter Two; Beri 1974). It would be expected that 3rd instar mines were encountered more frequently than 1st or 2nd instar mines, given approximately equal density. Older host stages have been shown to be located more rapidly by parasitoids of other agromyzids (Sugimoto 1977; Hendrikse *et al.* 1980; Minkenberg and van Lenteren 1986). The observed patterns of reproduction for both naive *O. cinerariae* and *H. varicornis* cannot be explained by differential encounter rates alone. For *O. cinerariae*, the similar emergence levels from each host instar suggests naive females may be discriminating against the more frequently encountered older host stages. In contrast, for *H. varicornis*, older host

stages were parasitized more frequently suggesting encounter rates may have influenced oviposition activity. However, the absence of progeny from 1st instar hosts indicates additional factors are involved. First instar *L. brassicae* may have gone undetected by *H. varicornis*, as has been described for *C. pentheus* (Sugimoto *et al.* 1988a) and *Kratochviliana* sp. (Sugimoto 1977). However, as *H. varicornis* - related mortality was observed in 1st instar hosts, it can be concluded that *H. varicornis* could detect this host stage. Therefore, the absence of progeny from 1st instar hosts indicates this stage was unsuitable. While factors which make a host acceptable for oviposition by parasitoids are poorly understood, one aspect considered important is host size (Vinson and Iwantsch 1980b). Offspring of *H. varicornis* are limited to the resources available in the host at oviposition. It is possible that first instar *L. brassicae* are too small for the successful development of *H. varicornis* and are thus rejected as suitable hosts. Alternatively, oviposition may have occurred in 1st instar hosts but resource limitation affected development, resulting in no emergences from this stage. It is uncertain if one or both of these hypotheses are correct. Data on the survival of parasitoid progeny on different host stages are unavailable. The implications of host size, the resource it represents and the strategy of host utilization followed by both parasitoid species are discussed further in Chapters Six to Eight.

Whether a female parasitoid was mated or not also influenced parasitism of host stages. Unmated females of hymenopterous parasitoids produce only sons while mated females can produce sons and daughters. Different host utilization strategies of mated and unmated females may indicate differential allocation of sexes between host stages. Mated *H. varicornis* parasitized 3rd instar hosts more frequently than their unmated counterparts in both choice and no-choice experiments. This suggests female offspring may be allocated more frequently to 3rd instar hosts. Greater parasitism was observed by mated than unmated *O. cinerariae* in no-choice experiments, but their strategies of host stage utilization were similar. Unmated females appeared less inclined to parasitize, either as a result of more frequent host

rejection or an alteration of their searching behaviour so that hosts are encountered less often. Modification of searching strategy has been observed in parasitoids which are egg limited (Prasad *et al.*, in prep). It is generally assumed that female offspring represent a better investment of reproductive energy than male progeny (Sandlan 1979; Charnov *et al.* 1981; King 1989). A strategy conserving reproductive potential until mated may represent a means of increasing overall fitness. The comparison of parasitism by mated and unmated females was made only for naive parasitoids. As an unmated female ages, the time available for her to realise her reproductive potential decreases. Progeny production would be expected to increase with age as females attempt to realise their diminishing potential. Results from these experiments did not support this hypothesis, with progeny production being similar between females of different ages. However, an effect of age may have been masked by using females that were not old enough to show increased reproductive rates. Further experiments investigating progeny production of both naive and experienced unmated parasitoids older than 6 days are needed before age can be dismissed as a factor influencing oviposition activity of unmated parasitoids.

Parasitism is only one means by which parasitoids may cause the death of a host. Host feeding or injury to a host by ovipositor probing may also result in mortality. Levels of parasitoid-related mortality were influenced by host instar. *H. varicornis* caused greater mortality of 3rd instar hosts than 1st or 2nd instars during the no-choice experiments. These mortality data were derived from the overall survival of hosts rather than the fate of individuals. It was not possible to distinguish hosts which had died from natural causes or by parasitoid activity. As many larvae were present within one leaf, crowding and competition for resources were potential mortality factors. Therefore, it cannot be assumed that the survival of an individual is independent of the fate of other individuals. Parasitoid-related mortality of 1st instar hosts may reduce the importance of crowding or resource competition as mortality factors by reducing the number of hosts present. This could then increase survival of

remaining hosts, effectively masking the presence of parasitoid-related host mortality. Consequently, the difference in 1st instar host mortality attributed to mated and unmated *H. varicornis* should be interpreted with caution. Increased survival of 1st instar hosts in cages with mated *H. varicornis* may actually indicate considerable parasitoid-related host mortality.

Whether hosts were fed upon by *H. varicornis* or injured by ovipositor probing is uncertain. Third instar hosts represent a larger food source than 1st or 2nd instars. It is probable that parasitoid-related mortality of 3rd instar hosts is primarily due to host feeding. If this is true, then it would be expected that 2nd instar hosts are fed on more than 1st instar hosts. As parasitoid-related mortality was greater on 1st than 2nd instar hosts, it is probable that host feeding is not the only parasitoid-related mortality factor operating. Mortality of 1st instars may be due to injury by ovipositor probing, as seen in other host-parasitoid systems (Sugimoto 1977; Burnett 1962).

Mortality resulting from *O. cinerariae* activity was present in both 1st and 3rd instar hosts during the no-choice experiment. This is similar to results observed for *H. varicornis*, and may again indicate that both host feeding and ovipositor probing are important mortality factors. In choice experiments with *O. cinerariae*, most parasitoid-related host mortality was again observed for 3rd instar hosts. However, survival of 1st instars was greater than expected. As discussed for *H. varicornis*, this may actually indicate parasitoid-related mortality leading to increased survival of remaining hosts. Increased survival of 2nd instar when in cages with active unmated *O. cinerariae* is probably an artefact of the small sample size (n = 5; Table 4.9).

Those cages with actively reproducing parasitoids did not show a different host mortality pattern than cages with inactive parasitoids. This suggests that ovipositing parasitoids were not increasing their rate of host feeding to assist with the

replenishment of eggs. However, results indicate that few eggs were actually oviposited by active naive females (Tables 4.4 and 4.12) so there may have been little need to replenish egg load.

The range of host stages encountered has been suggested to influence the oviposition activity of parasitoids (Charnov *et al.* 1981; Jones 1982; Charnov and Skinner 1985; Strand 1988). Presenting a choice of instars to naive *H. varicornis* and *O. cinerariae* did not influence the distribution of progeny between host stages. For both species, levels of progeny production on each instar were lower in choice situations than in no-choice experiments, reflecting the distribution of total reproductive effort over all 3 instars in choice situations.

In choice experiments, different instars were found in separate leaves. Therefore, the possibility exists that the first leaf encountered may have influenced the behaviour of the female parasitoid or that some host stages were not encountered. *O. cinerariae* females obviously searched two or more leaves, as shown by the majority of cages from choice experiments having parasitism on two or more leaves (Table 4.2). However, parasitism was only observed on one leaf for the majority of *H. varicornis* choice cages. If parasitism occurred on only the first leaf encountered then one would expect equal distribution of parasitoid emergences from all 3 instars. This was not observed with all emergences being from 3rd instars in cages with mated *H. varicornis* and three-quarters from 3rd instars in unmated cages. It can be assumed then that parasitoids of both species had the opportunity to encounter all host stages in choice cages.

While parasitism was largely unaffected by the range of host stages available, contrasting results were observed for levels of parasitoid-related host mortality between choice and no-choice experiments. For both species, more parasitoid-related mortality occurred for 1st and 3rd instar hosts in no-choice than in

choice experiments. Even when the density of each instar in both cage types is considered, less parasitoid-related mortality appears to have occurred in choice cages, particularly for *H. varicornis*. The lower density of 3rd instar hosts in choice cages would result in *H. varicornis* needing more time to locate this host stage, perhaps leaving less time for host feeding. Alternatively, naive parasitoids encountering a range of host stages may spend more time assessing relative host suitability, again leaving less time for activities which lead to host mortality.

Naive parasitoids appear to adopt strategies for conserving reproductive potential until experience with hosts has been gained. Unmated females further conserve their reproductive potential. However, the majority of a parasitoid's life is spent as an experienced, mated individual. Therefore, the host stage utilization strategy observed for naive and unmated females may not reflect the strategy followed by parasitoids for the majority of their life. Host stage utilization by experienced and mated parasitoids is investigated in Chapter Five.

CHAPTER FIVE

ATTACK OF HOST STAGES BY EXPERIENCED FEMALES OF *Hemiptarsenus varicornis* AND *Opius cinerariae*

5.1. ABSTRACT

Variation in the levels of reproduction by mated, experienced females of *Hemiptarsenus varicornis* and *Opius cinerariae* were determined on the different instars of their host, *Liriomyza brassicae*. In a situation where only one instar was offered, levels of reproduction were determined daily for the first 10 days of host exposure, and then every 5 days up to day 40. For *O. cinerariae*, parasitism levels were also measured daily for the first 10 days in situations where a choice between 1st and 2nd, or 2nd and 3rd instar hosts was available. Parasitism by both species increased during the first 5 to 6 days. For *H. varicornis*, reproduction declined after day 10 on both 2nd and 3rd instars. In contrast, *O. cinerariae* continued to reproduce at levels similar to the observed maximum for the remainder of the experiment. Both species produced the greatest number of progeny on 3rd instar hosts. Fewer *O. cinerariae* were reared from 1st instars while fewer *H. varicornis* emerged from 2nd instar hosts. Presenting a choice between host stages had little influence on the oviposition activity of *O. cinerariae*.

5.2. INTRODUCTION

Numerous factors may influence the host-stage utilization strategy of parasitoids, including host acceptability (Vinson 1976), encounter rates (Price 1972; Sugimoto 1977; van Alphen and Drijver 1982; Liu *et al.* 1984) and host defense (Taylor 1988; Allen 1990). In addition, the condition of the parasitoid will influence its oviposition activity, such as its age (van den Assem *et al.* 1984; Avilla and Albajes 1984; Wong *et al.* 1990), amount of previous host experience (van Lenteren 1976; Charnov and Skinner 1985) and mating status. Differential utilization of host stages were apparent for females of both *H. varicornis* and *O. cinerariae* having no prior host experience (naive; Chapter Four). However, these parasitoids produced few progeny suggesting their behaviour while naive would not reflect their overall strategy of progeny allocation.

It is reasonable to assume that most female parasitoids spend the majority of their life as experienced, mated individuals. Having previously encountered hosts, experienced parasitoids will have information enabling them to assess relative host suitability (Charnov and Skinner 1985). Thus, discrimination between host stages on the basis of suitability may occur, with parasitoids choosing to oviposit more frequently in the most suitable host stage. As a consequence of experienced parasitoid's longer periods of exposure to hosts, differences in encounter rates or handling times of host instars may also have a cumulative effect and influence the levels of parasitism observed on each host stage. Determining host-stage utilization of experienced, mated parasitoids is essential to understand the interaction between a parasitoid and its host.

Parasitism of the 3 instars of *Liriomyza brassicae* (Riley) by *Hemiptarsenus varicornis* (Girault) and *Opius cinerariae* Fischer was investigated over a 40 day period. This allowed the host stage utilization strategy by experienced

parasitoids to be determined and indicated how oviposition activity may change as a female ages. Comparisons of host utilization by naive (day 1 of these experiments) and experienced parasitoids were made.

5.3. MATERIALS AND METHODS

5.3.1. No-choice Experiments

Levels of parasitism on the three instars of *L. brassicae* were determined by caging a single female parasitoid with an excised *B. napus* leaf containing in excess of 10 *L. brassicae* larvae at the same stage of development (Appendix One). Experimental procedure was similar to that described in Chapter Three except that leaves were replaced daily for 40 days with fresh leaves containing the same host instar. Only mated parasitoids were used, which were naive at the start of the experiment. Parasitoid and host emergences and host mortality was recorded for days 1 to 10 and then every 5 days up to day 40 or when the parasitoid died, whichever came first. Cages of each host instar were replicated 20 times for each species.

5.3.2. Choice Experiments

For *O. cinerariae*, cages were established which contained two leaves, each with a different host instar. These leaves had either 1st and 2nd instars (choice experiment 1) or 2nd and 3rd instars (choice experiment 2), with 20 replicates of each combination. Leaves were changed daily for 10 days, with levels of parasitoid and host emergence and host mortality being determined for each day. Otherwise experimental procedures were as described for no-choice experiments.

5.3.3. Statistical analysis

Parasitoid emergences were compared by non-parametric ANOVA (Kruskal-Wallis test), using instar and day as the independent variables (SAS 1985).

Multiple comparisons were made by following the method outlined by Conover (1980).

5.4. RESULTS

5.4.1. No-choice Experiments

H. varicornis

Initial parasitoid reproduction was low on both 2nd and 3rd instar hosts (Table 5.1). On day 1, when the parent parasitoids were effectively naive, there was no significant difference in reproduction on host instars. More progeny were produced on 3rd than 2nd instar hosts on days 2 through 10 ($P < 0.05$). However, from day 15 to 40 there was again no significant difference in reproduction on the two instars. Estimates of realised fecundity during the experimental period indicated considerably more reproduction on 3rd instar than 2nd instar hosts. On both instars, reproduction increased during the first 10 days, reaching maximum levels from day 7 to 10 (Table 5.1). It then declined, approaching values similar to those initially observed.

O. cinerariae

Parasitoid reproduction was initially low on both 1st and 2nd instars, but greater on 3rd instar hosts ($P < 0.05$; Table 5.2). Greater progeny production on 3rd than 1st instar hosts continued up until day 35 ($P < 0.05$). On days 7 to 9, and 15 to 30, greater progeny production occurred on 2nd than 1st instar hosts ($P < 0.05$). Levels of reproduction on 2nd and 3rd instar hosts were generally similar except on day 10 where more occurred on 3rd instar hosts, and on day 35, where 2nd instar hosts showed a greater level of parasitism. No difference was observed for parasitism of each instar on day 40. Estimates of realised fecundity suggested that 2nd and 3rd instar hosts were subjected to much greater levels of parasitism than 1st instar hosts.

Table 5.1. Mean (± standard error) and range of *H. varicornis* emergences from cages with 2nd or 3rd instar hosts.

Instar	2nd			3rd		
Day	N	Mean ± standard error	Range	N	Mean ± standard error	Range
1	20	a 0.20 ± 0.16 x	0 - 3	20	a 0.60 ± 0.22 x	0 - 3
2	20	ab 0.15 ± 0.11 x	0 - 2	19	ab 1.05 ± 0.34 y	0 - 5
3	19	a-c 0.37 ± 0.22 x	0 - 4	19	bc 2.05 ± 0.49 y	0 - 7
4	17	a-d 0.76 ± 0.37 x	0 - 6	19	cd 3.26 ± 0.51 y	0 - 7
5	17	a-e 0.88 ± 0.38 x	0 - 6	18	de 5.33 ± 1.08 y	0 - 13
6	17	c-f 1.29 ± 0.42 x	0 - 5	18	c-f 4.06 ± 0.78 y	0 - 10
7	17	g 2.47 ± 0.55 x	0 - 7	18	g 7.67 ± 0.92 y	0 - 13
8	17	d-h 1.53 ± 0.40 x	0 - 5	18	gh 7.50 ± 1.05 y	2 - 17
9	17	e-i 1.59 ± 0.36 x	0 - 4	18	eg-i 7.17 ± 1.02 y	0 - 18
10	17	f-j 2.24 ± 0.48 x	0 - 6	18	d-j 5.33 ± 0.91 y	0 - 13
subtotal (1-10)	20	9.85 ± 1.92 x	0 - 26	20	40.00 ± 5.09 y	0 - 74
15	10	ac-k 1.40 ± 0.52 x	0 - 5	10	c-fjk 3.90 ± 1.27 y	0 - 11
20	10	a-el 0 ± 0 x	0	8	b-fj-l 3.00 ± 0.35 y	0 - 7
25	9	a-fhk-m 0.33 ± 0.24 x	0 - 2	6	abm 0.17 ± 0.17 y	0 - 1
30	8	a-fhk-n 0.50 ± 0.38 x	0 - 3	6	a-dfk-n 1.17 ± 0.65 y	0 - 4
35	7	a-ik-o 2.14 ± 1.06 x	0 - 8	6	b-fj-lno 3.00 ± 1.15 x	0 - 7
40	7	a-fhik-o 0.57 ± 0.30 x	0 - 2	6	a-cl-o 0.83 ± 0.48 x	0 - 3
Subtotal (11-40) *		20.00 ± 6.06			47.00 ± 11.33	
Total (1-40) **		29.85			87.00	

* Subtotal estimated by using four times the mean for each wasp to give the value for the preceding 4 days, then averaged for all wasps. For example, days 11 to 14 equal four times the mean for day 15.

** Total = subtotal (1-10) + estimated subtotal (11-40). Means with the same letter preceding or following them are not significantly different (P > 0.05; a to o, comparison between days for each instar; x or y, comparison between instars for each day; -, indicates that all letters between are relevant to that mean).

Table 5.2. Mean (± standard error) and range of *O. cinerariae* emergences from cages with 1st, 2nd or 3rd instar hosts.

Instar	1st				2nd				3rd			
Day	N		Mean ± standard error	Range	N		Mean ± standard error	Range	N		Mean ± standard error	Range
1	20	a	0.45 ± 0.26 x	0 - 5	20	a	0.35 ± 0.22 x	0 - 4	20	a	2.25 ± 0.75 y	0 - 9
2	20	ab	0.40 ± 0.40 x	0 - 8	20	ab	1.95 ± 0.84 xy	0 - 13	20	ab	4.65 ± 1.31 y	0 - 21
3	20	a-c	1.20 ± 0.57 x	0 - 10	20	a-c	1.50 ± 0.52 xy	0 - 7	18	a-c	4.17 ± 1.06 y	0 - 13
4	20	acd	2.60 ± 0.87 x	0 - 12	20	b-d	4.30 ± 1.28 xy	0 - 18	18	bd	8.50 ± 1.99 y	0 - 26
5	19	ac-e	2.47 ± 0.99 x	0 - 12	19	de	4.53 ± 1.36 xy	0 - 22	17	b-e	8.41 ± 1.95 y	0 - 22
6	17	df	4.35 ± 1.32 x	0 - 16	18	d-f	6.33 ± 1.67 xy	0 - 26	17	d-f	9.65 ± 1.44 y	0 - 20
7	14	ac-g	3.14 ± 1.20 x	0 - 11	17	fg	9.24 ± 1.59 y	0 - 21	17	d-g	12.41 ± 2.30 y	0 - 28
8	14	a-h	1.14 ± 0.43 x	0 - 5	17	e-h	7.71 ± 1.81 y	0 - 24	16	d-h	11.69 ± 2.15 y	0 - 28
9	14	d-i	2.36 ± 0.62 x	0 - 6	17	f-i	7.29 ± 1.15 y	0 - 18	16	d-i	9.19 ± 11.27 y	0 - 18
10	14	ac-j	2.43 ± 0.95 x	0 - 11	17	d-fh-j	5.18 ± 1.41 x	0 - 21	16	d-j	10.38 ± 11.57 y	0 - 23
Subtotal (1-10)	20		17.15 ± 3.74 x	0 - 54	20		43.10 ± 7.32 y	0 - 101	20		69.10 ± 11.24 y	0 - 152
15	8	a-eghjk	0.38 ± 0.38 x	0 - 3	7	d-k	6.29 ± 2.65 y	0 - 21	10	f-k	15.30 ± 2.71 y	4 - 29
20	4	a-l	2.00 ± 1.68 x	0 - 7	7	e-l	9.43 ± 3.01 y	0 - 24	10	bd-l	7.80 ± 1.07 y	1 - 13
25	4	a-m	0.50 ± 0.50 x	0 - 2	7	d-m	4.14 ± 0.94 y	1 - 7	8	d-m	9.88 ± 2.89 y	0 - 25
30	4	a-n	0.75 ± 0.48 x	0 - 2	7	e-n	6.86 ± 1.45 y	1 - 10	8	d-n	9.50 ± 2.20 y	1 - 17
35	2	c-jl-o	5.50 ± 3.18 xy	1 - 10	6	g-ik-o	11.17 ± 2.57 x	5 - 22	6	a-ceo	2.00 ± 0.93 y	0 - 6
40	2	c-jl-o	2.50 ± 0.35 x	2 - 3	6	d-o	5.50 ± 1.88 x	1 - 14	4	a-o	6.00 ± 2.86 x	1 - 14
Subtotal (11-40) *			16.00 ± 11.32				146.00 ± 40.84				211.00 ± 33.04	
Total (1-40) **			33.15				189.10				280.10	

* Subtotal estimated by using the mean for each wasp to give the value for the preceding 4 days then averaging for all wasps. For example, days 11 to 14 equal four times the value of day 15.

** Total = subtotal (1-10) + estimated subtotal (11-40). Means with the same letter preceding or following them are not significantly different (P > 0.05; a to o, comparison between days for each instar; x or y, comparison between instars for each day; -, indicates that all letters between are relevant to that mean).

On all 3 host instars, levels of reproduction underwent an increase during the first 5 to 6 days of host exposure (Table 5.2). The maximum parasitism of 1st instar hosts was reached by day 4 and similar, albeit low, levels were observed up until day 40. Parasitism of 2nd instar hosts reached a maximum by day 6 and continued undiminished until day 40. Reproduction on 3rd instar hosts was at a maximum level by day 4, continuing as such until day 35 when significantly fewer progeny were produced.

5.4.2. Choice Experiments

In the first choice experiment with *O. cinerariae*, total reproduction over the experimental period was greater in 2nd than 1st instar hosts (Table 5.3). Parasitism levels were greater on 2nd instar hosts on days 4, 5 and 10. More progeny were produced on 1st instar hosts on day 9. On both host instars, parasitism was infrequent initially but increased during the experiment.

In choice experiment 2, reproduction of *O. cinerariae* over the experiment did not vary with host instar (Table 5.4). Only day 7 showed a difference in levels of parasitism of instars, resulting from unusually low levels in 3rd instars. Again, reproduction was rare initially but increased over the experiment.

5.5. DISCUSSION

Progeny allocation by *H. varicornis* in these laboratory experiments corroborate the results obtained from the field. Only 2nd and 3rd instar hosts were parasitized, the latter suffering greater parasitism, which was also observed during field collections (Figure 3.2). Unfortunately, the scarcity of *O. cinerariae* in the field limited the comparison of progeny allocation by this species in field and laboratory conditions. However, in both situations, all three host stages were attacked. These

Figure 5.3. Mean (± standard error) and range of *O. cinerariae* emergences from cages where a choice of 1st or 2nd instar *L. brassicae* was present (choice experiment 1).

Instar	1st			2nd		
Day	N	Mean ± standard error	Range	N	Mean ± standard error	Range
1	20	a 0.15 ± 0.11 x	0 - 2	20	a 0.40 ± 0.21 x	0 - 4
2	20	b 1.05 ± 0.46 x	0 - 7	20	b 1.45 ± 0.49 x	0 - 4
3	20	bc 1.15 ± 0.46 x	0 - 8	20	ac 4.53 ± 1.25 x	0 - 18
4	20	b-d 1.15 ± 0.33 x	0 - 5	20	d 3.26 ± 1.16 y	0 - 15
5	19	b-e 1.16 ± 0.34 x	0 - 5	19	ac-e 3 00 ± 0.59 y	0 - 17
6	19	f 2.95 ± 0.77 x	0 - 13	19	f 3.41 ± 0.80 x	0 - 12
7	19	g 3.58 ± 0.71 x	0 - 12	19	fg 1.13 ± 0.47 x	0 - 18
8	17	gh 3.41 ± 0.72 x	0 - 9	17	dfh 2.53 ± 0.60 x	0 - 13
9	17	fi 5.59 ± 1.35 x	1 - 25	17	ac-ei 4.20 ± 0.83 y	0 - 9
10	17	fi 3.82 ± 1.10 x	0 - 13	17	fh 3.53 ± 1.34 y	0 - 17
Total	20	21.70 ± 3.19 x	1 - 50	20	35.90 ± 4.32 y	5 - 68

Means with the same letter preceding or following them are not significantly different ($P > 0.05$; a to i, comparison between days for each instar; x or y, comparison between instars for each day; -, indicates that all letters between are relevant to that mean).

Figure 5.4. Mean (± standard error) and range of *O. cinerariae* emergences from cages where a choice of 2nd or 3rd instar *L. brassicae* was present (choice experiment 2).

Instar	**2nd**				**3rd**			
Day	N		Mean ± standard error	Range	N		Mean ± standard error	Range
1	20	a	0.75 ± 0.36 x	0 - 7	20	a	0.40 ± 0.21 x	0 - 4
2	20	b	2.75 ± 1.00 x	0 - 14	20	b	1.45 ± 0.49 x	0 - 6
3	19	bc	1.74 ± 0.53 x	0 - 6	19	c	4.53 ± 1.25 x	0 - 18
4	19	b-d	2.16 ± 0.69 x	0 - 10	19	cd	3.26 ± 1.16 x	0 - 20
5	18	b-e	3.06 ± 0.82 x	0 - 10	18	c-e	3.00 ± 0.59 x	0 - 8
6	17	f	3.29 ± 0.74 x	0 - 10	17	c-f	3.41 ± 0.80 x	0 - 11
7	15	fg	5.40 ± 1.19 x	0 - 18	15	bg	1.13 ± 0.47 y	0 - 6
8	15	d-h	4.27 ± 1.08 x	0 - 14	15	bh	2.53 ± 0.60 x	0 - 7
9	15	bd-i	4.00 ± 1.18 x	0 - 14	15	c-fhi	4.20 ± 0.83 x	0 - 9
10	15	bd-i	3.47 ± 0.69 x	0 - 9	15	c-fhi	3.53 ± 1.34 x	0 - 20
Total	20		26.10 ± 4.10 x	1 - 54	20		23.40 ± 3.14 x	0 - 58

Means with the same letter preceding or following them are not significantly different ($P > 0.05$; a to i, comparison between days for each instar; x or y, comparison between instars for each day; -, indicates that all letters between are relevant to that mean).

strategies of progeny allocation appear to reflect the nutritional requirements of the parasitoids. The host range of the idiobiont, *H. varicornis*, is restricted to only those host stages large enough to support larval development, whereas all host stages provided sufficient resources for *O. cinerariae* larvae, by virtue of continued host development.

As distinct from naive parasitoids, experienced females of each species showed differential parasitism of host stages. Parasitism levels increased over the first few days of exposure to hosts, remaining at these levels for a considerable portion of the parasitoid's life. In all cases, an accurate indication of the different levels of parasitism between host stages could be gained within the first 10 days of the experiment.

More parasitoids emerged from 3rd instars than the other host stages for both *H. varicornis* and *O. cinerariae*. It is common for parasitoids of agromyzids to attack older host stages more frequently (Hendrickson 1975; Ibrahim and Madge 1979; Lema and Poe 1979; Sugimoto and Ishii 1979; Hendrikse *et al.* 1980; Chandler *et al.* 1988; Heinz and Parrella 1989). For *H. varicornis*, experienced parasitoids showed a strategy of host stage utilization that was similar yet more pronounced to what was observed for naive females (Chapter Four). Conflicting results were gained for naive females of *H. varicornis* from the experiments outlined in Chapter Four and from day 1 of the no-choice experiment presented here. While differential parasitism of host stages by naive *H. varicornis* was observed in the results of Chapter Four, this was not the case for day 1 of these experiments. Slightly different experimental conditions existed in each situation. The experiment outlined in Chapter Four involved 3 leaves per cage, while only one leaf was present in the no-choice experiments presented here. Having a smaller area on which to search for hosts, parasitoids in these experiments may have searched more intensively. Hence, more 2nd instar mines may have been encountered than in the experiment of Chapter Four. Mean levels of reproduction on

2nd instar hosts during these experiments were approximately double those observed from the experiment in Chapter Four, suggesting more hosts were encountered.

Experiments with naive *O. cinerariae* discussed in Chapter Four revealed no differential parasitism of host stages. Results for naive females presented in this chapter (day 1) confirm this behaviour, suggesting that naive parasitoids discriminate against the more frequently encountered older host stages. However, this was not observed for experienced females, with older host stages being parasitized more frequently (Table 5.2). This may represent a change in the rate of acceptance of these stages, or an improved searching or host-handling ability of the parasitoids with experience.

Observations of parasitoids of other agromyzids suggest that females use the host mine to locate larvae (Sugimoto 1977; Zucchi and van Lenteren 1978; Kato 1984; Sugimoto *et al.* 1986; Minkenberg and van Lenteren 1986). Sugimoto *et al.* (1988b) observed that visual and acoustic cues were important in determining which leaves were landed on by *Dapsilarthra rufiventris* (Nees). The white and linear mines of the host, *Phytomyza ranunculi* Schrank, were considered to be the visual cue, while sounds emitted by the feeding hosts comprised the acoustic cue. Tactile cues may be important for locating mines once on a leaf (Sugimoto 1977). It is uncertain how mines are located or detected by *O. cinerariae* and *H. varicornis*. If the cues used by these species are directly influenced by the length or area of the leaf mine, such as visual or tactile cues, then progeny allocation may also be proportional to mine length or area. However, additional factors may also be involved in host location, such as acoustic (e.g. sounds emitted by host) or chemical cues (e.g. volatiles released by host damage, feeding or frass). Different host stages may differ in the quality and/or quantity of the acoustic or chemical stimuli they produce, and these differences may vary disproportionately to mine length or area. Hence, the allocation of progeny by parasitoids which rely on these cues may not be proportional to the mine

measurements. While parasitism by *O. cinerariae* and *H. varicornis* increased in relation to host stage, this increase was not proportional to that observed for mine length or area (Chapter Two). Therefore, these parasitism results suggest that acoustic or chemical cues may be important components of the searching strategy of *O. cinerariae* and *H. varicornis*.

Although 3rd instar mines are probably encountered more frequently, parasitoids may also require the greatest time to locate the larvae within the mine and successfully oviposit. The far greater length of older stage mines would necessitate the parasitoid to travel further before encountering the host larva. Third instar larvae have shown escape tactics following the commencement of parasitoid attack (Chapter Four). While it is uncertain if other host stages behave similarly, 3rd instars still have the greatest distance over which to escape. Such behaviour may reduce the efficacy of parasitoid attack or, at least, prolong the time needed to successfully oviposit, thereby limiting the possible level of parasitism. Physical host defenses may also reduce the success of parasitoid oviposition (Taylor 1988; Allen 1990) but *L. brassicae* would be largely incapable of such a response (see Chapter Four).

Differences in the levels of parasitism observed may also be a result of discrimination against certain host stages. Discrimination may occur against both old or young host stages, depending on the rates of encounter. If host encounter rates were proportional to abundance, then the observed levels of parasitism may be attributed to more frequent rejections of 1st instar hosts. Conversely, if host instars were encountered at rates proportional to their mine lengths or areas, then discrimination against older host stages may have contributed to the levels of parasitism observed. There is no direct evidence of experienced females of either parasitoid species discriminating against host stages.

A further factor which may be important when considering parasitism levels is differential mortality between progeny developing on different host stages (Wellings *et al.* 1986). As parasitism levels were deduced from emergence data, these may not accurately reflect the oviposition patterns of the adult parasitoid. It is uncertain if differential mortality occurred during these experiments. For the koinobiont, *O. cinerariae*, it is unlikely that such mortality could explain the observed pattern of parasitoid emergences. *O. cinerariae* can complete development on all host stages. If physiological host defences influenced progeny development, then lower progeny survival in 3rd instar hosts would be expected. The idiobiont, *H. varicornis*, may have been unable to develop on some 2nd instar larvae as a result of resource limitation. Therefore, a greater number of *H. varicornis* ovipositions may have occurred on these host stages than indicated by emergence data.

Other studies of oviposition activity by parasitoids over a prolonged period include Ragusa (1974), Cloutier *et al.* (1981), Avilla and Albajes (1984), Minkenberg (1990) and Wong *et al.* (1990). *Dacnusa sibirica* Telenga oviposition on 3rd instar hosts at 15°C showed a similar pattern to that observed for both *O. cinerariae* and *H. varicornis*, with low levels initially followed by a rapid increase to a level that remained relatively stable for the remainder of the parasitoid's life (Minkenberg 1990). Cloutier *et al.* (1981) also showed low levels of progeny production initially by *Aphidius nigripes* Ashmead on all stages of it's host, *Macrosiphum euphorbiae* (Thomas). Reproductive rate then increased, remained stable for approximately 10 days, then declined gradually. Different experimental designs prevent direct comparison with other studies. However, in all cases there appears to be considerable reproduction by parasitoids within the first few days of encountering hosts. For *Biosteres tryoni* (Cameron), reproduction remained high up until day 20 (Wong *et al.* 1990). In contrast, for *Opius concolor* Szepligeti (Avilla and Albajes 1984) and *O. concolor siculus* (Ragusa 1974), peak reproduction was observed during the first 5 days of oviposition, after which it declined progressively.

These studies indicate different parasitoid species exhibit different reproductive strategies throughout their life. Furthermore, the work of Ragusa (1974) and Minkenberg (1990) suggests temperature is an important factor influencing oviposition. The different reproductive strategies observed here for *O. cinerariae* and by Ragusa (1974) and Avilla and Albajes (1984) for the related species, *O. concolor* and *O. concolor siculus* , may be partly due to the different experimental temperatures of these studies. The higher temperatures used by Avilla and Albajes (1984; 25°C) and Ragusa (1974; 22-28°C) would lead to shorter parasitoid lifespans and, correspondingly, more rapid oviposition.

It has previously been suggested that the range of host stages encountered will influence host utilization by parasitoids (Charnov and Skinner 1985). Results of the choice experiments indicate the range of host stages present had little influence on the levels of parasitism by experienced *O. cinerariae*. In the choice between 1st and 2nd instar hosts, *O. cinerariae* produced more progeny on 2nd instars, as was expected based on the conclusions of the no-choice experiment. When a choice was available between 2nd and 3rd instar hosts, there was no difference in the number of *O. cinerariae* progeny produced on each host stage. The no-choice experiments also indicated that overall reproduction by *O. cinerariae* over the first 10 days of exposure to these host stages did not vary. While parasitism levels appear unaffected by a range of host stages present, other aspects of host stage utilization, such as sex allocation, may be influenced. This is investigated in the following chapter.

CHAPTER SIX

SEX ALLOCATION BY *Opius cinerariae* AND *Hemiptarsenus varicornis*

6.1. ABSTRACT

Sex allocation by *Hemiptarsenus varicornis* and *Opius cinerariae* was investigated in relation to the larval instars of *Liriomyza brassicae* over the first 40 days of oviposition. When each host stage was offered separately, both species produced biased sex ratios. Sex allocation by *H. varicornis* was dependent upon the host instar attacked, although differential mortality of the sexes may have determined the sex ratio at emergence. *H. varicornis* produced strongly male-biased sex ratios on 2nd instar hosts. Generally unbiased sex ratios were produced on 3rd instar hosts between days 1 and 10, after which ratios became male-biased. Sex allocation by *O. cinerariae* during a no-choice experiment was not dependent upon the host instar offered. *O. cinerariae* frequently produced female-biased sex ratios during the first 10 days of oviposition on all three host instars. There was a shift towards greater male production on day 30 on 2nd instars and from day 15 onwards on 3rd instar hosts. The observed shifts towards male production by aged females of both parasitoid species suggested sperm depletion was occurring. Sex ratios produced on the first day of oviposition were frequently more male-biased than those observed on subsequent days. When a choice between 1st and 2nd instars hosts was offered to *O. cinerariae*, proportionately greater male production was observed in 2nd instars. When 2nd and 3rd instar hosts were offered simultaneously, *O. cinerariae* produced similar female-biased sex ratios on both stages. The consistent female-biased sex

g118

ratios produced by *O. cinerariae* may represent a fixed strategy to maximize average fitness when attacking a host with highly variable distribution and abundance.

6.2. INTRODUCTION

Hymenopteran parasitoids are arrhenotokous, being able to determine the sex of their offspring. By controlling the release of sperm stored in the spermatheca, females can determine if the egg is to remain unfertilised and develop into a haploid male, or be fertilized and develop into a diploid female (Flanders 1956). The ability to allocate sex to individual progeny provides for further plasticity in oviposition decisions. Differential allocation of sexes to host types and alteration of sex ratios in relation to host or parasitoid density have frequently been observed (Hamilton 1967; Charnov *et al.* 1981; Waage 1986; King 1987, 1989; Strand 1988).

A large body of theory has accumulated concerning how parasitoids should allocate sexes to maximize their fitness. Fisher (1930) first proposed that in randomly mating, dioecious populations where the cost of producing each sex is equal, natural selection would tend to produce 1:1 sex ratios. Later, Trivers and Willard (1973) suggested that manipulation of sex ratios in response to resource availability may be adaptive. More specifically for parasitoids, the influence of host quality (Clausen 1939; Bull 1981; Charnov *et al.* 1981; Charnov 1982) and the spatial structure of insect populations (Hamilton 1967; Werren 1980, 1983; Charnov 1982) or both (Green 1982; Werren 1984) on sex allocation has been examined. Host quality has frequently been measured in terms of host size, age or stage of development (Arthur and Wylie 1959; van den Assem 1971; Sandlan 1979; Charnov *et al.* 1981; Jones 1982; Avilla and Albajes 1984; van den Assem *et al.* 1984; Simbolotti *et al.* 1987; King 1987, 1988; de Jong and van Alphen 1989; Wong *et al.* 1990). These measures are generally correlated (Charnov *et al.* 1981; King 1987). Parasitoid sex

ratios have been shown to be influenced by host quality, at least circumstantially, with female-biased ratios frequently being produced on the larger, older or later host stages (Arthur and Wylie 1959; van den Assem 1971; Sandlan 1979; Charnov *et al.* 1981; Jones 1982; van Alphen and Thunnissen 1983; van den Assem *et al.* 1984; Kistler 1985; Luck and Podoler 1985; King 1988, 1989; de Jong and van Alphen 1989; Wong *et al.* 1990). Waage (1982a) hypothesized that host-size dependent sex ratios would only occur in parasitoids of growing hosts. His premise was that for parasitoids of non-growing hosts, host size at oviposition would not be a good indicator of the amount of larval resource. If this hypothesis is true, then idiobiont parasitoids should display host-size dependent sex ratios while koinobionts should not (King 1989). Available data indicate some koinobiont species do display host size dependent sex ratios, although less frequently than is observed for idiobionts (King 1989).

Hamilton (1967) proposed that the spatial structure of the host population could influence the sex ratio of parasitoids in his local mate competition (LMC) model. Certain spatial structures of host populations can lead to varying probabilities of inbreeding or outbreeding. Clumped distributions frequently lead to many progeny from the one female being present within a patch. Therefore, there is a high probability of outbreeding. However, in random distributions, patches are less discrete and parasitoids are less likely to emerge in proximity to one another. Consequently, there is a higher probability of males mating with non-siblings. In situations with considerable inbreeding, Hamilton (1967) suggested that females should produce only enough sons to fertilize all the daughters. Where outbreeding is more likely, such as with random distributions of hosts or multiple females ovipositing within a patch, competition amongst males for mates will be stronger and, hence, more males should be produced. Several studies have provided evidence supporting these hypotheses, often using gregarious parasitoids as examples (Waage and Lane 1984; Waage and Ng 1984; Werren 1984; Takagi 1985).

Proximate factors have also been shown to influence sex allocation. Sperm depletion can lead to a preponderance of male offspring late in the reproductive life of a mated female (Flanders 1956; Wong *et al.* 1990). Rapidity of oviposition has also been suggested to affect sex ratio, with frequent ovipositions increasing the rate of male production (Flanders 1956; van den Assem 1971).

Control of sex ratio requires assessment of the varying quality, density or distribution of hosts. In order for parasitoids to consider a host suitable for male or female progeny the relative quality of the host must be measured. The perceived quality of a host is likely to be influenced by information gained from hosts previously encountered (Charnov and Skinner 1985; Strand 1988). For example, a 2nd instar host may be considered as either large or small, depending on whether only 1st or 3rd instar hosts had been previously encountered. Therefore, it is important to have knowledge of a parasitoid's experience if its sex allocation strategy is to be interpreted.

A series of laboratory experiments was undertaken to investigate sex allocation by *Opius cinerariae* Fischer and *Hemiptarsenus varicornis* (Girault) on different instars of *Liriomyza brassicae* (Riley). Wasps were presented with one host instar only or a choice between two instars. Variation of sex ratios across the range of host stages encountered and the age and level of experience of the parasitoid were examined.

6.3. MATERIALS AND METHODS

Sex ratios were determined from emergence records of experiments discussed in Chapter Five. These were analysed to (i) investigate the variation of observed sex ratios from 1:1, using the chi-squared goodness of fit test or log-likelihood (G) test when sample sizes were small (Zar 1984); (ii) investigate if a

significant number of females produced biased sex ratios, using the Wilcoxon's paired-sample test (Zar 1984); and (iii) investigate relative rates of male and female production on different instar hosts, using the log-likelihood (G) test or Fisher's Exact Test (FET; SAS 1985). These three methods provided a comprehensive analysis of sex allocation, enabling strategies to be assessed in terms of the overall bias, general female behaviour and a comparison between strategies on different host instars.

Prior to goodness of fit testing, an overall sex ratio for each day was determined by calculating total male and female production by all females. In addition, sex ratios were determined for days 1 to 10, 1 to 40 and 15 and 40, by pooling daily totals. As parasitoid emergences were only sampled every fifth day after day 10, pooling data for days 1 to 40 required special handling. In these situations, the totals for every fifth day after day 10 were used as an indication of the oviposition during the previous four days. Thus, male production for days 11 to 14 was estimated to equal 4 times the male production on day 15, and so on. The Wilcoxon test indicated if most females followed similar strategies of sex allocation, thereby showing if sex ratios produced overall reflected the general behaviour of a species. Numbers of females producing biased sex ratios were analysed for each day and then for pooled totals of days 1 to 10, 1 to 40 and 15 to 40. Parasitoids which produced no offspring were ignored by this analysis. Log-likelihood or FET indicated differences between sex allocation strategies adopted by females in contact with different host instars. For these analyses, pooled totals were used for days 1 to 10, 1 to 40 and 15 to 40. Each choice experiment was analysed separately, except for a direct comparison of totals of male and female progeny produced during each experiment. For each instar, the sex allocation of parasitoids in choice and no-choice situations were compared by G tests.

6.4. RESULTS

6.4.1. No-choice Experiments

O. cinerariae

Sex ratios produced by *O. cinerariae* were frequently female-biased on all host stages. As the female aged, there was a tendency for sex ratios to become more male-biased, particularly where numerous progeny had been produced.

The sex ratios produced by *O. cinerariae* between days 1 and 10 (pooled) were female-biased, regardless of whether 1st (proportion males = 0.370), 2nd (prop. males = 0.342) or 3rd instar hosts (prop. male = 0.413) were attacked. During this period, the majority of females produced female-biased sex ratios on each host instar (Table 6.1). However, there was a significant difference in sex allocation on different host instars, with a greater proportion of males being produced on 3rd instars than 2nd instars (Figure 6.1A).

Sex ratios produced by *O. cinerariae* from days 15 to 40 (pooled) were female-biased on 1st (prop. male = 0.219) and 2nd instars (prop. male = 0.429), but male-biased on 3rd instar hosts (prop. male = 0.552). Throughout this period, a significant proportion of females produced female-biased sex ratios when offered 2nd instar hosts (Table 6.1). However, when offered either 1st or 3rd instars, neither male- nor female-biased sex ratios predominated. A greater proportion of males was produced on 3rd instars than either 1st or 2nd instar hosts (Figure 6.1B). Male production was also proportionately greater on 2nd than 1st instar hosts (Figure 6.1B).

Between days 1 and 40 (pooled) sex ratios produced by *O. cinerariae* were female-biased on 1st (prop. male = 0.307) and 2nd instar hosts (prop. male = 0.404). However, the sex ratio for this period was unbiased when only 3rd instars were offered (prop. male = 0.513). The majority of females produced female-biased

Table 6.1. Outcome of the Wilcoxon's paired-sample test investigating if a significant number of *O. cinerariae* females produced biased sex ratios on different *L. brassicae* instars during the no-choice experiments.

Day	Host Instar		
	1	2	3
1	NS	NS	NS
2	NS	NS	Female
3	NS	NS	Female
4	Female	Female	Female
5	Female	Female	Female
6	Female	Female	Female
7	NS	Female	NS
8	NS	Female	Female
9	NS	Female	Female
10	NS	Female	Female
15	NS	NS	NS
20	NS	Female	NS
25	NS	NS	NS
30	NS	NS	NS
35	NS	NS	NS
40	NS	NS	NS
1 - 10 (pooled)	Female	Female	Female
1 - 40 (pooled)	Female	Female	Female
15 - 40 (pooled)	NS	Female	NS

Female, indicates significantly more females produced female-biased sex ratios than male-biased (P < 0.05); NS, indicates the number of females producing female- or male-biased sex ratios was not significantly different (P > 0.05).

124

Figure 6.1. Total production of female (hatched area) and male (striped area) progeny by *O. cinerariae* on different *L. brassicae* instars during the no-choice experiment for (A) days 1 to 10 pooled, (B) days 15 to 40 pooled, and (C) days 1 to 40 pooled (* estimated total).

E, indicates expected levels of female production from contingency table analysis. For each graph, instars with the same letters (a to c) have proportion of male and female progeny that are not significantly different (P > 0.05).

A. Days 1 to 10 pooled

B. Days 15 to 40 pooled

C. Days 1 to 40 pooled*
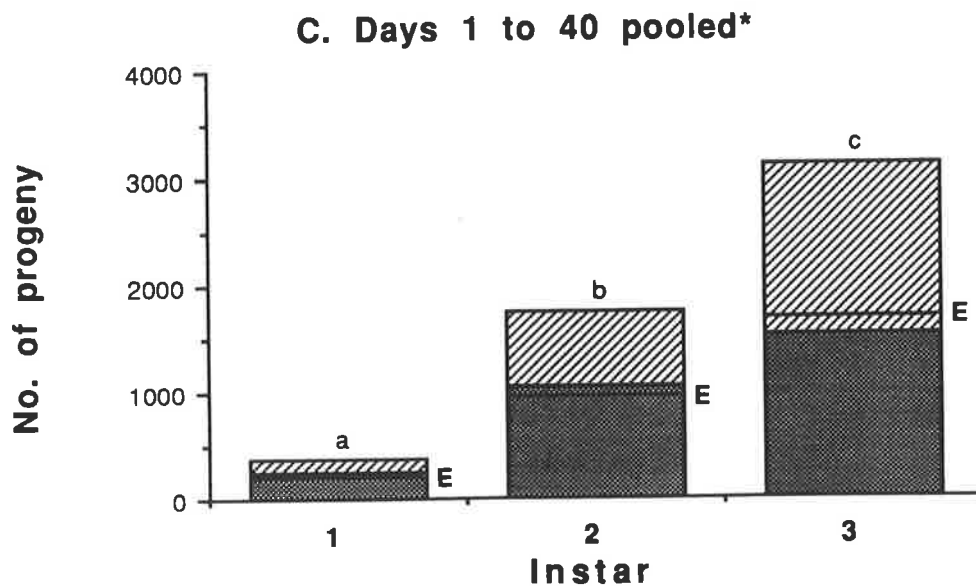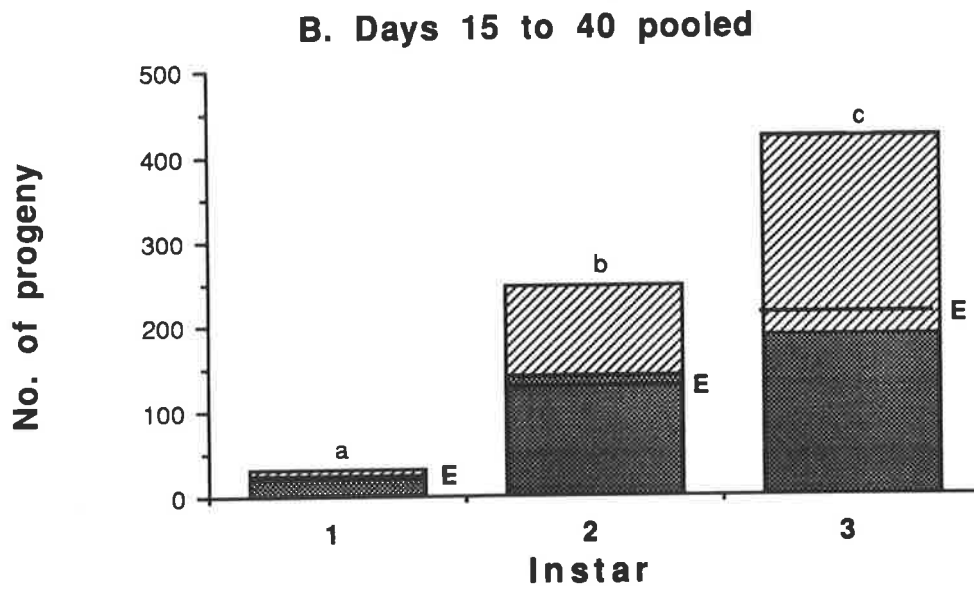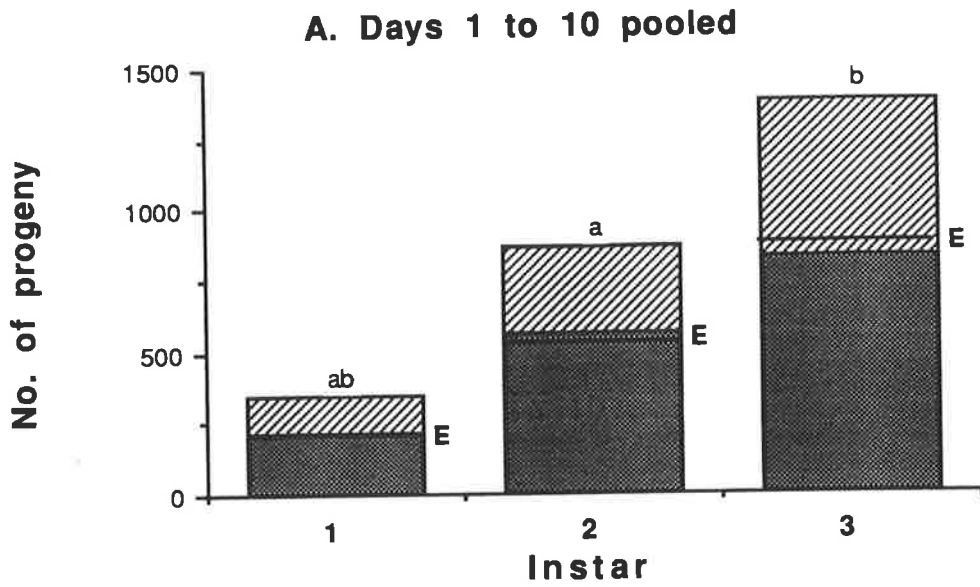
sex ratios on each host instar during this period (Table 6.1). Sex allocation again varied in relation to the host instar attacked with a greater proportion of males being produced on 3rd instars than either 1st or 2nd instar hosts (Figure 6.1C). Male production was also proportionately greater on 2nd than 1st instar hosts (Figure 6.1C).

On a per day basis, female-biased sex ratios were regularly produced by *O. cinerariae* on all 3 host instars up to day 10 (Figure 6.2). After day 10, sex ratios were highly variable on 1st instars, always being statistically unbiased. On 2nd instar hosts, female-biased sex ratios continued to be produced up to day 25. The following two samples were unbiased, while on day 40 a significantly male-biased sex ratio was observed. Those *O. cinerariae* offered 3rd instars produced a male-biased sex ratio on day 15, unbiased sex ratios on the following 2 samples, but male-biased sex ratios again on days 30, 35 and 40.

Analysis of the number of females producing biased sex ratios per day (Table 6.1) indicated that individual sex ratios were frequently female-biased, otherwise neither female- nor male-biased sex ratios predominated. Even though male-biased sex ratios were recorded from 2nd and 3rd instar hosts (Figure 6.2B,C), these were never produced by a significant proportion of the *O. cinerariae* females.

H. varicornis

Sex allocation by *H. varicornis* was strongly dependent upon the host stage offered. When only 2nd instar hosts were available, an extreme male-biased sex ratio was produced. However, when offered 3rd instar hosts, greater female production occurred with increasing male production being observed as the females aged.

Between days 1 and 10 (pooled), sex ratios produced by *H. varicornis* were male-biased on 2nd (prop. male = 0.954) and unbiased on 3rd instar

127

Figure 6.2. Proportion males (mean ± standard error) allocated by *O. cinerariae* to 1st (A), 2nd (B) and 3rd (C) instar *L. brassicae* sampled on the first 10 days of oviposition and then every 5 days until day 40. Females which produced no offspring were excluded from the calculation of the means.

A.

B.

C.

Day
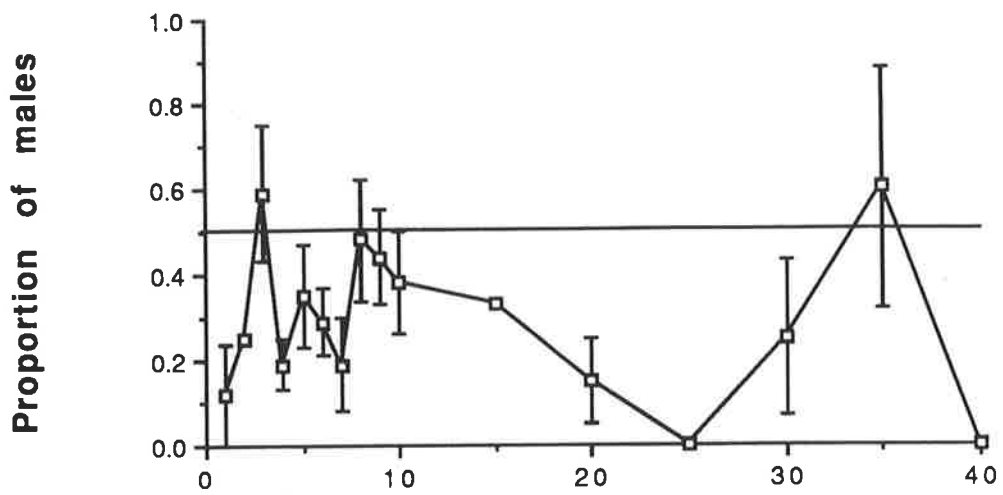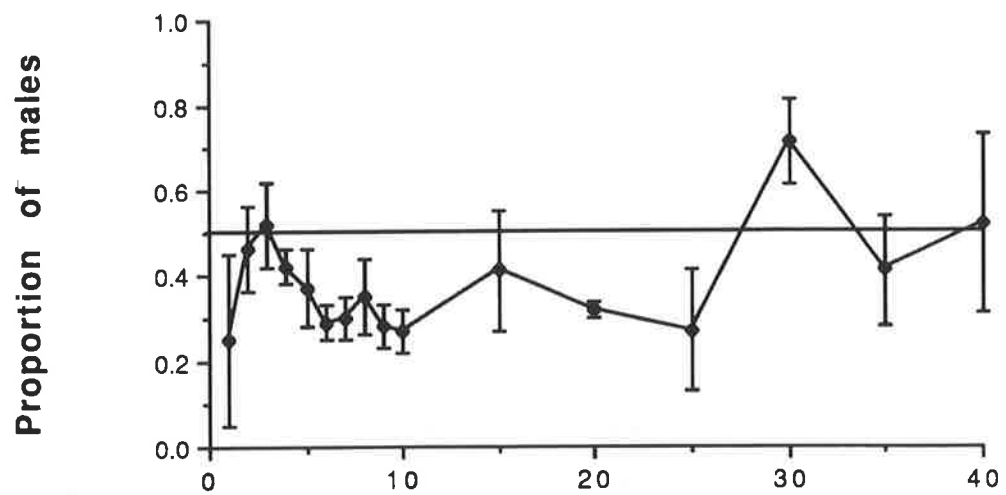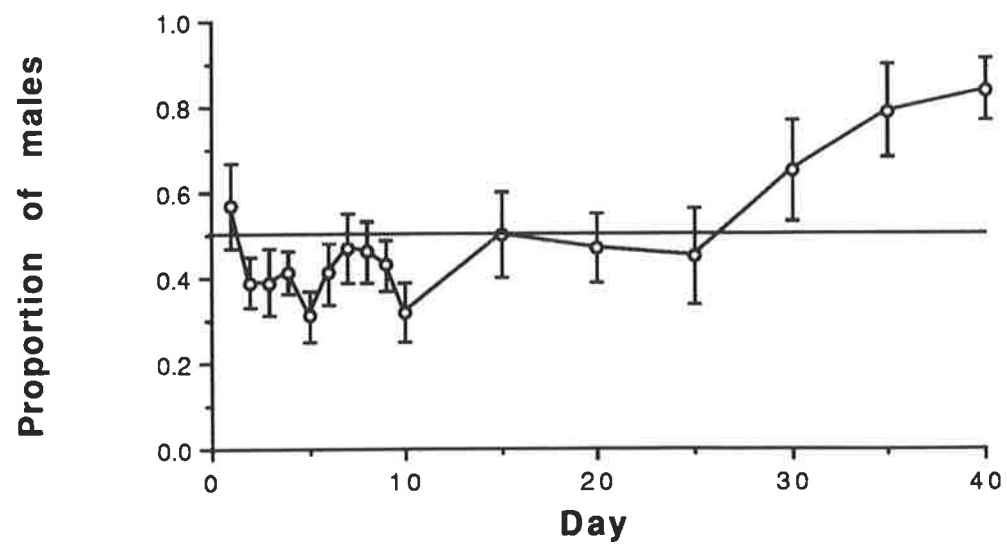
hosts (prop. male = 0.518). During this period, a significant proportion of the *H. varicornis* females produced male-biased sex ratios on 2nd instars, and female-biased sex ratios on 3rd instars (Table 6.2). These differences were further reflected in the allocation of sexes to progeny, with a greater proportion of males being produced on 2nd instar hosts (Figure 6.3A).

During days 15 to 40 (pooled), *H. varicornis* produced only male progeny on 2nd instar hosts and a strongly male-biased sex ratio on 3rd instar hosts (prop. male = 0.830). On both these host stages most *H. varicornis* females produced male-biased sex ratios (Table 6.2). A greater proportion of males were produced on 2nd than 3rd instar hosts (Figure 6.3B).

Sex ratios produced by *H. varicornis* between days 1 and 40 (pooled) were male-biased, regardless of whether 2nd (prop. male = 0.997) or 3rd instars (prop. male = 0.705) were attacked. Again, a significant proportion of the *H. varicornis* females produced male-biased sex ratios on 2nd instars. However, on 3rd instars, neither male- or female-biased sex ratios predominated. The production of males was proportionately greater on 2nd than 3rd instars (Figure 6.3C).

When overall sex ratio production per day was analysed for *H. varicornis*, only male-biased or unbiased ratios were observed on either host instar (Figure 6.4). On 2nd instar hosts, sex ratios were male-biased on every day sampled except day 20 where no progeny were produced (Figure 6.4A). However, when 3rd instars were offered, sex ratios were frequently unbiased during the first 10 days with only days 1 and 5 displaying male-biased sex ratios (Figure 6.4B). After day 10, male-biased sex ratios were frequently produced with an unbiased sex ratio occurring on day 25 only (Figure 6.4B).

Table 6.2. Outcome of the Wilcoxon's paired-sample test investigating if a significant number of *H. varicornis* females produced biased sex ratios on different *L. brassicae* instars during the no-choice experiments.

Day	Host Instar 2	Host Instar 3
1	NS	NS
2	NS	NS
3	NS	Female
4	Male	NS
5	Male	NS
6	Male	NS
7	Male	NS
8	Male	NS
9	Male	Female
10	Male	NS
15	Male	NS
20	NS	Male
25	NS	NS
30	NS	NS
35	NS	NS
40	NS	NS
1 - 10 (pooled)	Male	Female
1 - 40 (pooled)	Male	NS
15 - 40 (pooled)	Male	Male

Female, indicates significantly more females produced female-biased sex ratios than male-biased ($P < 0.05$); Male, indicates significantly more females produced male-biased sex ratios than female-biased ($P < 0.05$); NS, indicates the number of females producing female- or male-biased sex ratios was not significantly different ($P > 0.05$).

131

Figure 6.3. Total production of female (hatched area) and male (striped area)

progeny by *H. varicornis* on different *L. brassicae* instars during the no-choice

experiment for (A) days 1 to 10 pooled, (B) days 15 to 40 pooled, and (C) days 1 to

40 pooled (* estimated total).

E, indicates expected levels of female production from contingency table analysis.

For each graph, instars with the same letters (a to b) have proportion of male and

female progeny that are not significantly different (P > 0.05).
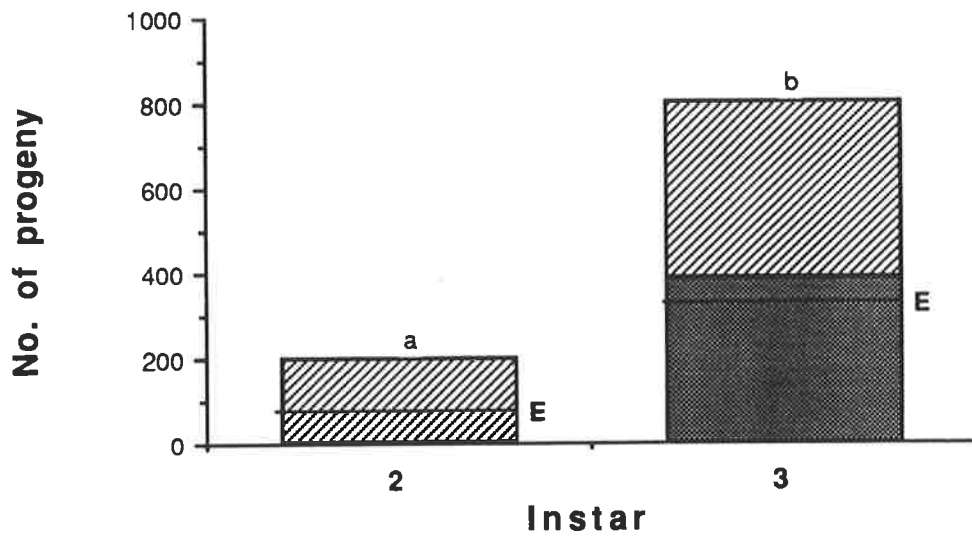
A. Days 1 to 10 pooled

B. Days 15 to 40 pooled
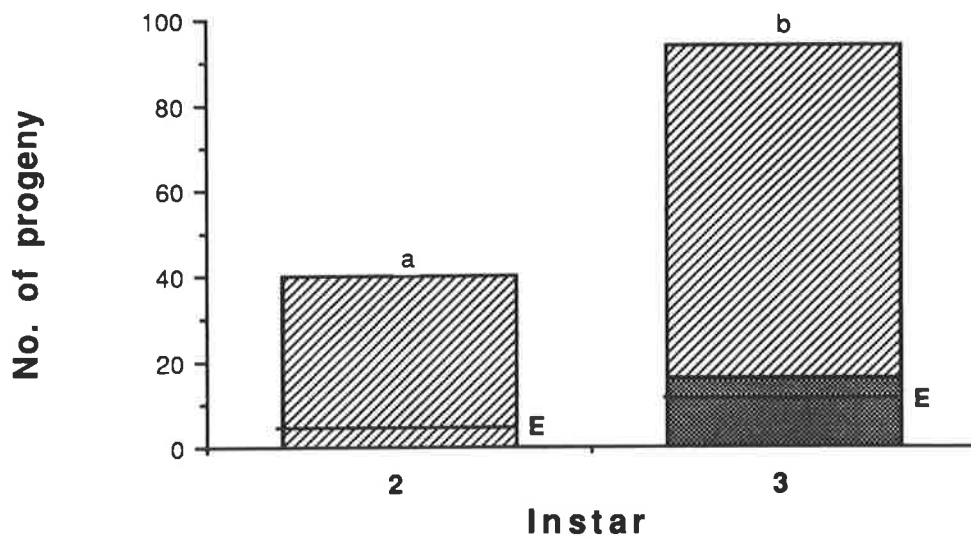
C. Days 1 to 40 pooled*
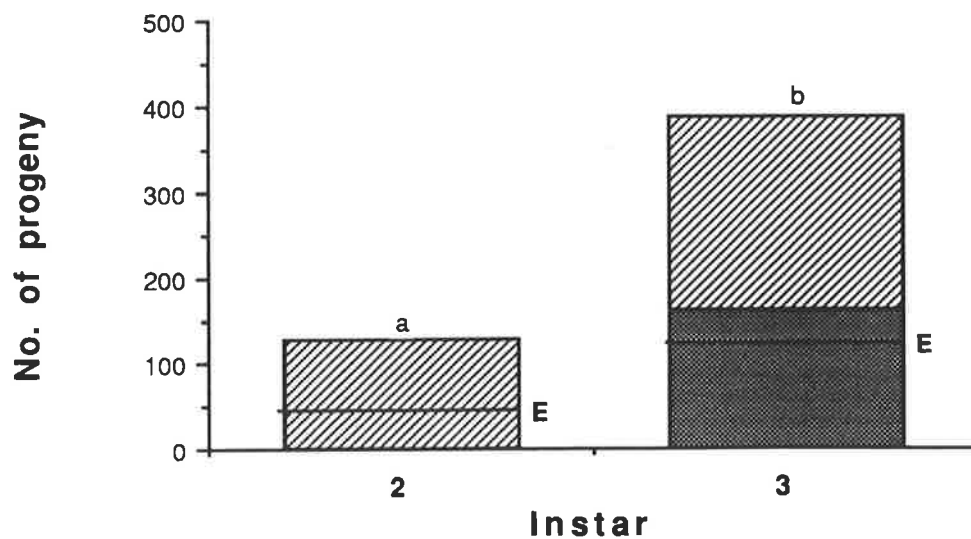
Figure 6.4. Proportion males (mean ± standard error) allocated by *H. varicornis* to 2nd (A) and 3rd (B) instar *L. brassicae* sampled on the first 10 days of oviposition and then every 5 days until day 40. Females which produced no offspring were excluded from the calculation of the means.

Dashed line indicates no offspring were produced on day 20.

A.

B.

135
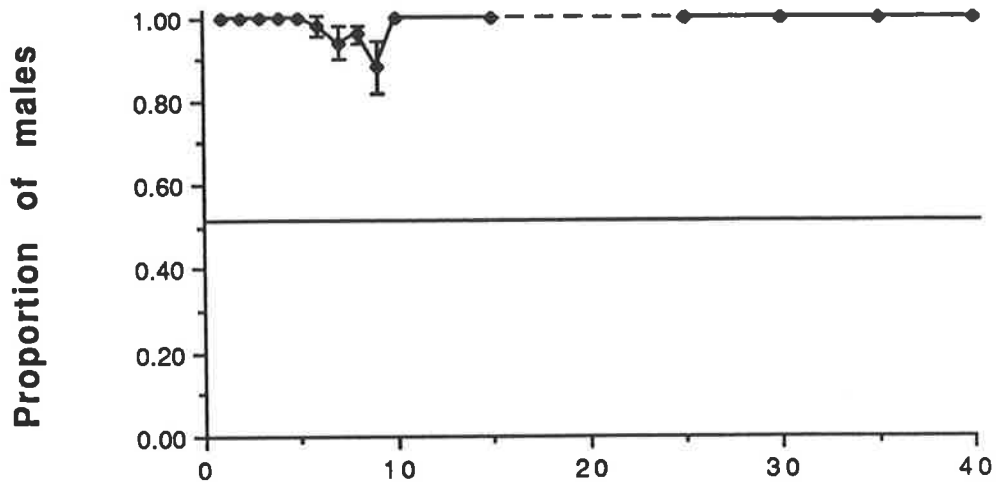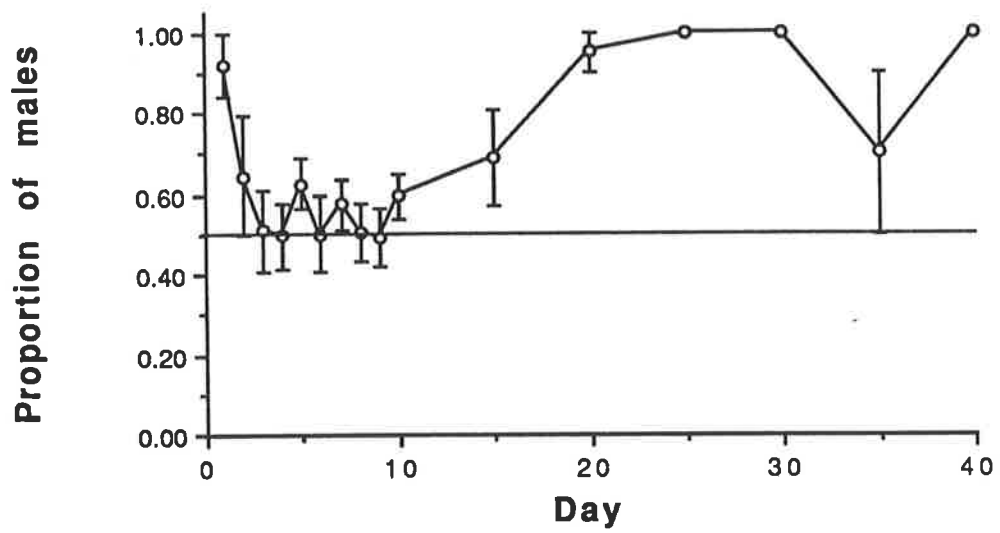
Analysis of the number of *H. varicornis* females producing biased sex ratios indicated a significant proportion produced male-biased sex ratios on 2nd instars on days 4 to 10 and then day 15 (Table 6.2). On other days sampled, neither male- or female-biased sex ratios predominated, probably resulting from only a small number of females producing progeny. When offered 3rd instar hosts, the number of females producing male- or female-biased sex ratios was not significantly different except on days 3, 9 and 20 (Table 6.2). Days 3 and 9 were characterized by a significant proportion of *H. varicornis* producing female-biased sex ratios, while on day 20 male-biased sex ratios were most common. This indicates that the overall male-biased sex ratios recorded from 3rd instars on some days resulted from the behaviour of only a portion of the *H. varicornis* reproducing.

6.4.2. Choice Experiments

Different strategies of sex allocation by *O. cinerariae* were observed from the two choice Experiments. Greater male production occurred during choice experiment 1 than 2, particularly on 2nd instar hosts.

O. cinerariae produced an unbiased sex ratio (days 1 to 10 pooled; instars pooled) when offered a choice of 1st and 2nd instar hosts (choice experiment 1; prop. male = 0.507). During this experiment, the sex ratios produced were female-biased on 1st instars (prop. male = 0.437) and male-biased on 2nd instars (prop. male = 0.550). Most *O. cinerariae* produced female-biased sex ratios during choice experiment 1 (days 1 to 10 pooled; instars pooled; Table 6.3). This was also true when sex ratios produced on 1st instar hosts were examined (days 1 to 10 pooled; Table 6.3). However, on 2nd instars the number of *O. cinerariae* producing male- or female-biased sex ratios was not significantly different (days 1 to 10 pooled; Table 6.3). A significantly greater proportion of males was produced on 2nd than 1st instars during choice experiment 1 (Figure 6.5A).

Table 6.3. Outcome of the Wilcoxon's paired-sample test investigating if a significant number of *O. cinerariae* females produced biased sex ratios on different *L. brassicae* instars during the choice experiments.

Day	Choice Experiment 1			Choice Experiment 2		
	1st Instar	2nd Instar	1st + 2nd Instar	2nd Instar	3rd Instar	2nd + 3rd Instar
1	NS	NS	NS	NS	NS	NS
2	NS	NS	NS	NS	NS	NS
3	NS	NS	NS	NS	NS	Female
4	Female	NS	Female	NS	Female	NS
5	Female	NS	NS	NS	Female	Female
6	NS	NS	NS	Female	NS	Female
7	Female	NS	NS	Female	NS	Female
8	NS	NS	Female	Female	Female	Female
9	Female	Female	Female	Female	Female	Female
10	NS	NS	NS	Female	Female	Female
1 - 10 (pooled)	Female	NS	Female	Female	Female	Female

Female, indicates significantly more females produced female-biased sex ratios than male-biased (P < 0.05); NS, indicates the number of females producing female- or male-biased sex ratios was not significantly different (P > 0.05).

137

Figure 6.5. Total production of female (hatched area) and male (striped area) progeny by *O. cinerariae* on different *L. brassicae* instars between days 1 and 10 (pooled) for the choice experiments. (A) Choice experiment 1, (B) Choice experiment 2, (C) Choice experiments 1 and 2 (instars pooled).

E, indicates expected levels of female production from contingency table analysis. For each graph, instars with the same letters (a to c) have proportion of male and female progeny that are not significantly different (P > 0.05).

A. Choice experiment 1
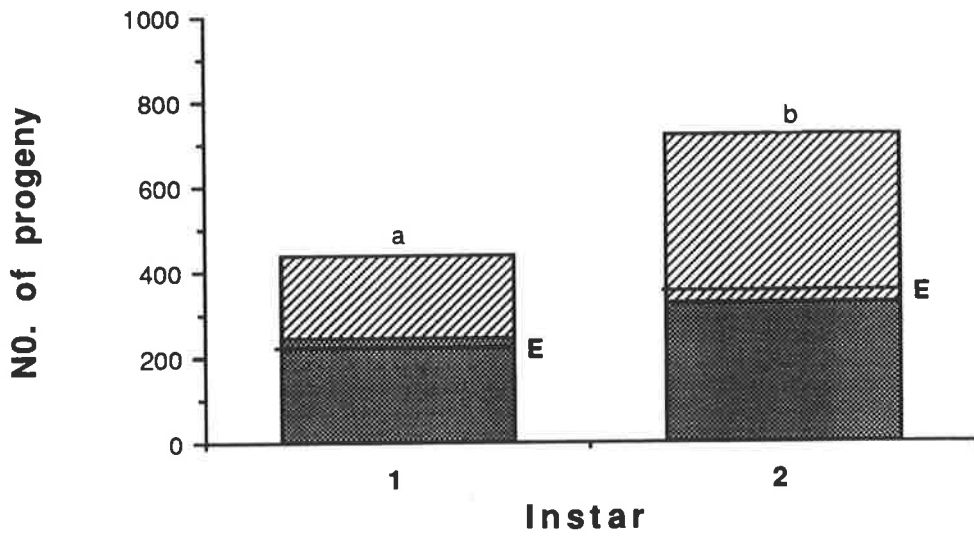
B. Choice Experiment 2
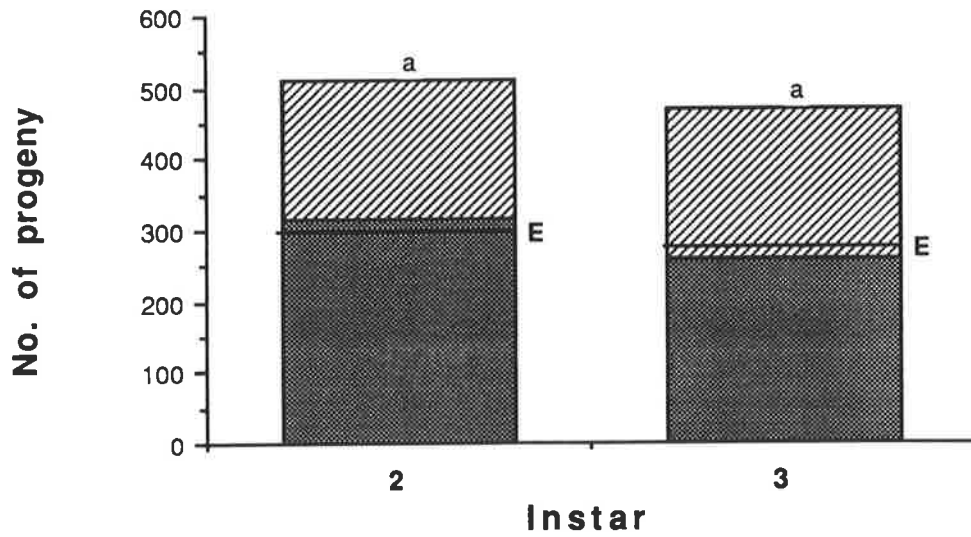
C. Comparison of Choice Experiments
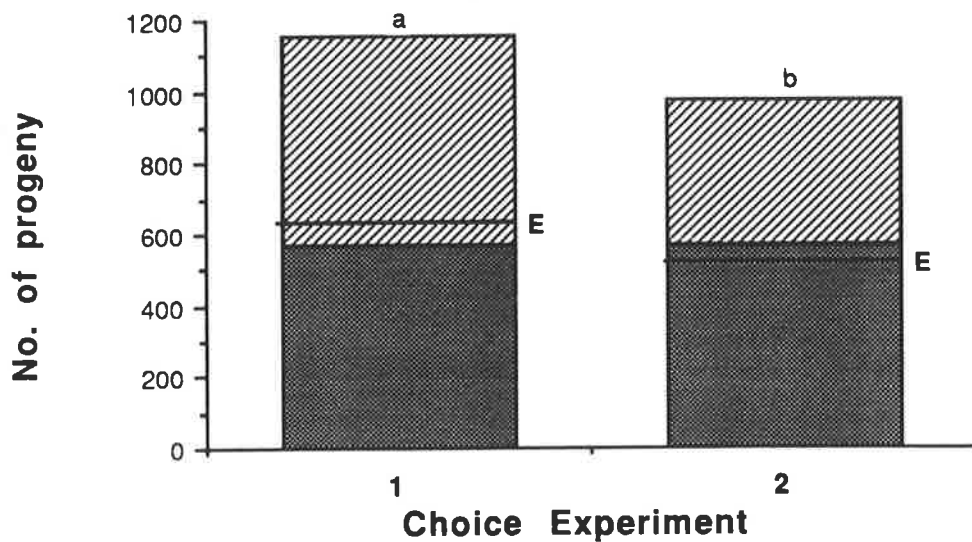
Analysis of the overall sex ratios produced per day during choice experiment 1 indicated unbiased sex ratios were consistently produced (Figure 6.6). On each instar, unbiased sex ratios were common. Only one female-biased sex ratio was produced on 1st instar hosts (day 4; Figure 6.6A) while one male-biased sex ratio was observed on 2nd instars (day 3; Figure 6.6B). More *O. cinerariae* produced female-biased rather than male-biased sex ratios on all hosts on 3 of the 10 days sampled (Table 6.3). A significant proportion of *O. cinerariae* produced female-biased rather than male-biased sex ratios on 1st instars on 4 of the 10 days (Table 6.3). On 2nd instars, female-biased sex ratios were more frequently produced on only 1 day (Table 6.3). The remaining days for each instar were characterized by the numbers of *O. cinerariae* producing male- or female-biased sex ratios not being significantly different.

When offered a choice of 2nd or 3rd instar hosts (choice experiment 2), *O. cinerariae* produced an overall female-biased sex ratio (days 1 to 10 pooled; instars pooled; prop. male = 0.414). On each instar separately, the sex ratio produced between days 1 to 10 (pooled) were also female-biased (1sts, prop. male = 0.384; 2nds, prop. male = 0.447). More *O. cinerariae* produced female-biased than male-biased sex ratios, regardless of whether each instar (pooled for days 1 to 10) was treated individually or combined (Table 6.3). There was no significant difference in the proportion of male offspring produced on 2nd or 3rd instars during choice experiment 2 (Figure 6.5B).

The sex ratio produced by *O. cinerariae* on all hosts on day 1 of choice experiment 2 was male-biased (Figure 6.6F). Unbiased sex ratios were produced on all hosts for days 2 to 5, after which sex ratios were female-biased. For each instar separately, sex ratios produced on day 1 were not significantly male-biased (Figure 6.6D,E). Female-biased sex ratios were observed for 4 days on 2nd instars but only 1 day on 3rd instar hosts. Initially, the proportion of *O. cinerariae* producing

Figure 6.6. Proportion males (mean ± standard error) allocated by *O. cinerariae* during the choice experiments. Choice experiment 1 - (A) 1st instars, (B) 2nd instars, (C) 1st and 2nd instars combined. Choice experiment 2 - (D) 2nd instars, (E) 3rd instars, (F) 2nd and 3rd instars combined. Females which produced no offspring were excluded from the calculation of the means.

Choice Experiment 1

A. 1st instar

B. 2nd instar

C. 1st and 2nd instars combined

Choice Experiment 2

D. 2nd instar

E. 3rd instar

F. 2nd and 3rd instars combined

Proportion of males

Day
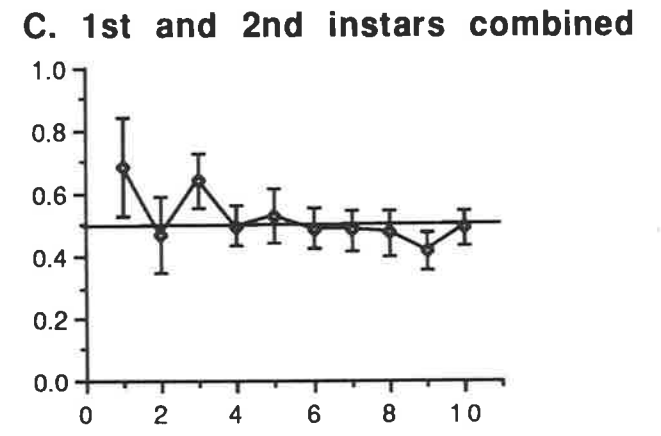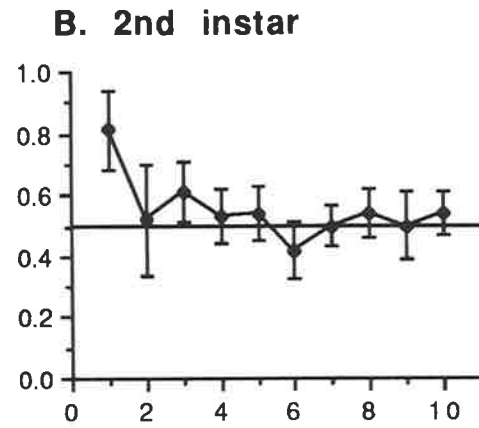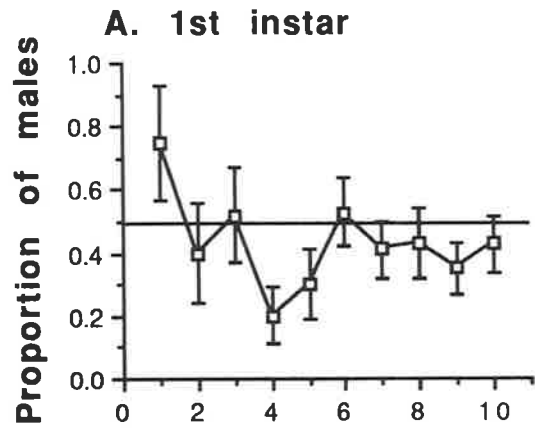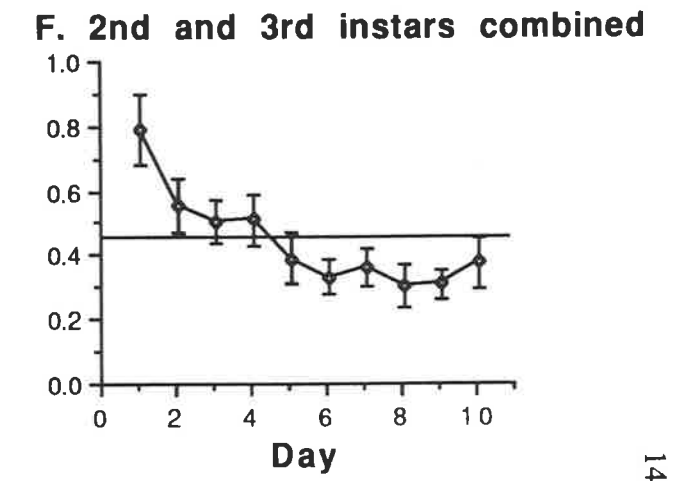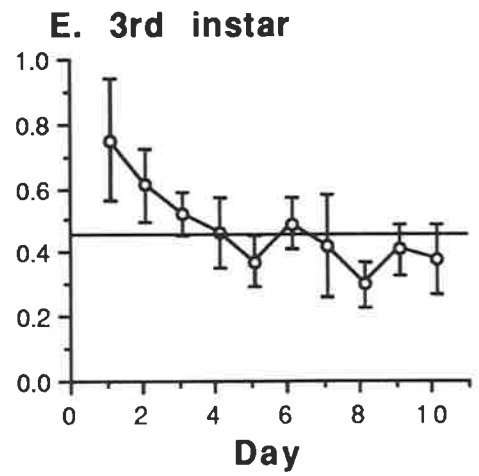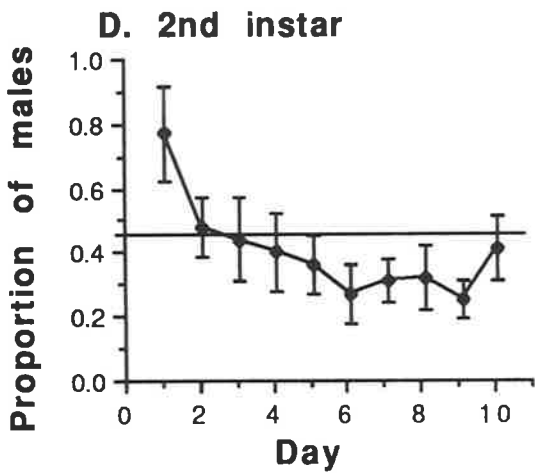
male- or female-biased sex ratios was not significantly different. However, on days 3 and 5 to 10, most parasitoids produced female-biased sex ratios (Table 6.3). On each instar, more *O. cinerariae* produced female biased than male-biased sex ratios on 5 of the 10 days sampled (Table 6.3).

A comparison of total production of progeny of each sex during the choice Experiments (days 1 to 10 pooled; instars combined) revealed a greater proportion of males were produced during choice experiment 1 than 2 (Figure 6.5C).

6.4.3. Comparison between no-choice and choice experiments

The sex allocation behaviour of *O. cinerariae* was similar in no-choice and choice Experiments when offered 1st or 3rd instar hosts. However, sex allocation to progeny produced on 2nd instar hosts was significantly different between the experiment types. The sex ratios produced on 2nd instars between days 1 and 10 (pooled) during the no-choice experiment and choice experiment 2 had significant female-biases as distinct from the male-bias produced during choice experiment 1. Female-biased sex ratios produced by individual *O. cinerariae* on 2nd instars were more common in the no-choice experiment and choice experiment 2 than choice experiment 1 (Table 6.1, 6.3). A greater proportion of males was produced on 2nd instars in choice experiment 1 than in either the no-choice experiment or choice experiment 2 ($P < 0.05$).

6.5. DISCUSSION

Both parasitoid species varied sex ratio in response to the host instar(s) offered. For *H. varicornis*, this response was strong in situations where only one instar was present. Although untested, it would be expected that a choice between 2nd and 3rd instar *L. brassicae* would show differential sex allocation in response to

host stage by *H. varicornis*. The strong male-bias in 2nd instar hosts accompanied by greater female production in 3rd instars observed for *H. varicornis* supports the assumption that female progeny gain more from developing in larger hosts (Charnov *et al.* 1981; Charnov and Skinner 1985; Waage and Godfray 1985; Strand 1988). This laboratory result conforms with the sex ratio of *H. varicornis* collected in the field which was also strongly male-biased on 2nd instars and unbiased on 3rd instars (Chapter Three).

While the sex ratios produced by *H. varicornis* are readily explained in terms of the host size model presented by Charnov *et al.* (1981), differential mortality may also have been a factor in determining the sex ratios at emergence. Being an idiobiont, resources available to *H. varicornis* progeny are limited by the size of the host at oviposition. Female parasitoids are generally thought to require more resources to complete development (Hurlbutt 1987). If this is the case for *H. varicornis*, then female progeny may have suffered greater mortality in 2nd instar hosts than male offspring. Such a process would skew sex ratios at emergence towards males even if primary sex ratios were approximately 1:1. However, under these circumstances a strategy allocating an approximately equal sex ratio to 2nd instar hosts would be selected against and should be rare amongst *H. varicornis*.

No choice situations indicated *O. cinerariae* favours female production regardless of host stage. However, a choice between 1st and 2nd instar hosts induced *O. cinerariae* to differentially allocate sexes, with a male-bias in 2nd instars and greater female production in 1st instars. Host-size dependent sex allocation theory predicts that female progeny should be allocated more frequently to larger hosts (Charnov *et al.* 1981; Charnov and Skinner 1985; Waage and Godfray 1985; Strand 1988); this prediction may not be valid for *O. cinerariae*. A shift towards male production in 2nd instar hosts by *O. cinerariae* suggests males may be most fit

when they develop on this host stage. A comparison of the fitness of male progeny from each host stage elucidated reasons for this strategy (see Chapter Seven).

The range of host sizes encountered has been suggested to influence sex allocation by parasitoids. Females encountering only one host size have been predicted to produce unbiased sex ratios (Avilla and Albajes 1984; Strand 1988), while differential allocation of sexes may occur if the female encounters a range of host sizes (Charnov and Skinner 1985; Strand 1988). This would indicate host size is determined on a relative rather than absolute basis (Strand 1988). For both parasitoid species studied here, biased sex ratios were consistently produced whether one or more host stages were offered. The strongly male-biased sex ratios produced by *H. varicornis* when only offered 2nd instar hosts does not support the above predictions. Similar results were presented by Jones (1982) and Luck and Podoler (1985), suggesting that some parasitoids use an absolute rather than relative measure of host size. Exclusively male offspring were initially produced by *H. varicornis* on 2nd instar hosts but, after several days, an increasing percentage of female progeny were produced (Figure 6.4A). Such behaviour has previously been observed for other species attacking sub-optimal hosts (Sandlan 1979; Avilla and Albajes 1984). This may represent a change in parasitoid strategy in an attempt to increase fitness when the expectation of encountering hosts suitable for female production is low. It also suggests that a shift from absolute measurement of host size (or suitability) to assessment of relative worth. Female biased sex ratios were produced by *O. cinerariae* when host stages were offered separately, again contrary to the prediction that unbiased sex ratios would be produced (Avilla and Albajes 1984; Strand 1988). The shift in sex allocation produced by *O. cinerariae* when offered a choice of instars (choice experiment 1) indicates host quality is measured on a relative basis.

It must be remembered that host stages, as distinct from host sizes, were offered in these experiments. While host stage is generally correlated with host

size (Charnov *et al.* 1981; King 1987), a range of host sizes would be present within one host stage. Therefore, sex ratio manipulation on the basis of host size may have gone undetected in no-choice experiments. Furthermore, both *O. cinerariae* and *H. varicornis* are polyphagous (Kleinschmidt 1970; Spencer 1973; Johnson 1987; Waterhouse and Norris 1987; Chapter Three). If the sizes of other host species vary considerably from that of *L. brassicae*, then differential responses to host stages of *L. brassicae* may not completely reflect the sex-allocation strategies of these parasitoids (Arthur and Wylie 1959; King 1989) The sizes of larval stages of other known host species for these two parasitoids appear similar to *L. brassicae* (Kleinschmidt 1970; Spencer 1973). However, the entire host range of both species is unknown.

Overall female-biased sex ratios, as produced by *O. cinerariae*, have also been observed for other species (Gerling and Bar 1971; Charnov *et al.* 1981; Jones 1982; van Alphen and Nell 1982; Avilla and Albajes 1984; Donaldson and Walter 1984; van den Assem *et al.* 1984; Werren 1984). There are several possible explanations for the female-biased sex ratios produced by *O. cinerariae*. Firstly, they may have resulted from differential mortality of the sexes. If male offspring were allocated more frequently to smaller hosts, greater mortality of this sex may have occurred as a result of insufficient resources to complete development. Evidence suggests that this explanation does not apply to *O. cinerariae* . Being a koinobiont, host resources should not be a limiting factor for development. Furthermore, both male and female progeny developed successfully from 1st instar *L. brassicae*. In most hymenopteran parasitoids, females are larger than males and would require a larger resource to develop successfully (Hurlbutt 1987). Therefore, it is unlikely that more male than female progeny would die as a result of resource limitation in 1st instars. Also, this would not explain the female-biased sex ratios in 2nd or 3rd instar hosts. Consequently, this bias would appear to be directly related to the sex allocation by females at oviposition.

Another possible explanation is that the female-biased sex ratios of *O. cinerariae* represent a strategy selected for by LMC (Hamilton 1967). LMC is thought to be found in association with clumped host distributions (Waage 1982b; Strand 1988). In host patches visited by a single female, there would be a strong chance of sibling mating and parasitoids would be expected to produce female-biased sex ratios (Hamilton 1967; Werren 1980, 1983). Such conditions were present in my experiments. Each cage was effectively a single patch containing numerous hosts. The experimental female was the first and only parasitoid to visit that patch and may, therefore, only have produced enough males to successfully mate all her female offspring. While the experimental design and the female-biased sex ratios produced by *O. cinerariae* conform with the predictions of LMC theory, this does not indicate that LMC is an important selection pressure upon sex allocation of this parasitoid. Demonstration of the importance of LMC would require *O. cinerariae* being shown to manipulate sex ratios in response to variation in host and parasitoid density. Considering the variability of host distribution and abundance (Chapter Two) and the low density of *O. cinerariae* (Chapter Three), it is unlikely that this parasitoid regularly encounters previously parasitized hosts or conspecific females in the field. Therefore, LMC does not appear to be a significant selection pressure on sex allocation behaviour of *O. cinerariae*.

Parasitoids of hosts with highly variable distributions and abundance may not be exposed to consistent selection pressures. Fixed strategies which tend to maximize the average fitness over all host population structures may prove the most adaptive. Slight female-biased sex allocation may represent one such strategy. The fitness of males will depend on their ability to successfully obtain mates whereas for females fitness can be roughly equated with the number of hosts parasitized. In patchily distributed, low density parasitoid populations, not all males may obtain mates while most females will successfully locate and parasitize hosts. Therefore, the fitness of males will be more variable than that of females and, in such circumstances, the

average return from the production of male progeny may be lower than for female production. Consequently, female-biased sex ratios may represent a strategy whereby parasitoids can maximize fitness when attacking highly variable host populations.

Other conditions may favour the production of female-biased sex ratios. If male fitness was greater than female fitness from large hosts and parasitoids manipulated sex ratios to favour male production in larger hosts, then an overall female-biased sex ratio would be expected (Bull 1981; Waage 1986). Waage and Godfray (1985) and Godfray (1986) suggested sexual asymmetries may also favour the production of female-biased sex ratios. This may be as a result of an asymmetric response, such as differential mortality, or an asymmetric effect, where one sex has a detrimental effect upon the development of the other (Godfray 1986; Strand 1988) Asymmetric responses in larval parasitoid mortality have already been discussed and considered unlikely to be present. As *O. cinerariae* is a solitary parasitoid it is also unlikely that sexes may influence the development of each other. There is no evidence to suggest that male eggs require a greater reproductive effort than female eggs. The relative fitness of male and female offspring from large hosts is compared in Chapter Seven.

The pattern of variation of sex ratios over time for both species suggests that sperm depletion or reduced sperm viability may be important factors in determining sex ratios produced by aged females. As females in these experiments were only mated once, depletion of their sperm reserves could have lead to a shift towards male production. On 3rd instar hosts, sex ratios became more male-biased after day 10 for *H. varicornis* and after day 25 for *O. cinerariae*. From Chapter Five we know that both species produced most progeny on 3rd instar hosts. It is reasonable to expect that the chance of sperm depletion would increase in relation to the number of female progeny produced. Stored sperm may also lose viability over time (Flanders 1956; Abdelrahman 1974a). However, this reduction in viability would be

similar in parasitoids attacking each host stage and would not explain why only parasitoids attacking 3rd instar hosts showed greater male production late in life. While these results may indicate sperm depletion over the long-term, it is also possible that shorter-term sperm depletion may have influenced sex allocation. Parasitoids which oviposit in numerous hosts in quick succession often produce a high proportion of, or exclusively, male offspring after the first few ovipositions (Flanders 1956; Wiackowski 1962; Abdelrahman 1974b; Mackauer 1976). This has been attributed to the inability of the spermathecal gland to secrete enough sperm activating fluid at high rates of oviposition so that, after the first few fertilizations, all eggs pass through the oviduct unfertilized (Flanders 1956). In cages with numerous, readily-located host larvae it is possible that intervals between oviposition were short. This would be most likely on 3rd instar hosts where most progeny were produced (Chapter Five). Short-term unavailability of sperm may have contributed to the greater frequency of male *O. cinerariae* offspring in 3rd instar hosts compared to either 1st or 2nd instars (Figure 6.1). Experiments investigating sex allocation in relation to the speed of host finding and oviposition by *O. cinerariae* are needed to support this argument.

Sex ratios produced by naive females (i.e. on day 1) were often markedly different from sex ratios produced on the following days. In many cases, these were more male-biased than observed subsequently (Figures 6.2C, 6.4B, 6.6A-F). Greater production of male progeny on the first day of oviposition has also been observed by Mackauer (1976), Avilla and Albajes (1984), van den Assem *et al.* (1984) and Wong *et al.* (1990). This behaviour may be associated with inexperience or represent a physical constraint, such as functional virginity for a short period following mating (Mackauer 1976).

Waage's (1982a) prediction that idiobionts should display host size dependent sex ratios while koinobionts should not is partly supported by the data presented here. The sex ratios of the idiobiont, *H. varicornis*, exhibited strong host

stage dependency while those of the koinobiont, *O. cinerariae*, were less dependent on host stage. The premise that host stage at oviposition would not be a good indicator of resource availability for koinobionts is investigated in Chapter Seven.

Control of sex ratios in response to host stages indicates parasitoids are able to discriminate between hosts on the basis of their suitability for development of each sex. Progeny production and sex allocation have frequently been shown to vary in relation to host factors, such as size, age or stage of development. How these factors are assessed by parasitoids remains poorly understood. Egg parasitoids, such as *Trichogramma* sp., have been shown to alter clutch size in relation to the duration of their initial transit across the exposed surface area of the egg (Schmidt and Smith 1987). However, the mechanisms by which parasitoids of larval stages assess host size are unknown. Strand (1988) suggested *Habrobracon (=Bracon) hebetor* (Say) may measure host length by repeatedly walking the length of the host larva before ovipositing. Hosts differ in quality as a result of their physiological or nutritional state yet little information is available concerning parasitoid detection of these factors. Variation in host quality is reflected in the fitness of parasitoids and consequently may influence selection pressures on host utilization strategies. Various measures of fitness in relation to host stage are reported in Chapter Seven. Understanding how parasitoids of larval host stages assess and discriminate on the basis of host quality remains a challenging and important area of investigation.

CHAPTER SEVEN

RELATIVE FITNESS OF PARASITOIDS DEVELOPING FROM DIFFERENT HOST INSTARS

7.1. ABSTRACT

The relative fitness of *Opius cinerariae* and *Hemiptarsenus varicornis* developing on different instars of their host, *Liriomyza brassicae*, was estimated. Fitness components measured included developmental rate at three constant temperatures, body size, longevity, the number of mature oocytes at emergence for females and the mating success of males. For *O. cinerariae*, only size increased directly with the host instar on which development began. Different developmental rates, longevities and rates of mating success were observed for *O. cinerariae* progeny from different instars, but these did not increase directly with instar. Egg load at emergence was not influenced by the host instar on which development began. Females lived longer and males developed faster at high temperatures (25°C); otherwise sex did not influence fitness. In contrast, body size, longevity, egg load at emergence and mating success of *H. varicornis* increased directly with the host instar attacked. Only developmental rate was not influenced by host instar. Female *H. varicornis* derived greater fitness benefit than males by developing on larger hosts. The consequences of the host instar attacked on subsequent fitness is discussed in relation to host utilization theory, and the phenology and behaviour of these species in the field.

7.2.   INTRODUCTION

Natural selection theory predicts that parasitoids should evolve a strategy of host-stage utilization that maximizes fitness (Charnov and Skinner 1985). In situations where densities of different host stages are similar, the host stage providing greatest fitness should be the most heavily parasitized (Charnov 1982). Host stage may also have a differential effect on fitness of the sexes (Charnov *et al.* 1981; Charnov and Skinner 1985). Therefore, differential allocation of sexes to different host stages should occur where one sex achieves greater fitness compared to the other in a particular host stage (Charnov 1982; Waage and Godfray 1985). Parasitoids frequently attack more than one host stage (see Chapter Four). Furthermore, some host stages suffer greater levels of parasitism than others and are selectively used for the development of one sex more so than the other (see Chapters Four to Six). Progeny having greater reproductive fitness than others will pass on more of their parent's genes to the next generation, including any genes which affect the parent's strategy of host utilization. Therefore, these genes should become more common within the parasitoid population, such that strategies of host utilization maximizing fitness should become dominant.

Critical to testing such an hypothesis is an appropriate measure of relative fitness of each sex developing from each host instar. Numerous features of an individual may be measured to give an indication of its relative fitness. These include its developmental time, size, longevity and fecundity. Such features may be interdependent and, for various species, different combinations may serve as the best estimate of fitness.

The suitability of hosts for development of solitary parasitoids often varies with the developmental stage, age or size of the host (Vinson and Iwantsch 1980b). Consequently, parasitoids frequently take longer to develop in some host stages/ages/sizes than others (Arthur and Wylie 1959; Puttler 1961; Fox *et al.* 1967;

Legner 1969; Vinson and Barras 1970; van den Assem 1971; Smilowitz and Iwantsch 1973; Miles and King 1975; Nechols and Tauber 1977; Beckage and Riddiford 1978; Flanders and Oatman 1982; Nealis *et al*. 1984; Hopper 1986; King 1988; de Jong and van Alphen 1989). Development time can influence fitness in several ways. Risk of predation, multiple-, super- or hyper-parasitism may increase with the duration of the period spent developing within or adjacent to the host. Similarly, short development times may give individuals a competitive advantage over cohorts which take longer to develop (Wilson 1961; King 1987). However, shorter developmental times have been correlated with smaller individuals (Arthur and Wylie 1959; Abdelrahman 1974a). Male parasitoids usually have a slightly shorter developmental time than their female counterparts and are often smaller as a result (van den Assem 1971; Charnov *et al*. 1981; Hurlbutt 1987).

The size of solitary parasitoids is often correlated with the size of the host (Arthur and Wylie 1959; Miles and King 1975; Lawrence *et al*. 1976; Jowyk and Smilowitz 1978; Sandlan 1979; Charnov *et al*. 1981; Nealis *et al*. 1984; Samson 1984; Liu 1985; Hopper 1986; Opp and Luck 1986; Reeve 1987; King 1988; de Jong and van Alphen 1989; Gunasena *et al*. 1989). At least one species, *Spalangia endius* Walker, has been shown not to become larger when attacking larger hosts (Donaldson and Walter 1984). Females are usually larger than males developing from the same size host (Jowyk and Smilowitz 1978; Charnov *et al*. 1981), although not always (Legner 1969; Nealis *et al*. 1984; Hurlbutt 1987).

Parasitoid size has frequently been shown to be an indicator of other fitness measures, such as longevity and fecundity. Larger parasitoids often live longer (Jackson 1966; Wylie 1966; Shiga and Nakanishi 1968; van den Assem 1971; Abdelrahman 1974a; Sandlan 1979; Charnov *et al*. 1981; Waage and Ng 1984) and hence have a greater opportunity to oviposit (for females) or mate (for males). Female size is frequently positively correlated with egg or offspring production (King and

Hopkins 1963; Velthuis *et al.* 1965; Iwata 1966; Jackson 1966; Shiga and Nakanishi 1968; van den Assem 1971; Benson 1973; Lawrence 1981; Narasimhan 1984; Nealis *et al.* 1984; Waage and Ng 1984; Takagi 1985; Opp and Luck 1986; Allen 1989). This may result from larger females possessing more eggs (Iwata 1966), living longer (Sandlan 1979), being more successful in locating and attacking hosts (Rotheray *et al.* 1984) or a combination of the above (van den Assem 1971). Male reproductive success may also increase with body size through an improved mating ability (van den Assem 1976, 1986; Grant *et al.* 1980; Jones 1982), greater short-term sperm availability (Barrass 1961) and a longer life (van den Assem 1971; Charnov *et al.* 1981). Many authors have assumed that the relative fitness of females increases more with size than it does for males (Sandlan 1979; Charnov *et al.* 1981; Avilla and Albajes 1984; van den Assem *et al.* 1984; Werren 1984; Opp and Luck 1986; Simbolotti *et al.* 1987; de Jong and van Alphen 1989; King 1989). Relatively few studies have compared the increment in male and female reproductive ability in relation to the host size in which development began (van den Assem 1971; Jones 1982; de Jong and van Alphen 1989), despite this relationship being a crucial assumption in sex ratio theory (Charnov *et al.* 1981; Werren 1984).

The relative fitness of male and female progeny developing from each instar of *Liriomyza brassicae* (Riley) was estimated for both *Hemiptarsenus varicornis* (Girault) and *Opius cinerariae* Fischer. Development time, size, longevity, egg load at emergence (females) and mating success (males) were used to estimate progeny fitness. By comparing the relative fitness of progeny from each instar it was possible to evaluate the host utilization strategies observed in Chapters Four to Six.

7.3. MATERIALS AND METHODS

7.3.1. Development

Duration of progeny development for *O. cinerariae* and *H. varicornis* was measured at 3 constant temperatures (15, 20 and 25°C) and 12L:12D hr photoperiod. Parent wasps were allowed to oviposit into one of the three host instars at 20°C and within 24 hr of oviposition, leaves containing parasitized hosts were transferred to either 15 or 25°C or left at 20°C. Parasitoids were reared as described previously (Chapters Three and Four). Sample sizes (Table 7.1) were dependent upon several factors, including the frequency at which each sex was allocated to different instars and degree to which leaves remained viable for long periods at low temperatures. In an attempt to gain a sufficient sample size for each group, the experiment was replicated in time. A mixed model analysis of variance (ANOVA; SAS 1985) was used to determine the effect of temperature, sex, host instar at oviposition and experimental replication on the developmental time of each species Where necessary, multiple comparisons were made using the Student-Newman-Kuels (SNK) multiple range test (SAS 1985). Lower thresholds for development of each species were estimated by the temperature-intercept method (Andrewartha and Birch 1954).

7.3.2. Longevity

Parasitoid longevity was measured by placing a single newly-emerged parasitoid which had developed at 25°C into a glass vial (50 x 18 mm). Each vial contained a smear of honey and numerous punctures in the lid to provide aeration. These wasps were held at 20°C and checked daily for mortality. Upon death, the hind tibia length was measured for each parasitoid. Up to twenty individuals of each sex from each instar were used for *O. cinerariae*. For *H. varicornis*, the longevity of up to twenty individuals of both sexes from third instar hosts and males from second instar hosts was recorded. However, there was insufficient development of female *H. varicornis* from 2nd instar hosts to assess their longevity. The effect of host instar,

Table 7.1. Sample sizes of developmental time data for each sex of *H. varicornis* and *O. cinerariae* at 3 constant temperatures.

Species	Temperature °C	Sex	Instar on which development began		
			1	2	3
H. varicornis	15	Female	-	-	46
		Male	-	4	35
	20	Female	-	-	9
		Male	-	8	16
	25	Female	-	4	36
		Male	-	16	29
O. cinerariae	15	Female	12	32	35
		Male	13	39	32
	20	Female	25	45	35
		Male	18	51	35
	25	Female	38	43	64
		Male	38	42	60

sex and size on parasitoid longevity was determined by ANOVA (SAS 1985), with multiple comparisons being made using the SNK multiple range test.

7.3.3. Egg Load

To determine the egg load of parasitoids emerging from different host stages, newly-emerged females which had developed at 25°C were frozen in gelatine capsules for dissection at a later stage. Prior to dissection, the wasps were thawed for 5 minutes in two drops of insect saline on a microscope slide and their hind tibia length was measured. The reproductive system was then removed and ovarioles separated using fine needles and forceps. Ovarioles were stained with two drops of methylene blue (0.3% in 50% ethanol) for 10 minutes, covered with a coverslip (50 x 22 mm) and mature oocytes counted. Up to twenty females from each instar were dissected for each species. The influence of host instar on parasitoid egg load was determined by ANOVA using hind tibia length as a covariate (SAS 1985). Where applicable, multiple comparisons were made using the SNK multiple range test.

7.3.4. Mating Success

The relative mating success of male parasitoids from different host instars was determined by observing male mating behaviour in glass cylinders (70 x 25 mm) with both ends covered by fine mesh. Experiments were always conducted between 1400 and 1800 hours at a constant temperature of 20°C. A single virgin female which had developed from a 3rd instar host at 25°C was placed into the mating cylinder and left undisturbed for at least 2 hours. Males were then introduced into the cylinder and timing begun. An encounter was considered to have occurred when the male exhibited courtship behaviour as a direct result of female proximity. If no encounter between sexes had occurred within 10 minutes of male introduction the pair was separated. If an encounter occurred within 10 minutes, a stop watch was started and the time and number of encounters until successful mating recorded. If no mating had occurred within 10 minutes of the first encounter then the pair was separated. Forty pairings

were set up for males from each host instar for each species, except *H. varicornis* males from 1st instar hosts. The hind tibia length was measured for all individuals of both sexes used in the mating success experiments. Log-likelihood (G) tests were used to compare the number of successful matings (total and on the first encounter) between males from different host instars. The influence of the host instar on which development began and male size on (i)the time to the first encounter, (ii) the number of encounters prior to mating or (iii) the time to mate were determined using a mixed model ANOVA (SAS 1985). Where needed, multiple comparisons were made by the SNK multiple range test. Pearson's correlation coefficient (SAS 1985) was used to determine if a significant relationship existed between male size and courtship variables. The courtship of each species was described briefly.

7.3.5.   Size

The influence of host size on parasitoid size was determined by pooling hind tibia measurements made in the previous sections for each sex from each host instar. The influence of sex and the host instar attacked on parasitoid size was determined by ANOVA (SAS 1985), with multiple comparisons made using the SNK multiple range test. Hind tibia length was considered a reliable estimate of overall size and was positively correlated with head capsule width (Appendix Four).

7.4.   RESULTS

Except where noted, all values are given as mean ± standard error.

7.4.1.   Development

Development of *O. cinerariae* from egg to adult took approximately 45 days at 15°C, 22 days at 20°C and 16 days at 25°C. *H. varicornis* development required approximately 35 days at 15°C, 18 days at 20°C and 12 days at 25°C. The

low frequency of female production by *H. varicornis* in 2nd instar hosts made it difficult to obtain data on developmental time of this group at 15 and 20°C (Table 7.1). There was no replication effect so data were pooled for each temperature-sex-instar combination. Lower temperature thresholds for development were estimated to be between 7 and 10°C for *O. cinerariae* and 9°C for *H. varicornis* (Table 7.2).

The developmental rates of both species display positive linear relationships with temperature ($p < 0.001$; Figures 7.1, 7.2). For *O. cinerariae*, temperature interacted with the host instar attacked and sex ($p < 0.001$). At 15°C, *O. cinerariae* developed faster in 3rd than either 1st or 2nd instars. However, at 25°C, this was reversed with progeny in 2nd instars having the fastest developmental rate. The difference in developmental rate between sexes increased with temperature, males developing faster than females.

Neither sex nor the host instar attacked influenced the developmental rate of *H. varicornis*. There was no significant interaction between temperature and either class variable.

7.4.2. Size

Lengths of *O. cinerariae* hind tibia ranged from 0.3000 to 0.575 mm. For *H. varicornis*, hind tibia length ranged from 0.225 to 0.600 mm. For both parasitoid species, measurements of hind tibia did not vary significantly between experiments. Therefore, data for each sex and host instar attacked were pooled.

Size of *O. cinerariae* varied with the host instar in which development began ($p < 0.05$; Figure 7.3A). Multiple comparisons revealed individuals that developed from 3rd instars were larger than those developing from 1st instar hosts ($p < 0.05$), but those from 2nd instar hosts did not differ from either 1st or 3rd instars. There was no relationship between sex and the size of *O. cinerariae*.

Table 7.2. Lower temperature thresholds (°C) for development of *O. cinerariae* and *H. varicornis*.

| Species | Host Instar attacked | | | |
	1	2	3	Total
O. cinerariae	8.9	9.1	7.6	8.4
H. varicornis	-	-	-	9.4

160

Figure 7.1. Developmental rates (mean ± standard deviation) of *O. cinerariae* at three constant temperatures for males (□) and females (●) which began development in (A) 1st instar, (B) 2nd instar and (C) 3rd instar *L. brassicae* larvae.

1st instars, male developmental rate = -0.0364 + 0.0040temperature, R^2 = 0.997; female developmental rate = -0.0338 + 0.0037temperature, R^2 = 0.995.

2nd instars, male developmental rate = -0.0393 + 0.0042temperature, R^2 = 0.998; female developmental rate = -0.0351 + 0.0038temperature, R^2 = 0.996.

3rd instars, male developmental rate = -0.0291 + 0.0037temperature, R^2 = 0.976; female developmental rate = -0.0267 + 0.0034temperature, R^2 = 0.964.

A.   1st instars
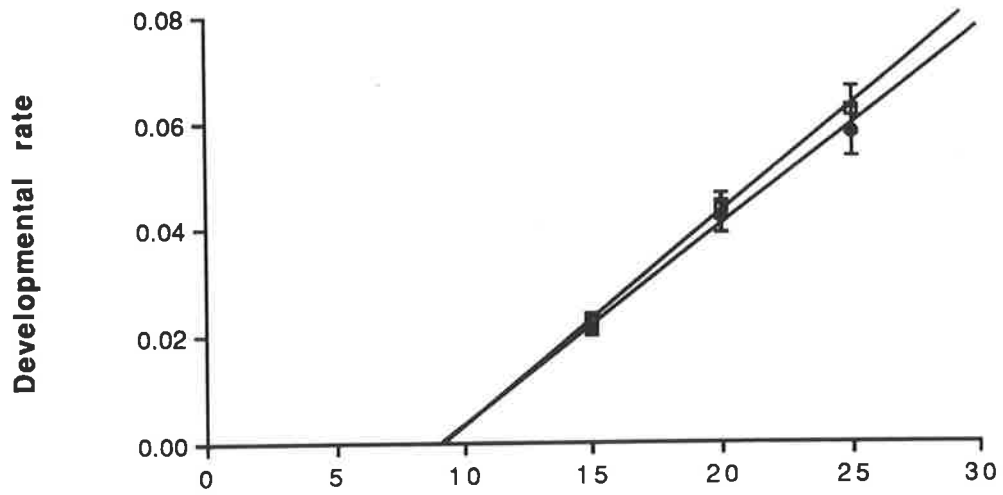
B.   2nd instars
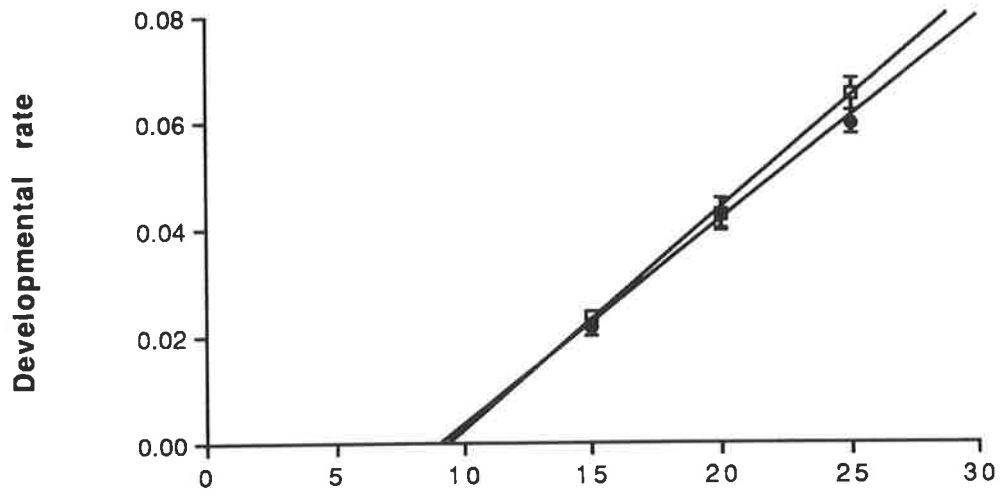
C.   3rd instars
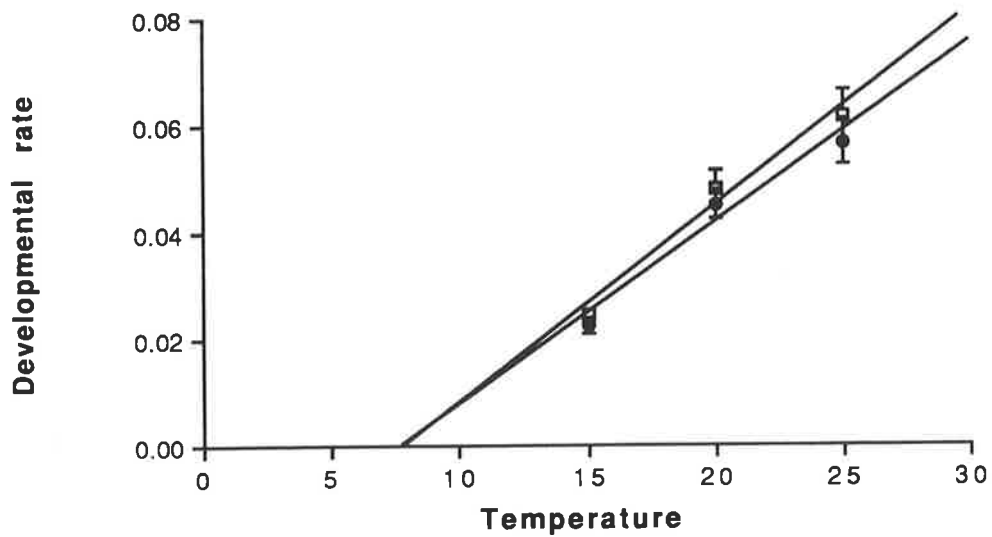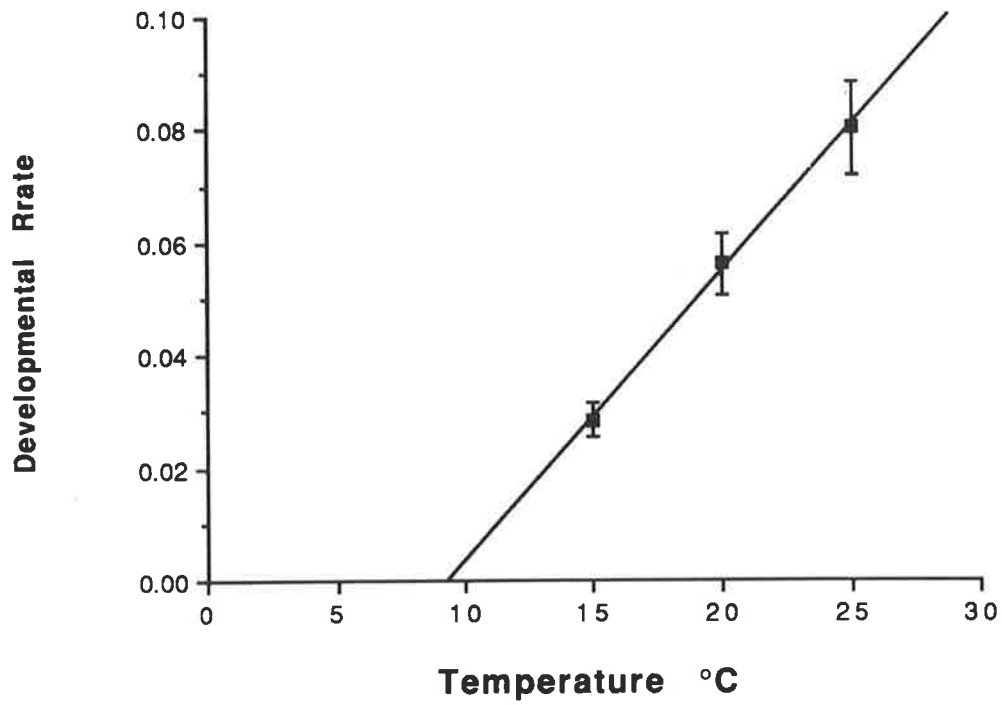
Figure 7.2. Developmental rate (mean ± standard deviation) of *H. varicornis* at three constant temperatures.

developmental rate = -0.0482 + 0.0052temperature, R^2 = 0.999.

Figure 7.3. Hind tibia lengths (range and mean ± standard error, boxed) of (A) *O. cinerariae*, (B) female *H. varicornis* and (C) male *H. varicornis* in relation to the host instar on which development began.

A.
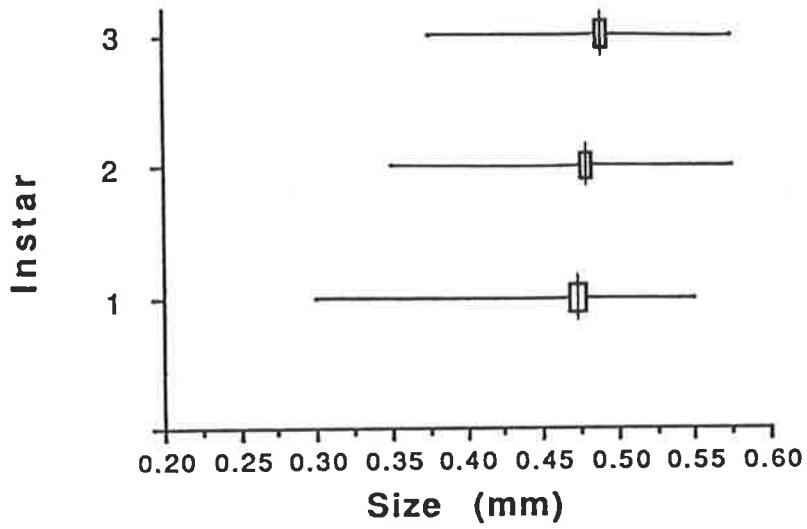
B.
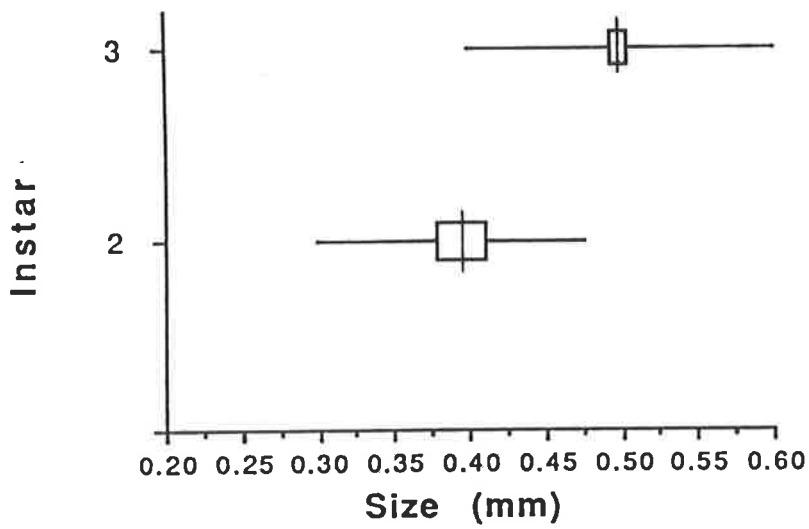
C.
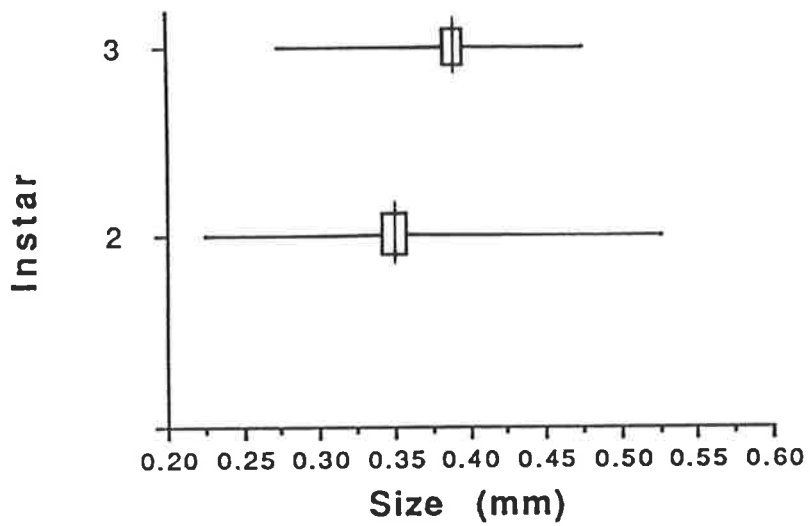
The size of *H. varicornis* varied with the host instar on which development occurred and with sex, there being a significant interaction between these factors (P < 0.005; Figure 7.3B, C). Multiple comparisons revealed that females from 3rd instars were larger than males from 3rd instars and both sexes from 2nd instars (P < 0.05). Males from 2nd instars were smaller than either females from 2nd instars or males from 3rd instars, while the latter two did not vary significantly in size.

7.4.3. Longevity

The longevity of *O. cinerariae* ranged from 38 ± 3 days for males from 2nd instar hosts up to 86 ± 5 days for females from 1st instars. For *H. varicornis*, longevity ranged from 33 ± 4 days for males from 2nd instars up to 49 ± 6 days for females from 3rd instars.

The lifespan of *O. cinerariae* varied with host instar (P < 0.05) and sex (P < 0.001), but the interaction between these variables was not significant (Figure 7.4A). Female *O. cinerariae* lived longer than males. Individuals developing from 3rd instars did not differ significantly in longevity from those developing on 1st or 2nd instars. However, individuals from 1st instars lived longer than those developing from 2nd instars (P < 0.05). Size did not influence the longevity of *O. cinerariae*.

The longevity of *H. varicornis* was greater for individuals from 3rd instar hosts than 2nd instar hosts (P < 0.005; Figure 7.4B). No significant difference was found between the mean longevity of the sexes of *H. varicornis*. Size did not influence the longevity of *H. varicornis*.

7.4.4. Egg Load

Both *O. cinerariae* and *H. varicornis* emerged with few mature oocytes. Newly emerged *O. cinerariae* had between 4 and 24 mature oocytes, while
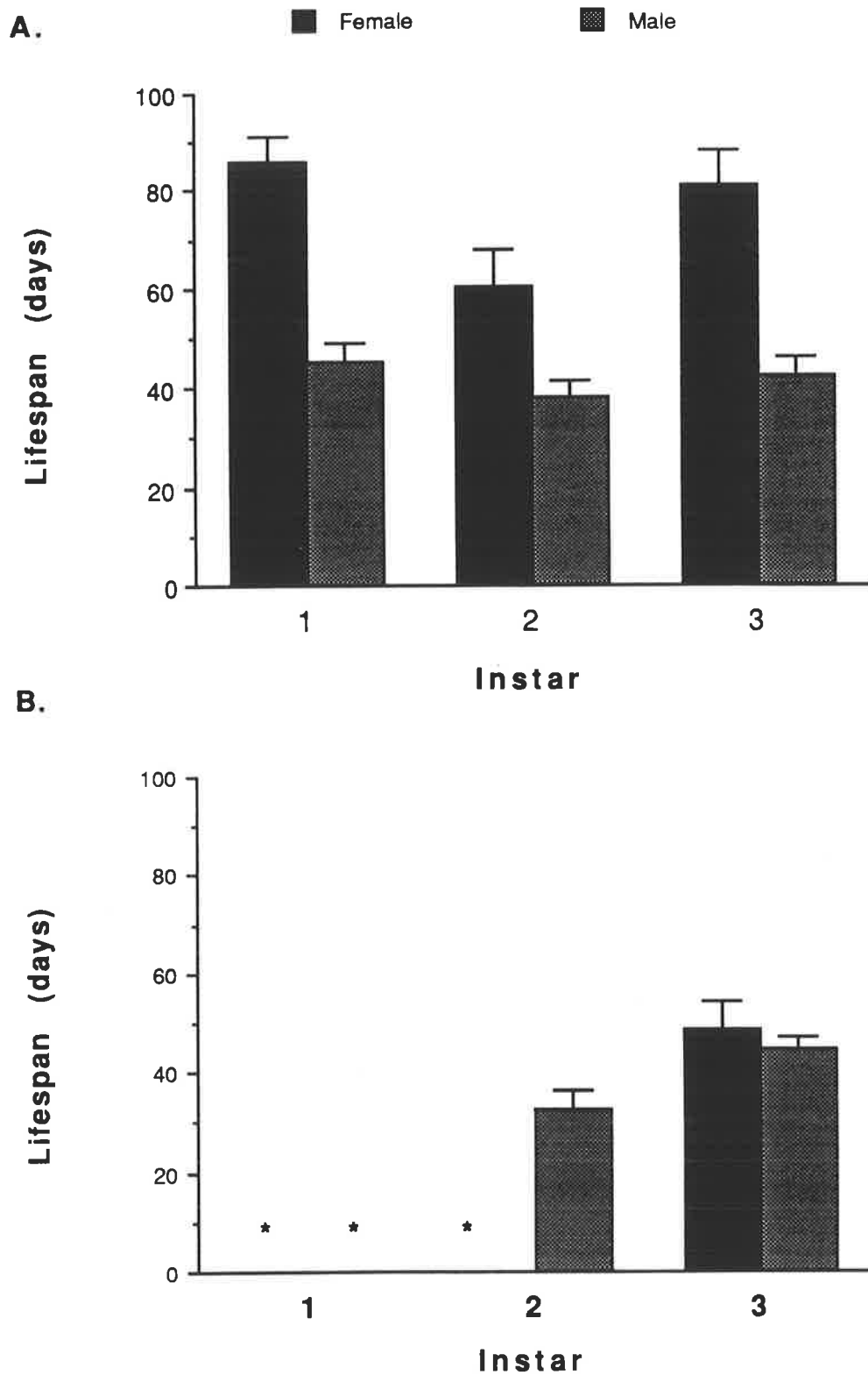
Figure 7.4. Longevity (mean ± standard error) for (A) *O. cinerariae* and (B) *H. varicornis* in relation to the instar of *L. brassicae* on which development began.

* Insufficient individuals emerged to investigate longevity of these classes.

H. varicornis had up to 3, but frequently no, mature oocytes upon emergence. Mature oocytes of *H. varicornis* were 0.311 ± 0.006 mm long, this being approximately twice as long as mature oocytes of *O. cinerariae* ($\bar{x}$ = 0.151 ± 0.001 mm).

The number of mature oocytes of newly emerged *O. cinerariae* did not vary significantly with the host instar on which development began (Table 7.3). However, egg load at emergence did show a positive linear relationship with female size (Figure 7.5) For *H. varicornis*, females developing on 3rd instars emerged with more mature oocytes than females developing on 2nd instar hosts (P < 0.001 ; Table 7.3). There was no relationship between the size of *H. varicornis* females and the number of eggs with which they emerged.

7.4.5.   Mating Success

7.4.5.1. *O. cinerariae*

In *O. cinerariae*, courtship began by males detecting a female within a distance of approximately one centimetre. A male then began prolonged bouts of wing fanning while moving towards the female. This movement was not direct, but commonly involved frequent turning, creating a zig-zag path. When close to the female, the male would mount quickly from the posterior, grasping the female's thorax with his fore-legs. The male then curved his abdomen around to make contact with the female's genitalia. Copulating males alternately dropped then raised their antennae in a rhythmic fashion. Female's exhibited no obvious sign of receptivity other than not moving away as the male approached. Mating ceased when the female began moving during copulation, dislodging the male. Occasionally males commenced wing fanning without females being nearby, making frequent turns and moving in directions other than towards the distant female. This was presumed to indicate the male's detection of remnant sex pheromone and was not recorded as courtship or an encounter.

Table 7.3. Mean number of mature oocytes upon emergence (± standard error) for *O. cinerariae* and *H. varicornis* in relation to the *L. brassicae* instar on which development began.

Species	Instar on which development began		
	1	**2**	**3**
O. cinerariae	9.07 ± 1.11[a]	10.95 ± 0.69[a]	11.15 ± 1.02[a]
H. varicornis	-	0.10 ± 0.10[a]	1.05 ± 0.22[b]

For each species, means followed by the same letter are not significantly different (P > 0.05).
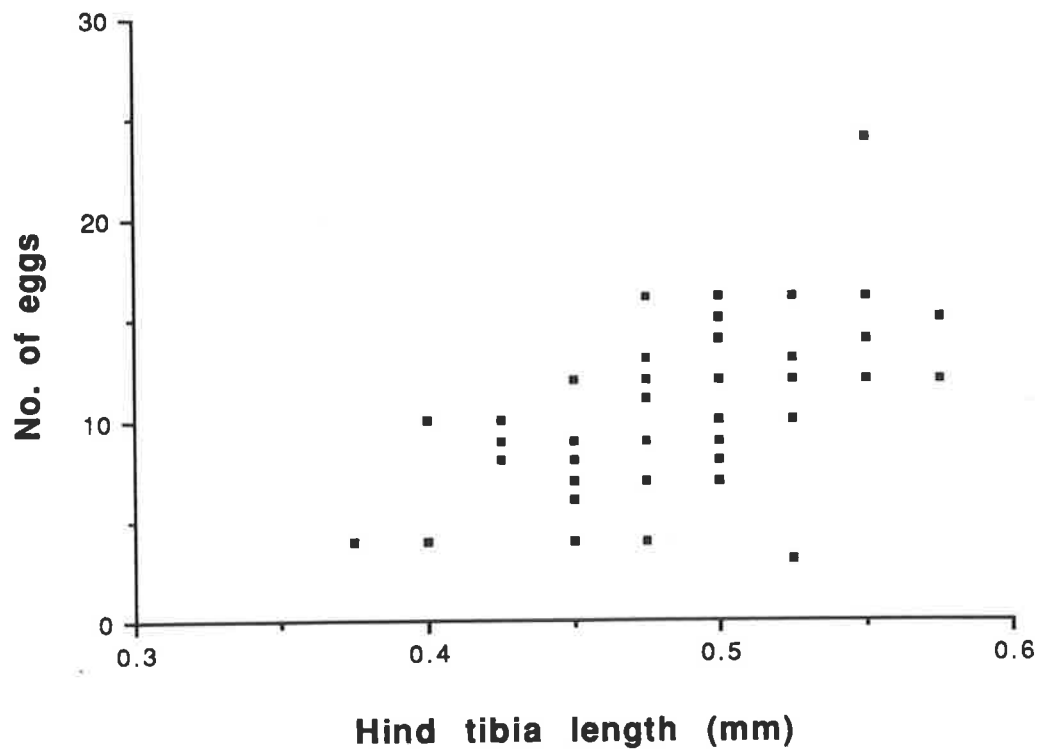
Figure 7.5. Number of mature eggs in relation to body size (hind tibia length) of *O. cinerariae.*

Pearson's correlation coefficient, r = 0.603 , (P < 0.01).

The chance of a male *O. cinerariae* successfully mating was dependent upon the host instar in which it's development began (P < 0.05; Table 7.4). Males developing from 2nd instar hosts were more likely to mate than those developing from 1st instar hosts (P < 0.05). However, the chance of mating did not differ significantly between males from 3rd instar and those from 1st or 2nd instar hosts. The mating success rate of *O. cinerariae* males on their first encounter with a female was also dependent on host instar (P < 0.05; Table 7.4). More males from 2nd instars mated successfully on their first encounter with a female than males from 1st or 3rd host instars (P < 0.05), while the latter two stages did not differ significantly. For males which mated on their first encounter with a female, the time to mate was influenced by the interaction between the host instar on which development began and male size (P < 0.05). There was no relationship between size and time to mate for males from 1st or 3rd instar hosts or when males from all instars were considered, but males from 2nd instars showed a negative relationship between size and time to mate (Figure 7.6). Size of *O. cinerariae* females offered to males did not vary significantly throughout these mating experiments.

The host instar in which development began did not influence the chance of male *O. cinerariae* encountering females throughout these experiments (Table 7.4). The time prior to the first encounter or the number of encounters prior to mating were not influenced by the host instar from which the males development began or the size of the *O. cinerariae* males. Accordingly, the time to the first encounter did not influence whether a male successfully mated.

7.4.5.2. *H. varicornis*

Courtship by *H. varicornis* was initiated when a male detected a female within a distance of approximately one centimetre. The male then exhibited short bursts of wing fanning while darting towards the female. He then mounted her

Table 7.4. Results of mating success experiment for *O. cinerariae*.

Variable	Host Instar In Which Male Development Began		
	1	**2**	**3**
Number of males tested	40	40	40
No. males which encountered females	18	26	28
Mean time to first encounter for all males (minutes)	1.23 ± 0.32	1.75 ± 0.32	2.18± 0.40
Mean no. encounters prior to mate	1.92 ± 0.42	1.13 ± 0.07	1.75 ± 0.29
No. males which mated (total) *	13	23	20
Mean time from first encounter to mate for all males (seconds)	29.43 ± 13.48	28.55 ± 13.49	38.45 ± 16.51
No. males which mated on the first encounter *	8	20	13
Mean time from first encounter to mate for males which mated on first encounter (seconds)	12.25 ± 2.94	13.80 ± 1.40	20.08 ± 3.21

*   Differences between host instars were statistically significant ($P < 0.05$).
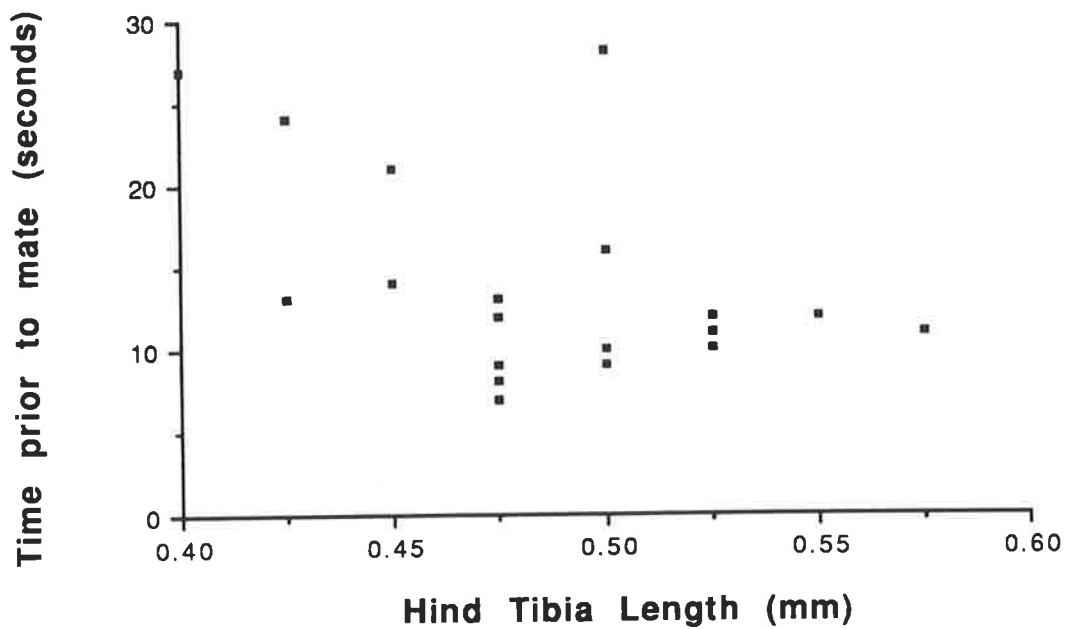
Figure 7.6. Relationship between body size (hind tibia length) and the time prior to

mating for male *O. cinerariae* which began development in 2nd instar *L. brassicae*

larvae and successfully courted females on their 1st encounter.

Pearson's correlation coefficient, r = -0.468, (P < 0.05).

posteriorly and commenced a "rocking back and forth" motion, maintaining contact between the female's antennae and his own while keeping his body horizontal. This phase lasted from several seconds up to several minutes. The female signalled receptivity by lowering and spreading her wings while raising her abdomen. The male responded by moving back along the female and curving his abdomen to bring their genitalia into contact. Females halted copulation by moving and dislodging the male.

The host instar on which *H. varicornis* developed influenced the chance of a male mating ($P < 0.05$; Table 7.5). Males from 3rd instars mated more frequently than males from 2nd instar hosts. The time prior to a male's first encounter was influenced by the host instar from which they developed ($P < 0.01$; Table 7.5), and the interaction between host instar and whether they successfully mated ($P < 0.01$), but not by their size. Males from 2nd instars which did not mate took longer to encounter a female than those from 2nd instars that did mate, or all males from 3rd instars that had an encounter ($P < 0.05$).

Male *H. varicornis* from 2nd and 3rd instar hosts had similar chances of encountering a female. The success rate of mating on the first encounter with a female was not influenced by host instar (Table 7.5). The time to mate or the number of encounters prior to successful mating were not influenced by either the host instar from which males developed or male size. No significant difference was found between the size of males that successfully mated and those that did not from each host instar. Unfortunately, *H. varicornis* females offered to males from 2nd instars were smaller than those offered to 3rd instar males ($P < 0.05$) but the mean size of females encountered or mated did not differ significantly between males from the two host instars.

Table 7.5. Results of mating success experiments for *H. varicornis.*

Variable	Host instar On Which Males Developed	
	2	**3**
Number of males tested	40	40
No. males which encountered females	32	34
Mean time to first encounter for all males (minutes) *	3.97 ± 0.56	2.32 ± 0.35
Mean time to first encounter for males which mated (minutes)	2.73 ± 0.69	2.65 ± 0.40
Mean time to first encounter for males which did not mate (minutes)	5.06 ± 0.79	1.25 ± 0.56
Mean no. encounters prior to mate	1.40 ± 0.16	1.50 ± 0.19
No. males which mated *	15	26
Mean time from first encounter to mate for all males (seconds)	46.40 ± 16.10	85.04 ± 26.68
No. males which mated on the first encounter	10	18
Mean time from first encounter to mate for males which mated on the first encounter (seconds)	19.90 ± 2.93	28.56 ± 6.59

* Differences between host instars were statistically significant ($P < 0.05$).

7.5.   DISCUSSION

The existence of differential increments in the fitness of progeny developing from different host sizes/ages/stages and of different sexes is an underlying assumption of the host-size sex ratio model described by Charnov *et al.* (1981) and Charnov (1982).   Females are assumed to gain more in terms of fitness than males when developing on larger, older or later stage hosts (Sandlan 1979; Charnov *et al.* 1981; Avilla and Albajes 1984; van den Assem *et al.* 1984; Werren 1984; Opp and Luck 1986; Simbolotti *et al.* 1987; de Jong and van Alphen 1989; King 1989). Consequently, the host-size sex ratio model predicts greater production of female progeny on those hosts, with male production concentrated on smaller hosts.   Such a strategy was observed for *H. varicornis* (Chapter Six) but not for *O. cinerariae*. Estimated relative fitness of progeny of each sex from each host instar provides an explanation of the different strategies adopted by these two parasitoid species.

Female progeny of *H. varicornis* had greatest relative fitness when developing on 3rd instar hosts.   They were larger, lived longer and carried approximately 10 times more mature eggs at emergence than females from 2nd instars. As few female progeny of *H. varicornis* emerged from 2nd instars, estimating relative fitness of these individuals was not possible.   However, it was clear that these individuals were less fit than females which developed from 3rd instar hosts.   Male progeny developing from 3rd instar hosts also had a fitness advantage, as they were larger, lived longer and were approximately twice as successful at mating compared with males from 2nd instar hosts.   Comparison of the relative fitness of the sexes indicated female *H. varicornis* progeny gained more by developing on a 3rd instar host than male offspring.   Therefore, the sex allocation strategy of *H. varicornis* would tend to maximize fitness, providing an example of a host-stage dependent strategy as predicted by Charnov *et al.* (1981).

Different host stages varied markedly in the quantity of resource they represent for developing progeny of the idiobiont, *H. varicornis*. Third instar hosts provided a larger resource, allowing *H. varicornis* to attain a greater size. The large variability in the recorded size of *H. varicornis* indicated the importance of host size on progeny development (Figure 7.3). Increases in other fitness components with the host instar attacked, such as longevity and egg load, may reflect greater nutrient reserves in the fat body or other storage tissues of progeny developing from 3rd instars. These reserves could then be mobilized to assist in the maturation of eggs or prolonging life in situations with limited food availability. How the host instar on which development began influenced mating success is not easily understood. An aspect of courtship which appears related to mating success is the speed at which males can locate females. This behaviour was not dependent upon size and probably represented some host instar effect upon male ability to detect cues emitted by females. There was a trend for males from 3rd instar hosts to have a longer courtship from first encounter to successful mating. The longest portion of the courtship procedure was the male "rocking" phase prior to the female signalling receptivity. Males from 3rd instar hosts may have been able to effectively stimulate females longer, thereby successfully mating with those females that would have rejected other males.

The components of *O. cinerariae* fitness were often not directly related to host instar in which development began. Developmental rates of *O. cinerariae* were influenced by temperature, gender and the host instar attacked. As other fitness components were measured for progeny that developed at 25°C, developmental rate at this temperature is most relevant to this discussion. There appeared to be trade-offs between fitness components such that a low level of fitness indicated by one component was compensated for by an improved performance of another component. Progeny which began development in each host stage derived the greatest benefit for at least one of the fitness components measured. For example, progeny which began development in 1st instars lived the longest but had the lowest

mating success. Those which began development in 2nd instars had the fastest developmental rate and, for males, the best chance of obtaining a mate, but they also had the shortest lifespan. The largest progeny were those that began development in 3rd instars but these also had the slowest developmental rate. Size was the only fitness component for *O. cinerariae* which showed a direct relationship with host stage. This suggests that older host stages do represent a larger quantity of resource for some koinobiont species, contrary to the hypothesis of Waage (1982a). Solitary koinobiont species frequently reduce the growth rate of their hosts while having little influence on developmental time (Slansky and Scriber 1985; Slansky 1986). While *O. cinerariae* was shown to influence developmental time of hosts, the growth rate was not investigated. However, if a reduction in growth rate for parasitized hosts did occur, then 1st instar hosts parasitized by *O. cinerariae* would achieve a smaller final size than later parasitized hosts, consequently influencing the size of the emerging parasitoid.

Only two fitness components showed differential gains between the sexes with the host instar attacked. Female progeny from all host instars lived longer than males, but males developed faster at 25°C. The instar of *L. brassicae* attacked had differential effects upon the various fitness components measured. However, the contribution of each of these components to the overall fitness of an individual is uncertain and, consequently, the relationship between the host instar attacked and *O. cinerariae* fitness remains unclear. The lack of clear fitness gains to one sex in a particular host instar may explain why sex allocation by *O. cinerariae* was similar in all host stages. *O. cinerariae* appears not to comply with the assumption of Charnov *et al.* (1981) that females gain more in terms of fitness by developing in larger hosts. Therefore, it is not surprising that its sex allocation strategy was generally not host-stage dependent.

The only evidence of sex ratio manipulation by *O. cinerariae* in response to host instar was the shift towards male production on 2nd instar hosts in the choice test between 1st and 2nd instar *L. brassicae* larvae (Chapter Six). The measures of fitness indicated that *O. cinerariae* males developing from 2nd instars had a reproductive advantage over those which began development in 1st instars. The observed shift in sex allocation may reflect a strategy of maximizing the fitness realised through male progeny. If so, this is a novel observation requiring closer examination. The influence of the host instar attacked on the subsequent mating ability of male progeny is difficult to understand. The characteristics of a 2nd instar host that provide for an increase in mating ability may include its nutritional or physiological condition. However, the understanding of the interaction between these aspects of hosts and their parasitoids is poor (Strand 1986). Closer examination of courtship may identify the step(s) at which males from 2nd instar hosts gain an advantage over other males. Size was shown to be important in influencing the length of courtship for males from 2nd instars (Figure 7.6), and the absence of similar correlations for other males may be due to smaller sample sizes. It is interesting to note that the faster development of progeny in 2nd instar hosts would further strengthen the male reproductive fitness by giving them a temporal advantage over males from other instars (Wilson 1961; King 1987).

The evaluation of fitness for the progeny of each species has implications for the behaviour and phenology of these parasitoids in the field. *H. varicornis* required a higher temperature to commence development (Table 7.2) than either *O. cinerariae* or *L. brassicae* and, consequently, is most common during the warmer parts of the year (Figure 3.1; Appendix Two). The developmental time of *H. varicornis* was also shorter than that required by *L. brassicae*, except at high temperatures (25°C). These features may partly explain its abundance within the parasitoid complex of *L. brassicae*, particularly during summer. In contrast, *O. cinerariae* required a similar temperature to *L. brassicae* for development to occur, although this fluctuated depending on the host instar that was attacked (Table 7.2).

Developmental rates of *O. cinerariae* were slower than those for *L. brassicae* at low (15°C) and high (25°C) temperatures, but faster at moderate temperatures (20°C). These features may contribute to *O. cinerariae* abundance primarily during the milder parts of the year (Figure 3.1; Appendix Two) and its much lower abundance than *H. varicornis*.

For both parasitoid species, the longevity estimated by the laboratory experiments probably overestimates the actual lifespan of these wasps in the field. Furthermore, longevity was only investigated at 20°C yet temperature may have had a differential effect on the longevity of progeny developing from each host instar. The longer lifespan recorded for females of both species would provide greater opportunity to distribute eggs and, thus, realize their reproductive potential. However, the capacity of males to find mates may be limited by their shorter lifespan.

Both *O. cinerariae* and *H. varicornis* carried few mature eggs upon emergence, indicating they are synovigenic species. Fewer eggs were found in the newly emerged *H. varicornis* but these eggs were about twice as long as those of *O. cinerariae*, representing over double the volume. As both parasitoids have a similar body size, the number of mature eggs that *O. cinerariae* could carry was greater than that of *H. varicornis* . Female *O. cinerariae* were observed to carry up to 24 eggs which is sufficient to prevent egg limitation given the average level of reproduction of this species (Chapters Four and Five). However, as *H. varicornis* was only observed to carry up to 3 eggs, it is possible that the low levels of reproduction found for this species (Chapters Four and Five) represents limitation of oviposition by the rate of egg maturation. The rate of egg maturation following emergence for both species is unknown but it is probably influenced by the rate of host feeding (Chapters Three and Four). This behaviour has been shown to provide nutrients essential for egg maturation (De Bach 1943; Bartlett 1964).

While numerous features of courtship were measured, many of these (i.e. time to first encounter, number of encounters prior to mating, time to mate) were probably influenced by the design of the cage used. Field (1990) indicated the importance of appropriate cage design in studying parasitoid mating behaviour. The most relevant measure to the field situation is the number of males that successfully obtained a mate on their first encounter with a female. In the field, a female which is not persuaded to mate on the first encounter may disperse and never be relocated. Consequently, those males that are able to successfully court females on the first encounter would have a greater fitness.

It must be remembered that other factors may influence mating success and, therefore, male fitness. In these experiments, the age, size and mating status of the female were controlled. However, females in the field vary in age and size, and may already be mated. Previously-mated females of some parasitoid species have been shown never or rarely to mate again (Vinson 1972; Abdelrahman 1974a; van den Assem 1986). Furthermore, aged virgin females may be less likely to mate than younger wasps (Flanders 1943; Rao and de Bach 1969) The ability of males to assess the receptivity of a female could reduce wasted courtship effort (van den Assem 1986). In addition, males probably vary in their ability to locate mates. These attributes could be further indicators of male fitness.

O. cinerariae and *H. varicornis* appear to have different selection pressures influencing their host-stage utilization. The fitness of *H. varicornis* varies in relation to the host instar attacked and, accordingly, this species exhibits host-stage dependent progeny and sex allocation. In contrast, the fitness of *O. cinerariae* varies less with the host stage attacked. This parasitoid displayed host-stage dependent levels of reproduction, but sex allocation was only influenced when a range of host stages was available (Chapter Six). Therefore, these parasitoids provide an interesting example of two species adopting different strategies to utilize the same host.

CHAPTER EIGHT

HOST UTILIZATION BY *Hemiptarsenus varicornis* AND *Opius cinerariae* IN RELATION TO RECENT THEORY

8.1. INTRODUCTION

Over the last three decades the technique of mathematical modelling has been applied to the question of parasitoid oviposition behaviour with increasing frequency. These models assume certain fitness relationships between hosts and parasitoids, spatial structures of their populations, and physiological or behavioural states of the parasitoid. In doing so, various strategies of progeny and sex allocation have been shown to be adaptive (Hamilton 1967; Charnov *et al.* 1981; Charnov 1979, 1982; Werren 1983, 1984; Iwasa *et al.* 1984; Green 1982; Charnov and Stephens 1988; Mangel 1989). This approach can lead to a better understanding of the selection pressures exerted upon parasitoid behaviour in natural systems as well as predict responses which are otherwise difficult to conceptualize.

A certain level of generality is inherent in the modelling process. In order to understand the natural systems being investigated and generate predictions about them, some simplification of these complex systems is essential. The trade-off for this simplification is the generality of models dealing with host utilization by parasitoids (Waage 1986). Frequently, the predictions of these models overlap so that ovipositional behaviour may be explained by two or more models. This generality is unfortunate as it reduces the ability to directly test these models. One way in which to overcome this problem is to combine models, producing more precise predictions.

This approach has been adopted by Green (1982) and Werren (1984). However, further understanding can be gained by using natural examples, rather than simulations, to test these models. At present, there is an imbalance between the number of models which have been generated to describe parasitoid oviposition and actual studies testing the assumptions and predictions of these models (Orzack and Parker 1986; Waage 1986). My study partially redresses this imbalance by investigating progeny and sex allocation, and host feeding by *Hemiptarsenus varicornis* (Girault) and *Opius cinerariae* Fischer.

Few studies have compared the strategies adopted by two or more parasitoid species attacking the same host (van Alphen 1980; Luck *et al.* 1982; Luck and Podoler 1985; Opp and Luck 1986; Allen 1989). Here I do not attempt to generate new models based upon the oviposition behaviour of these two species, but to test the validity and conclusions of existing models. First I provide a summary of theoretical models which consider host utilization by parasitoids in the framework of natural selection. A brief summary of the oviposition behaviours of *H. varicornis* and *O. cinerariae* and the factors found to influence them are provided in the following section. This information is then used to evaluate the various host utilization models. Approaches to understanding parasitoid oviposition decisions other than that of natural selection (Charnov and Skinner 1985) are discussed next. Finally, general conclusions are presented, relating my laboratory studies to the abundance of *H. varicornis* and *O. cinerariae* on natural host populations. Avenues for future research are suggested.

8.2. SUMMARY OF RELEVANT MODELS

8.2.1. Which host types to attack?

Optimal foraging theory was used by Charnov and Stephens (1988) to develop several predictions about host selection by solitary parasitoids. Their model dealt specifically with synovigenic species (i.e. those parasitoids which continue to mature eggs throughout adult life) that randomly encountered hosts and where egg production was negatively related to mortality. It was assumed that a parasitoid should behave in a way that maximizes its fitness over time and that its behaviour is fixed over this interval (Stephens and Krebs 1986; Charnov and Stephens 1988; Mangel 1989). Based on these assumptions, the theory predicted that (i) host types are either always accepted or always rejected; (ii) hosts are ranked in terms of the fitness gained by attacking them; and (iii) acceptance of a given host type did not depend upon the rate at which it was encountered (Charnov and Stephens 1988).

Mangel (1989) referred to the approach of Charnov and Stephens (1988) as rate-maximization theory and criticized their assumptions that parasitoid behaviour was static and independent of the state of the parasitoid. He used a state-variable model to investigate the same problem as Charnov and Stephens (1988). The underlying assumptions were similar except that parasitoid behaviour was considered to be dynamic and dependent upon the state of the parasitoid. This model predicted that acceptance of host types will be influenced by the state of the parasitoid. For instance, parasitoids with small egg holding capacities, low egg production rates or long lifespans were more likely to act as optimal foragers, attacking a small set of optimal host types. In addition, it showed that the level of similarity between host types in terms of the fitness they confer will influence the utilization of those hosts. However, in accordance with rate-maximization theory, the rate of encountering a host type was shown to have little effect upon the acceptance of that host type.

Iwasa *et al.* (1984) used a dynamic analytical model to investigate the influence of egg load and mortality upon oviposition behaviour of solitary parasitoids. However, their model dealt with proovigenic parasitoids (i.e. those that emerge with their full complement of mature eggs) and, consequently, was not considered relevant to understanding oviposition by synovigenic species, such as *H. varicornis* and *O. cinerariae*.

8.2.2.   Whether to oviposit or host feed?

Jervis and Kidd (1986) developed models that incorporated host feeding by parasitoids. Their approach assumed that the only important constraints were energetic ones and that a parasitoid's fitness could be measured as the number of eggs laid during its lifetime. The proportion of hosts used for oviposition was predicted to drop as the rate of host encounters decreased. As the amount of energy derived from feeding on a single host increased or the costs incurred in egg development and oviposition decreased, the proportion of encounters devoted to oviposition was predicted to increase. Conversely, as the costs of searching or maintaining the adult parasitoid increased, the proportion of hosts used for oviposition was expected to decrease.

8.2.3.   Which sex to allocate?

The local mate competition model (LMC), first described by Hamilton (1967), predicted that parasitoid populations with a high degree of sib-mating should have a female-biased sex ratio. This bias should decrease as the chance of sib-mating within the population decreased (i.e. the foundress number within the patch increased). Assumptions of this model were: (i) hosts have a clumped distribution or parasitoids are gregarious, such that localized mating groups are present; (ii) males do not disperse upon emergence but remain within a patch or near their host to inseminate female siblings; (iii) males are able to mate repeatedly; and (iv) synchronous

development of broods; and (v) females are able to identify hosts or host patches already parasitized and modify sex allocation accordingly.

Nunney and Luck (1988) investigated the effects of relaxing several of the assumptions of the LMC model. They showed that when male dispersal occurs, the chance of sib-mating is reduced and the optimum female-biased sex ratio (as predicted by LMC) is lowered. If broods are assumed to develop asynchronously, then males from later-arriving females will be at a competitive disadvantage to males from early-arriving females. Consequently, later-arriving females should produce fewer male progeny, creating a female bias greater than predicted by Hamilton (1967). These effects were explained in terms of local parental control (LPC) acting through sons (LMC) or through daughters (sib-mating).

Charnov (1979) and Charnov *et al.* (1981) predicted that parasitoids would manipulate sex ratio in response to host size. If sex A gained more in relative fitness than sex B when oviposited in a large host, then sex A should be allocated more frequently to large hosts. It is generally assumed that the increase in relative fitness is greater for females than males when developing on larger hosts. The host-size model predicted more female progeny in large hosts, while overall sex ratios would tend to be male-biased (Bull 1981; Waage and Godfray 1985). This model assumes that female parasitoids are able to distinguish between different host sizes and that the parasitoid population was outbreeding. It was presumed that any host character correlated with size, such as host age or instar, would similarly influence parasitoid sex ratio as long as the same fitness relationships existed (Charnov *et al.* 1981).

The LMC and host-size (quality) models described above were combined into a single model by Werren (1984). Assuming that females derive a greater fitness benefit than males by developing from large hosts, the model predicted deviation from Hamiltonian sex ratios (i.e. the sex ratio dictated by LMC and

inbreeding) under certain circumstances. When a single host size was parasitized, the model predicted production of a Hamiltonian sex ratio. Any deviations from this sex ratio were predicted to be slight when small hosts comprised either a low or high proportion of total hosts parasitized. However, when an intermediate proportion of small hosts was parasitized, sex ratio was predicted to increase from Hamiltonian levels. The extent of this increase was shown to be influenced by the relative fitness of daughters from small hosts. As the relative fitness of small daughters declined, the sex ratio was expected to increase.

Other models of progeny and sex allocation by parasitoids have dealt primarily with gregarious species and, hence, are of little or no relevance to the behaviour of *H. varicornis* and *O. cinerariae*. These include those models pertaining to clutch size (Charnov and Skinner 1984, 1985; Skinner 1985; Godfray 1987) or the role of sexual assymetries in sex allocation (Godfray 1986).

At present, there has been little overlap between theory dealing with progeny or sex allocation for solitary parasitoids, with the exception of Green (1982). Consequently, these two processes will be discussed separately in the following sections.

8.3. SUMMARY OF FACTORS INFLUENCING HOST UTILIZATION BY *H. varicornis* AND *O. cinerariae*

As a result of the laboratory experiments discussed in the preceding chapters, several aspects of the biology of *H. varicornis* and *O. cinerariae* were observed which are pertinent to a discussion of their progeny and sex allocation. Following is a short summary of these observations.

8.3.1. *H. varicornis*

1. Most progeny were allocated to 3rd instar hosts with fewer to 2nd instars. First instars were never successfully parasitized by *H. varicornis* .

2. Sex ratios were female-biased or unbiased on 3rd instar hosts while strongly male-biased on 2nd instars.

3. Fitness was strongly dependent upon the host stage attacked, with progeny developing on 3rd instar hosts having the greatest relative fitness (Appendix Five). Presumably, there would have been little difference in the fitness gained by attacking a late 2nd instar or an early 3rd instar host, but the average contribution to fitness by each stage differed markedly.

4. Both sexes gained more in terms of relative fitness by developing on larger hosts. However, this increment was greatest for female progeny, as they were larger and lived longer. Furthermore, at emergence females from 3rd instar hosts had 10 times the egg load of those from 2nd instars, while males from 3rd instars only mated twice as frequently as their counterparts from 2nd instars.

5. Females carried few mature eggs at emergence. While egg limitation was not clearly demonstrated, *H. varicornis* certainly has a lower realised (and probably potential) fecundity than *O. cinerariae*.

6. Longevity in laboratory experiments with unlimited food indicated a female lifespan of around 40 to 50 days. Actively reproducing females frequently lived this long, indicating that reproduction had a minor effect upon longevity.

7. Greatest levels of destructive host feeding were observed on 3rd instar hosts.

8. Female age and experience influenced the oviposition behaviour of *H. varicornis*.

9. It is a polyphagous parasitoid with a geographic distribution spanning several continents.

8.3.2. *O. cinerariae*

1. Reproduction occurred on all host instars, with greatest levels occurring on 3rd and lowest on 1st instars.

2. Female-biased sex ratios were regularly produced on each host instar.

3. Fitness was not strongly dependent upon the host stage in which development began. While body size increased directly with the host instar attacked, other fitness components did not show this relationship (Appendix Five). At present, the contribution of each fitness component to overall fitness is uncertain. However, it appears that trade-offs between fitness components occur when development begins in different host stages and that relative fitness varies less for *O. cinerariae* than *H. varicornis*.

4. It is not possible to determine which sex gains the most by developing from larger hosts. The fitness of females varied little as a result of beginning development in different host stages. Male fitness was more variable with those from 2nd instars displaying greatest mating ability.

5. *O. cinerariae* carried more mature eggs at emergence than *H. varicornis* and also had a greater realised (and probably potential) fecundity.

6. Mean longevity of females from 3rd instar hosts was found to be over 80 days in a laboratory experiment with unlimited food. Actively reproducing *O. cinerariae* females lived for shorter periods, suggesting reproduction had a detrimental effect upon female lifespan.

7. Greatest levels of destructive host feeding were observed on 1st and 3rd instar hosts.

8. Female age and experience influenced progeny and sex allocation by *O. cinerariae*.

9. It is polyphagous species and is probably less widely distributed than *H. varicornis*.

8.4. TESTS OF PREDICTIONS OF MODELS

8.4.1. Progeny Allocation

The results obtained for *H. varicornis* and *O. cinerariae* support many of the predictions of progeny allocation models. *H. varicornis* appears to function as a rate-maximizer more so than *O. cinerariae*. The former species possesses many of the characteristics predicted to be associated with rate-maximization, such as small egg holding capacity, low rate of egg production and long lifespan. Consequently, its reproductive capacity was more limited than *O. cinerariae* and the most profitable strategy of progeny allocation would be to selectively deposit its few eggs in larger hosts. Fitness measures clearly indicated that 3rd instar hosts provided for greatest *H. varicornis* fitness and this stage was most frequently used for oviposition. Lower levels of reproduction occurred on 2nd instar hosts, which also afforded lower fitness. First instar hosts may have been associated with extremely low fitness and, apparently, were not utilized as hosts. This would require discrimination against these hosts. Acceptance rates following encounters with each host stage were not determined and any discussion of discrimination between host instars by *H. varicornis* is speculative. It is also possible that encounter rates influenced progeny allocation by *H. varicornis*. Although theory predicts that the rate of encounter with a given host type will not influence acceptance of that host type, this does not preclude any involvement of this process in determining progeny allocation. If acceptance rate upon encounter was the same for each host instar, then more progeny would be allocated to the most frequently encountered stage.

O. cinerariae attacks all host stages and, therefore, does not appear to function as a rate-maximizer. In comparison to *H. varicornis*, it carries more eggs, has a shorter reproductive life (though not "potential" lifespan) and greater rate of egg production. These features conform with those predicted by Charnov and Stephens (1988) and Mangel (1989) for species which do not act as rate-maximizers. Analysis

of the relative fitness of *O. cinerariae* revealed little variation in the intrinsic value of each host instar for development. Mangel (1989) showed that when rankings of hosts were very close, as they appear to be here, rate-maximization may fail as a predictor of oviposition decisions. If each host stage confers a similar relative fitness, then selective allocation of progeny to these host stages would not further maximize fitness. Consequently, any host stage may be suitable and accepted as such. Under these circumstances, reproductive potential would be the most limiting factor. Lifespan rather than egg load appeared to limit the reproductive potential of *O. cinerariae*. During their lifespan, most oviposition occurred in 3rd instars. As this host stage was bigger and had mined a larger area of leaf surface (Chapter Two), it would be expected that this host stage was also the most frequently encountered. Therefore, progeny allocation by *O. cinerariae* may be explained by differential encounter rates with each host instar during the limited lifespan of the adult female.

These results suggest that the relationship between host instar and resulting fitness is of great importance in determining a parasitoid's strategy of progeny allocation. Most models assume that different host types will vary with regards to the contribution they make towards fitness. When this is true, parasitoids can be shown to attack a restricted, but approaching, optimal set of host types. However, if such variation is not present or only slight, then the set of host types attacked will be less restricted and may also prove optimal.

8.4.2. Host Feeding

No-choice experiments revealed the greatest levels of destructive host feeding by *H. varicornis* and *O. cinerariae* on 3rd instars, which also proved to be the stage on which most oviposition occurred. The model of Jervis and Kidd (1986) predicted that host feeding should increase as the costs incurred by egg development or oviposition increased. Therefore, those parasitoids attacking 3rd instar hosts, having

the greatest rate of reproduction, would also have required more regular host feeding. However, several aspects of these data must be considered. Firstly, increases in destructive host feeding on 3rd instar hosts were only observed during no-choice experiments. This may be attributed to lower reproductive rates in choice experiments. Secondly, destructive host feeding on 1st instars by *O. cinerariae* was also high. However, host feeding on 1st instars may be over-represented as a result of not distinguishing between actual feeding and mechanical injury during oviposition. Finally, these experiments dealt with naive parasitoids only. Therefore, examination of host-feeding strategies by experienced females would be required to fully test the predictions of Jervis and Kidd (1986).

8.4.3. Sex Allocation

Models of sex allocation assume the parasitoid population has a high degree of inbreeding (LMC; Hamilton 1967), females derive greater fitness from large hosts than do males (host-size; Charnov *et al.* 1981), or both (Werren 1984). The extent of inbreeding in populations of *H. varicornis* and *O. cinerariae* is unknown. Their host, *L. brassicae*, is patchily distributed, both on a large scale (host plant patches) and a small scale (between leaves). However, establishment of localized mating populations with frequent sib-mating for solitary parasitoids would also require the production of numerous progeny within a patch and a low foundress number. Both these features are more likely to be found in *O. cinerariae* populations than in *H. varicornis*. *O. cinerariae* is more fecund and it has a lower population density in the field. Hence, fewer foundresses are likely to locate a patch and, once in a patch, more offspring are likely to be produced.

Variation between the sexes in terms of the fitness derived from attacking large hosts is greater for *H. varicornis* than *O. cinerariae*. *H. varicornis* females clearly profited more by developing from larger hosts than their male

counterparts. Furthermore, females developing from 3rd instars had greater relative fitness than females from 2nd instars. In contrast, there appeared to be little variation in relative fitness between *O. cinerariae* of either sex from any host instar.

These trends are reflected in the sex allocation behaviour of these two species. *H. varicornis*, for which host size (stage) had a large influence upon fitness (particularly for females), displayed host-size dependent sex ratios as described by Charnov *et al.* (1981). There was no suggestion that LMC may be an important selection pressure for this species. Werren (1984) suggested Hamiltonian sex ratios would be produced when only one host size was attacked. No-choice experiments described in Chapters Four to Six presented only one host stage to parasitoids, yet *H. varicornis* never produced Hamiltonian sex ratios. Host stages represent a range of host sizes (see Chapter Two) and this may have prevented production of female-biased sex ratios. Alternatively, Hamiltonian sex ratios may not be adaptive for this species and, therefore, no selection for such a strategy may have occurred.

O. cinerariae, for which host size had little influence on fitness, did not display host-size dependent sex ratios. Instead, its sex allocation resembled that predicted by LMC models (Hamilton 1967). These were not strongly female-biased sex ratios even though foundress number on the experimental patches was one. This suggests a slight LMC effect, possibly indicating a weak selection pressure for female-biased sex ratios as a result of frequent male dispersal (Nunney and Luck 1988). Werren (1984) predicted that when an intermediate proportion of small hosts were attacked, an increase in male production would occur. In a choice test between 1st and 2nd instars, *O. cinerariae* displayed increased male production on 2nd instars. However, the LMC/host-size model described by Werren (1984) predicted greater male production on the small hosts (i.e. 1st instars). Relative fitness measures indicated males from 2nd instars had greater reproductive fitness than males from 1st instars, while females from these instars did not differ. Therefore, in this situation, the

assumption that females will derive greater fitness benefit than males by developing on the large hosts may not be valid and sex ratio manipulation appears to have maximized fitness realised through male offspring.

LMC requires sex ratio shifts to occur in relation to the chance of sib-mating, which in turn will be influenced by the number of hosts and parasitoids in a patch. My study did not investigate the response of parasitoid sex allocation to changes in host or parasitoid densities. Therefore, alternative hypotheses for female-biased sex ratios in *O. cinerariae* must be considered. Parasitoids must gather information about the host population in order to modify their sex allocation strategy. The costs involved in this information gathering phase may sometimes outweigh the benefits of producing an optimum sex ratio (Stephens 1987). Therefore, if the cost of assessing the host population is high, natural selection may favour those individuals which bypass this process and produce a sex ratio not influenced by the structure of the host population or the presence of previously parasitized hosts in a patch. When the cost of gathering information is low, such as in patches with high host densities or conspecific females, a shift in sex ratio may be adaptive. However, if the occurrence of patches which can be readily assessed is irregular in time and/or space, then consistent selection for shifts in sex allocation would not be present. Within the Adelaide region, *L. brassicae* populations were highly variable with respect to location, density and abundance. Thus, selection pressures on sex allocation may also have been variable. It is possible that the slight female-biased sex ratios produced by *O. cinerariae* represent a fixed strategy, giving greatest average fitness over all host and parasitoid densities.

As with progeny allocation, these results indicate that the relationship between the host instar at oviposition and fitness is an important determinant of sex allocation. For these two species, LMC, if present, may only have influenced sex ratio when relative fitness had a weak relationship with host instar. Estimating the

impact of the host instar upon fitness should be a high priority when attempting to understand oviposition decisions of parasitoids. Furthermore, how the various fitness components contribute to lifetime fitness of parasitoids of each sex and how the quality and/or quantity of host resources influences each component remain largely unanswered questions, despite their importance to understanding parasitoid behaviour.

8.5. OTHER APPROACHES TO UNDERSTANDING HOST UTILIZATION OF *H. varicornis* AND *O. cinerariae*

Charnov and Skinner (1985) suggested four complementary approaches to understand oviposition decisions of parasitoids. The previous sections of this chapter have dealt primarily with the natural selection or "ultimate" approach and the various theoretical models which have been generated to explain parasitoid behaviour. However, findings from the three other approaches remain to be discussed.

Experimental work did not concentrate on the proximate approach suggested by Charnov and Skinner (1985). Nevertheless, results suggested that some proximate behaviour may be important in determining which host stages are attacked, which stages are fed upon and how sexes are allocated. Host location behaviour, particularly encounter rates, are thought to be an important determinant of the level of parasitization each host stage suffers. The rate of reproduction may be implicated in the frequency of host feeding. Sperm depletion was likely to have influenced sex ratios produced by older females which were reproductively active.

Aspects of the development/ontogeny approach (Charnov and Skinner 1985) were dealt with by investigating differences in progeny and sex allocation by naive and experienced females. Both the age and experience of the

195

female were found to influence oviposition decisions. Fewer progeny were produced by females when naive than when experienced. This suggests conservation of reproductive potential until the range of hosts present has been assessed and optimal allocation of progeny can be executed. Naive females also tended to produce male-biased sex ratios but this was not statistically proven. Male-biased sex ratios produced by naive females may have resulted from females remaining functionally virgin for a short period following mating. Older females also frequently produced male-biased sex ratios but, as discussed above, this was probably a result of sperm depletion.

The final approach of Charnov and Skinner (1985) involved phylogenetic and evolutionary constraints upon host utilization of parasitoids. This approach was also not a main focus of my study. Investigation of the biology of *H. varicornis* and *O. cinerariae*, both by field studies and through the literature, indicated these species are polyphagous. Furthermore, *H. varicornis* is widely distributed. It is uncertain if these species have had a long association with *L. brassicae* or if this host is a recent addition to their range. Consequently, the behaviour observed throughout this study may not be a result of selection favouring optimal utilization of *L. brassicae*. If considerable evolution of these species has occurred on another host with a different relationship between fitness and host instar, then their ability to manipulate behaviour in relation to different instars of *L. brassicae* may be limited. Such considerations are difficult to test but should be borne in mind when discussing selection pressures acting upon *H. varicornis* and *O. cinerariae*.

8.6.   GENERAL CONCLUSIONS

Laboratory studies of progeny and sex allocation by *H. varicornis* and *O. cinerariae* reveal the latter to be the more fecund species. Generally female-biased sex ratios linked with this greater progeny production suggests *O. cinerariae*

should be the more abundant species on field populations of *L. brassicae*. However, this is not the case, with *H. varicornis* being the more abundant of the two species. *H. varicornis* has a shorter developmental time which may partly counteract the advantage *O. cinerariae* has in fecundity. Also, the idiobiont, *H. varicornis*, would be competitively superior to *O. cinerariae*, being the victor in any cases of multiparasitism. In addition, *H. varicornis* was found to utilize alternate hosts in the Adelaide region and, thus, may have a larger resident population. The abundance of these two parasitoids may also be affected by interactions with other members of the parasitoid complex.

The relevance of laboratory investigations to field situations has often been questioned. By their nature, laboratory studies frequently place parasitoids into conditions far removed from those usually encountered in the field. Therefore, the behaviour observed may reflect a response to these extreme circumstances rather than indicating the "normal" behaviour of a species . In my study, parasitoids were offered many more hosts than they could parasitize in the time available. Such high host densities only occasionally occurred in the field. Nevertheless, there was a striking similarity between the progeny and sex allocation of *H. varicornis* from field (Chapter Three) and laboratory studies (Chapters Four to Six). However, additional important factors which influence parasitoid behaviour were not investigated, primarily as a result of time constraints. These include host patch location, searching strategies, and ovipositional responses to variations in host density. Knowledge of such behaviour would be desirable for a more complete understanding of the oviposition decisions of these two parasitoids.

Despite these limitations, my study highlights several aspects of parasitoid biology which deserve further consideration. Comparison between young or old, naive and experienced, and mated or unmated females of both species revealed significant differences in the strategy of host utilization adopted. This clearly indicates

that the state of the parasitoid is vitally important in determining its ovipositional activity. In field situations, parasitoids of various ages, levels of experience and mating status are interacting with host populations. Therefore, models and experimental studies dealing with uniform groups of parasitoids give only a limited indication of their oviposition decisions.

The control of progeny and sex allocation by *H. varicornis* and *O. cinerariae* requires assessment of hosts on the basis of their suitability for progeny, and then male or female, development. How parasitoids of larval host stages assess hosts is largely unknown. Studies such as this clearly demonstrate behavioural responses on the basis of host characteristics. However, detailed examination of the behaviour leading up to acceptance or rejection of a host is required to ascertain what sort of cues are used to discriminate between hosts and how they are detected. Leafminers represent amenable organisms with which to test such questions as the interval between mine detection and actual oviposition is frequently protracted, containing numerous actions that may be involved in detection and assessment of hosts.

Host sizes/ages/stages were shown to have a differential impact on the various fitness components, such as developmental rate, size, longevity, egg load and mating success. Variation in the quantity of host resources were demonstrated for both idiobiont and koinobiont parasitoids. Moreover, hosts may vary greatly in quality independent of the quantity of resources. How this variation in host quality and/or quantity interacts with parasitoid development to influence variation in fitness has rarely been investigated. In particular, the underlying physiological processes remain to be elucidated. These may be relatively simple to determine for such features as parasitoid size or egg load. The relationship between host resources and the mating success of male progeny is likely to be more complex.

Knowledge of the host utilization behaviour of parasitoids has important consequences for the future biological control of insect pests. Predicting a parasitoid's performance on host populations will become more accurate once variability in parasitoid behaviour is understood. Host characteristics may be manipulated to achieve sex ratios that will increase the efficiency of culturing parasitoids for experimental and mass-release purposes. Furthermore, evaluating the role of host quality and/or quantity in determining fitness may lead to further improvements in parasitoid rearing methods. As well as these practical applications, host utilization by parasitoids has proven to be a profitable avenue for the exploration of the adaptive significance of behavioural decisions. Comprehensive studies of further parasitoid species and the interpretation of their host utilization strategies in terms of natural selection theory will enable the development of reproductive models which are more relevant to natural situations. Understanding how and why parasitoids adopt particular reproductive strategies will prove a challenging but rewarding area for future research.

APPENDIX ONE

METHODS FOR REARING *Liriomyza brassicae* IN THE LABORATORY

The methods used to rear *L. brassicae* evolved in conjunction with experiments revealing the life history, biology and environmental requirements of this leafminer (Chapter Two). Various rearing methods and associated activities are outlined and the experiments which used the emerging flies noted in this Appendix. All rearing of *L. brassicae* took place at 25(±2)°C and 12L:12D photoperiod unless otherwise stated.

A1.1. METHOD ONE

A1.1.1. Rearing

Initial rearing methods involved placing mated female flies in a lantern globe (Maxbilt Trading Co. Lantern Glass 285, approximately 1 litre capacity) which had been inverted over a *Brassica napus* (rape) seedling in a 100 mm pot. Fine mesh was placed over the top of the globe and held in place with a rubber band (Figure A1.1). Plants were changed daily and flies replaced when needed. Infested plants were transferred to a large, cylindrical plastic container (Shannon Designs, Australia, 260 x 150 mm, 4 litre capacity) which had four circular, mesh covered holes (diameter 60 mm) in the side for aeration. Observations were made regularly until 3rd instar mines were present. Leaves with mines were then excised and transferred to large plastic petri dishes (Kayline, diameter 150 mm) containing moistened filter paper (Whatman no. 1, diameter 12.5 mm). Emerged larvae and pupae were collected daily and transferred to glass or plastic vials. Adult flies which emerged were removed from these vials, separated into sexes and transferred to cylindrical plastic containers (100 x 40 mm) with honey smeared on the side and small holes punctured in the lid to
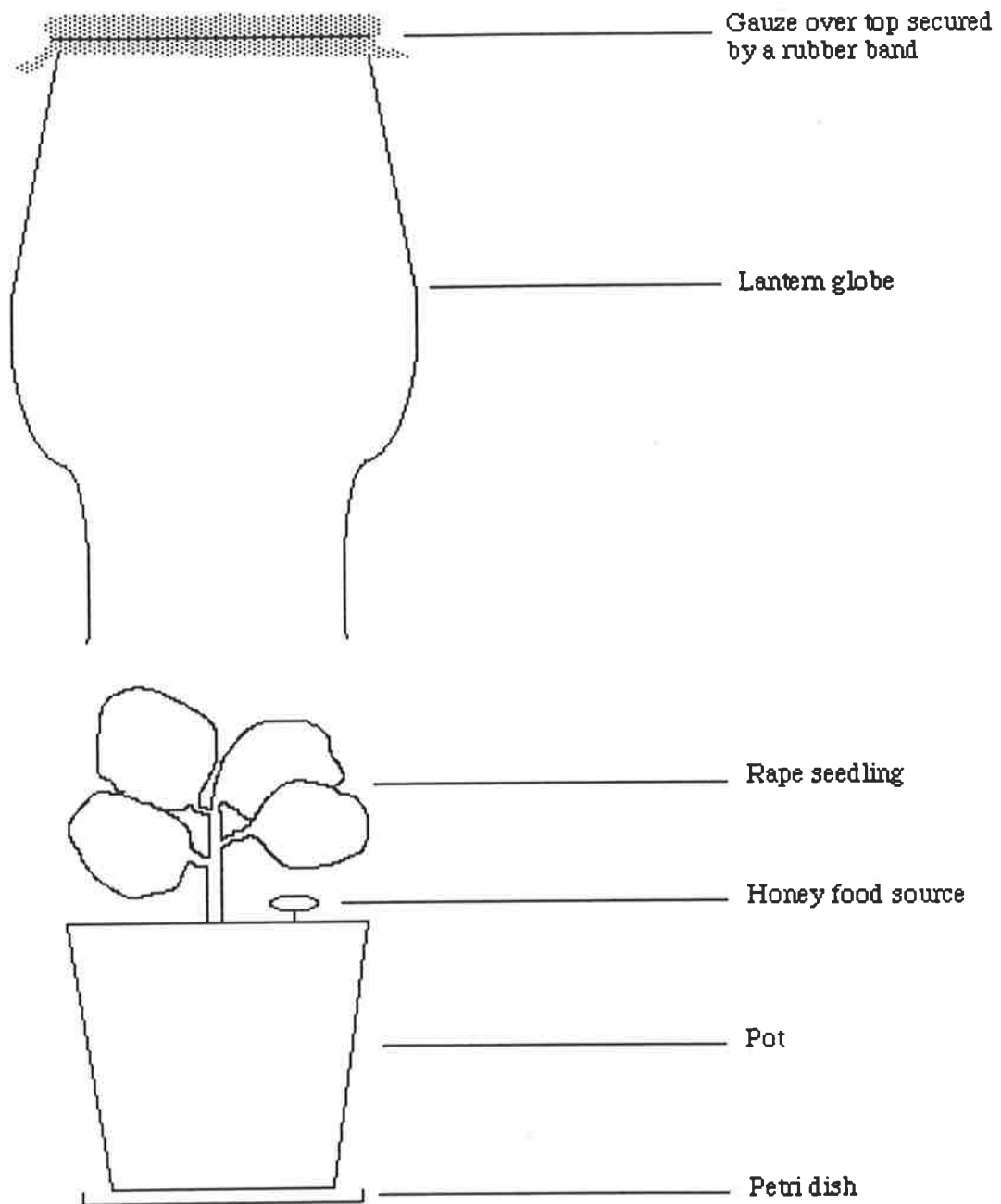
Figure A1.1. Caging system used for ovipositing *L. brassicae* during rearing method 1.

provide aeration. Approximately 20 adults were placed in each container. Adults were maintained at 15(±2)°C , 12L:12D photoperiod until used.

Flies reared by this method were used in the experiments described in Chapter Two.

A1.1.2. Additional Activities

Rape was grown from seed in sterilized recycled soil . Germination took place in seedling trays kept in a shadehouse under field conditions and watered as required. Seedlings at the first leaf stage were transplanted into pots (diameter 100 mm) and watered as required. Plants with 4 or more fully developed leaves were considered suitable for laboratory use. From 1990 onwards, plant growth took place in a glasshouse.

Mated *L. brassicae* females were obtained by placing several males and females together in a lantern globe, the top enclosed with fine mesh. The globes were placed on a piece of white paper and kept at room temperature A fluorescent light (Crompton 15W, 45 mm, white) was placed approximately 150 mm above the globes to concentrate fly activity at the top. When pairs started to mate, other flies were removed. Mating pairs were left undisturbed until they separated.

Due to insufficient resources, several types of vials were used to hold *L. brassicae* pupae. These were glass (50 x 18 mm or 50 x 12 mm) or plastic (40 x 15 mm). To determine if the size or material of the vial influenced the survival of flies, a small experiment was conducted comparing *L. brassicae* mortality between the 3 vial types. Ten newly pupated flies randomly selected from the *L. brassicae* culture were placed in 10 vials of each type (total n = 300). Vials were maintained until all flies had emerged or at least 10 days had elapsed; all surviving flies emerged within 9 days. There was no significant difference in mortality rate between vial types

(P > 0.05; Table A1.1). Therefore, it was concluded that any combination of these vials could be used for rearing and experimental purposes.

In order to refine rearing techniques, knowledge of the oviposition activity of flies throughout the day was considered desirable. To determine if there was considerable diurnal variation in oviposition activity, a small experiment was initiated where the number of feeding and egg punctures made by *L. brassicae* females during five time periods (0800-1100, 1100-1400, 1400-1700 and 1700-0800 hours) were counted for five days. Space and plant supply allowed only two flies to be tested simultaneously. After two replicates (n = 4 flies), the experiment was terminated as it was felt the time investment needed to satisfactorily complete it was unwarranted. Initial results indicated no pronounced variation in oviposition activity throughout the day (Figure A1.2), although slightly more oviposition appeared to occur between 1100-1700 hours than at other times. Considerably more feeding punctures than egg punctures were produced, particularly between 1700-0800 hours. Similar results were observed by Oatman and Michelbacher (1958) for *L. sativae*.

A1.2. METHOD TWO

For large scale experiments, such as those discussed in Chapters Four to Seven, insufficient quantities of infested plants could be created by rearing method 1. Modifications were made to the rearing technique in order to expand the output.

Twenty rape seedlings, grown by the methods described above, were placed within a wooden-framed cage (length 660 mm, height 300 mm, and depth 500 mm) at approximately 0900 hours every week day. The top, one long side and the two short sides of this cage were enclosed by fine mesh. The other long side was covered by transparent plastic sheeting. The cage was placed on an aluminium tray and was illuminated by means of a single fluorescent light tube (Philips 20W, 60 mm,

Table A1.1. Comparison of *L. brassicae* pupal mortality when stored in three vial types.

Vial type	Mean ± standard error
Glass 50 x 18 mm	2.20 ± 0.44
Glass 50 x 12 mm	2.00 ± 0.47
Plastic 40 x 15 mm	2.20 ± 0.53

A.

B.
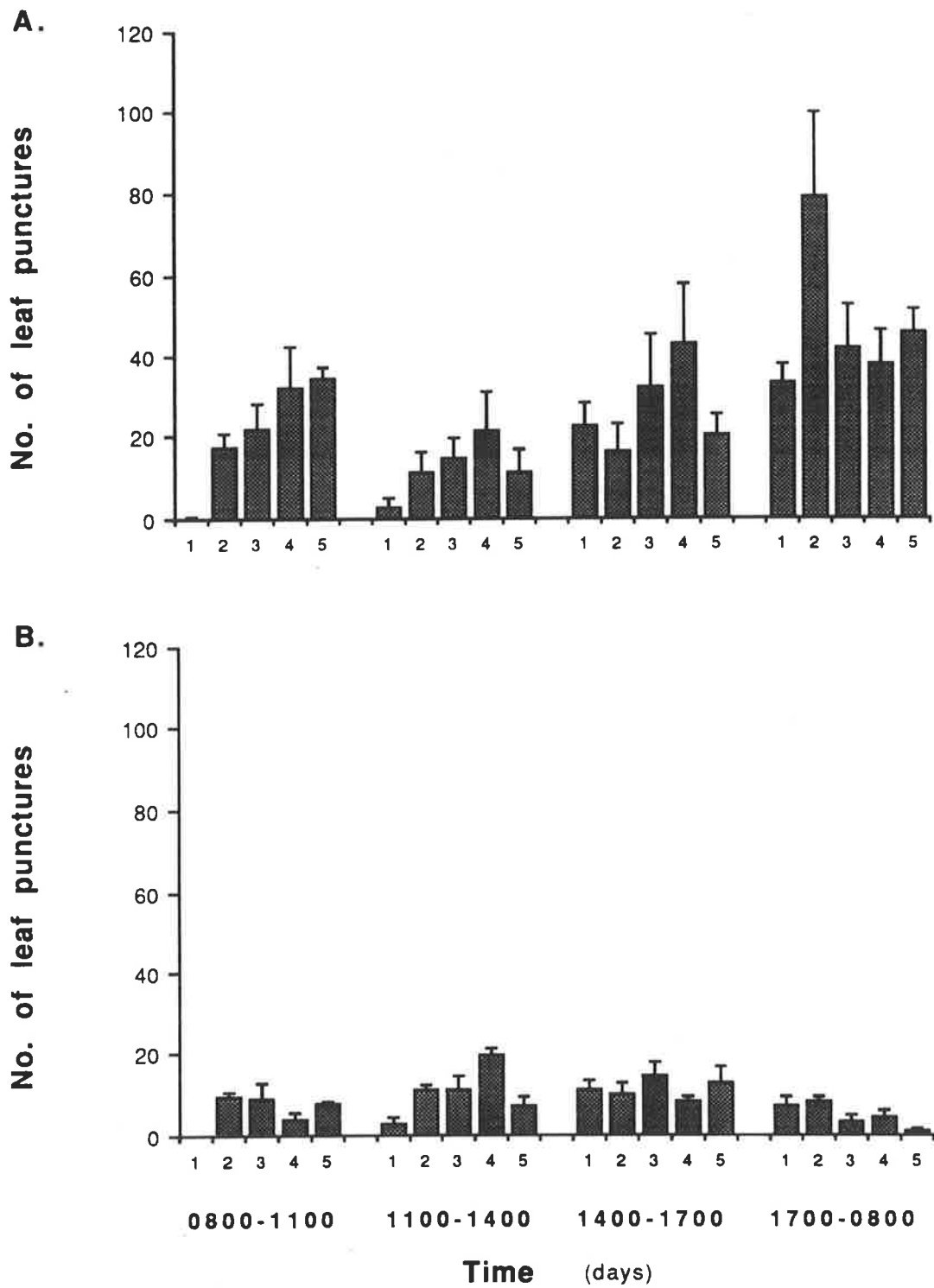
Figure A1.2. Number of (A) feeding and (B) egg punctures (mean ± standard error) produced by *L. brassicae* between 0800-1100, 1100-1400, 1400-1700 and 1700-0800 hours for the first 5 days of oviposition.

white) elevated 300 mm above its upper surface. In excess of 100 adult *L. brassicae* (approximately 1:1 sex ratio) were placed in the cage. Plants were replaced at approximately 1700 hours by between 3 to 5 plants to act as overnight feeding sites. The fly population in the cage was replenished every 7 days.

Those plants which had been removed from the cage were labelled, placed in plastic trays and held at 15, 20 or 25°C, depending on the experimental requirements. It was found that by moving plants between controlled temperature rooms a constant supply of 1st, 2nd and 3rd instar L. *brassicae* larvae would be available. Leaves not used for experimental purposes were placed in a rectangular plastic container (Decor, Australia, no. 253, 4 litre capacity) lined with paper towelling. These were maintained at 15°C until larval emergence when prepupae or pupae were collected and placed into vials until adult emergence. Adults of both sexes were placed together into an aerated cylindrical plastic container (British Plastics Pty Ltd, Medium, No. 532), provided with honey and kept at 20°C until needed or death. It was found that enough adults would mate successfully under these conditions to sustain the culture.

APPENDIX TWO

LOWER DEVELOPMENTAL THRESHOLDS CALCULATED FROM DEVELOPMENTAL TIMES OF *Liriomyza brassicae* PUBLISHED BY BERI AND CHANDRA (1983)

Table A2.1. Developmental times from Beri and Chandra (1983) and calculation of lower developmental thresholds for various life stages of *L. brassicae* using their data.

Temperature °C	Development time in hours (days)						
	Egg	1st instar	2nd instar	3rd instar	Larval (total)	Pupal	Total (egg - adult)
10	241 (10.04)	-	-	-	-	-	-
15	155 (6.46)	80 (3.33)	66 (2.75)	73 (3.04)	219 (9.13)	630 (26.25)	1004 (41.83)
20	121 (5.04)	66 (2.75)	55 (2.29)	64 (2.67)	185 (7.71)	408 (17.00)	714 (29.75)
25	74 (3.08)	53 (2.21)	43 (1.79)	60 (2.50)	156 (6.50)	251 (10.46)	481 (20.04)
30	72 (3.00)	40 (1.67)	35 (1.46)	53 (2.21)	128 (5.33)	222 (9.25)	422 (17.58)
35	72 (3.00)	-	-		-	-	-
Estimate	0.00536	-0.0169	0.0185	0.212	0.0290	-0.0320	-0.0105
Temperature	0.0105	0.0197	0.0219	0.0078	0.00516	0.0048	0.0023
R squared	0.898	0.960	0.991	0.991	0.986	0.968	0.980
Lower Threshold °C	-0.51	0.86	-0.84	-27.20	-5.62	6.67	4.56

APPENDIX THREE

ABUNDANCE OF PARASITOIDS ATTACKING *Liriomyza brassicae* IN THE ADELAIDE REGION AS SHOWN BY FIELD SURVEYS FROM 1989 TO 1991

Table A3.1. Numbers of parasitoids collected during field sampling 1988-89. Data pooled from all sites.

		Parasitoid species						
Collection no.	Date	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv
1	13/7/88	-	-	-	-	-	-	-
2	9/8/88	-	1	-	-	-	-	-
3	8/9/88	-	1	-	-	-	-	-
4	5/10/88	-	-	6	2	-	-	1
5	9/11/88	-	1	-	7	12	-	-
6	14/12/88	-	-	-	2	2	-	-
7	14/1/89	2	1	-	-	-	-	5
8	2/2/89	1	-	-	-	-	4	1
9	22/2/89	-	-	-	1	1	6	-
10	16/3/89	-	1	-	-	-	4	2
11	13/4/89	-	1	6	1	-	8	-
12	16/5/89	-	4	-	-	-	3	1

Parasitoid species : Oa, *Opius atricornis* ; Oc, *Opius cinerariae* ; Cp, *Chrysocharis pubicornis* ; Cl sp., *Closterocerus* sp.; Ch sp., *Chrysonotomyia* sp.; Z sp., *Zagrammasoma* sp.; Hv, *Hemiptarsenus varicornis*.
-, no individuals of a species collected.

Table A3.2. Numbers of parasitoids collected (percentage of total parasitism) during field sampling 1989-91.

Collection no.	Date	Plant	Area	Parasitoid species							
				Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U
1	26/10/89	Tm	Ww	-	-	-	-	-	-	-	-
			At	0	0	0	0	0	0	0	0
			Mi	-	-	-	-	-	-	-	-
		Rr	Ww	0	0	0	0	0	0	2 (100)	0
			Dt	-	-	-	-	-	-	-	-
			Mi	-	-	-	-	-	-	-	-
		Cm	Os	0	0	1 (100)	0	0	0	0	0
			Ma	0	0	0	0	0	0	0	0
			Sm	0	0	1 (100)	0	0	0	0	0
		So	Dt	-	-	-	-	-	-	-	-
			Sl	-	-	-	-	-	-	-	-
			Dy	-	-	-	-	-	-	-	-
2	28/11/89	Tm	Ww	0	0	0	0	0	0	0	0
			At	0	0	0	0	0	0	0	1 (100)
			Mi	0	0	0	0	0	7 (100)	0	0
		Rr	Ww	0	0	0	0	0	8 (42)	2 (11)	9 (47)
			Dt	0	0	0	0	0	3 (100)	0	0
			Mi	0	0	0	0	0	0	0	0
		Cm	Os	0	0	0	0	2 (50)	1 (25)	1 (25)	0
			Ma	0	0	0	0	0	0	0	0
			Sm	0	0	0	0	0	2 (100)	0	0
		So	Dt	-	-	-	-	-	-	-	-
			Sl	0	0	0	0	0	0	0	0
			Dy	0	0	0	0	0	0	0	0

Collection no.	Date	Plant	Area	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U
3	3/1/90	Tm	Ww	1 (9)	0	0	0	9 (82)	0	1 (9)	0
			At	0	0	0	0	0	0	1 (100)	0
			Mi	0	0	1 (100)	0	0	0	0	0
		Rr	Ww								
			Dt								
			Mi								
		Cm	Os	0	0	0	0	0	0	1 (50)	1 (50)
			Ma	-	-	-	-	-	-	-	-
			Sm	0	0	0	0	1 (4)	10 (42)	7 (29)	6 (25)
		So	Dt	0	0	0	0	0	2 (15)	11 (85)	0
			Sl	0	0	0	0	0	6 (38)	9 (56)	1 (6)
			Dy	0	0	0	0	0	18 (39)	8 (17)	20 (44)
4	25/1/90	Tm	Ww	6 (46)	0	0	0	5 (38)	0	0	2 (16)
			At	3 (100)	0	0	0	0	0	0	0
			Mi								
		Rr	Ww								
			Dt								
			Mi	0	0	0	0	0	0	3 (75)	1 (25)
		Cm	Os	0	0	0	0	0	0	1 (50)	1 (50)
			Ma	0	0	0	0	0	5 (45)	2 (18)	4 (37)
			Sm	0	0	0	0	0	23 (59)	6 (15)	10 (26)
		So	Dt	0	0	1 (6)	1 (6)	1 (6)	1 (6)	13 (72)	1 (6)
			Sl	0	1 (6)	0	0	1 (6)	5 (28)	5 (28)	6 (32)
			Dy	0	0	0	0	0	34 (45)	10 (13)	32 (42)

Collection no.	Date	Plant	Area	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U
5	22/2/90	Tm	Ww	0	0	0	0	0	1 (100)	0	0
			At	0	0	0	0	0	0	0	0
			Mi								
		Rr	Ww								
			Dt								
			Mi	2 (40)	0	0	0	0	0	3 (60)	0
		Cm	Os	0	0	0	0	0	0	0	0
			Ma	0	0	0	0	0	7 (41)	6 (35)	4 (24)
			Sm	0	0	0	0	0	4 (44)	4 (44)	1 (12)
		So	Dt	0	0	0	0	0	1 (7)	11 (73)	3 (20)
			Sl	0	0	0	0	0	5 (71)	1 (14)	1 (14)
			Dy	0	0	0	0	0	6 (75)	0	2 (25)
6	14/3/90	Tm	Ww	-	-	-	-	-	-	-	-
			At	-	-	-	-	-	-	-	-
			Mi								
		Rr	Ww								
			Dt								
			Mi	0	0	0	0	0	1 (17)	2 (33)	3 (50)
		Cm	Os	0	0	0	0	0	0	0	0
			Ma	0	0	0	0	0	4 (57)	0	3 (43)
			Sm	0	0	1 (7)	0	0	4 (27)	6 (40)	4 (27)
		So	Dt	0	0	0	0	0	9 (56)	4 (25)	3 (19)
			Sl	0	0	0	0	0	1 (100)	0	0
			Dy	0	0	0	0	0	3 (100)	0	0

Collection no.	Date	Plant	Area	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U
7	6/4/90	Tm	Ww								
			At								
			Mi								
		Rr	Ww								
			Dt								
			Mi	0	2 (50)	0	0	0	0	2 (50)	0
		Cm	Os	0	0	0	0	0	1 (100)	0	0
			Ma	0	0	0	0	0	11 (85)	0	2 (15)
			Sm	0	0	0	0	0	6 (75)	0	2 (25)
		So	Dt	0	0	0	0	0	7 (64)	1 (9)	3 (27)
			Sl	0	0	0	0	0	0	0	0
			Dy	0	0	0	0	0	2 (40)	0	3 (60)
8	2/5/90	Tm	Ww								
			At								
			Mi								
		Rr	Ww	0	0	0	0	0	0	0	0
			Dt								
			Mi	0	1 (50)	0	0	0	0	1 (50)	0
		Cm	Os	0	0	0	0	0	0	0	0
			Ma	0	0	0	0	0	3 (43)	0	0
			Sm	0	0	0	0	0	0	0	0
		So	Dt								
			Sl	0	0	0	0	0	0	0	0
			Dy	0	1 (11)	0	0	0	0	7 (78)	1 (11)

Collection no.	Date	Plant	Area	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U
9	25/6/90	Tm	Ww								
			At								
			Mi								
		Rr	Ww								
			Dt								
			Mi	-	-	-	-	-	-	-	-
		Cm	Os	0	0	0	0	0	1 (33)	2 (67)	0
			Ma	0	0	0	0	0	0	1 (100)	0
			Sm	0	0	0	0	0	0	0	0
		So	Dt								
			Sl								
			Dy								
10	24/10/90	Tm	Ww								
			At								
			Mi								
		Rr	Ww	0	0	0	0	0	0	0	0
			Dt								
			Mi	-	-	-	-	-	-	-	-
		Cm	Os	-	-	-	-	-	-	-	-
			Ma	0	0	0	0	0	0	0	0
			Sm	0	0	0	0	0	0	0	0
		So	Dt								
			Sl								
			Dy								

Collection no.	Date	Plant	Area	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U
11	30/11/90	Tm	Ww	0	0	0	0	0	0	0	0
			At	1 (14)	0	0	0	0	3 (43)	0	3 (43)
			Mi								
		Rr	Ww	-	-	-	-	-	-	-	-
			Dt	0	0	0	0	0	0	0	0
			Mi	0	0	0	0	0	0	0	0
		Cm	Os	0	0	0	0	0	0	0	0
			Ma	0	0	0	0	0	0	0	0
			Sm	0	0	0	0	0	0	0	0
		So	Dt	-	-	-	-	-	-	-	-
			Sl	0	0	0	0	0	0	0	0
			Dy	0	0	0	0	0	0	0	0
12	9/1/91	Tm	Ww	0	0	0	0	2 (100)	0	0	0
			At	0	0	0	0	0	0	0	0
			Mi								
		Rr	Ww	-	-	-	-	-	-	-	-
			Dt	-	-	-	-	-	-	-	-
			Mi	0	1 (100)	0	0	0	0	0	0
		Cm	Os	0	0	0	0	0	1 (33)	2 (67)	0
			Ma	0	0	0	0	0	0	0	0
			Sm	-	-	-	-	-	-	-	-
		So	Dt	0	0	0	0	0	1 (25)	0	3 (75)
			Sl	-	-	-	-	-	-	-	-
			Dy	0	0	0	0	0	3 (75)	0	1 (25)

Collection no.	Date	Plant	Area	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U.
13	6/2/91	Tm	Ww	0	0	0	0	0	0	0	0
			At	0	0	0	0	0	0	0	0
			Mi								
		Rr	Ww	-	-	-	-	-	-	-	-
			Dt	0	0	0	0	0	0	0	0
			Mi	0	0	0	0	0	0	0	0
		Cm	Os	-	-	-	-	-	-	-	-
			Ma	-	-	-	-	-	-	-	-
			Sm	-	-	-	-	-	-	-	-
		So	Dt	0	0	0	0	1 (100)	0	0	0
			Sl	-	-	-		-	-	-	-
			Dy	0	0	0	0	0	4 (67)	0	2 (33)
14	4/3/91	Tm	Ww								
			At	-	-	-	-	-	-	-	-
			Mi	-	-	-	-	-	-	-	-
		Rr	Ww	-	-	-	-	-	-	-	-
			Dt	0	0	0	0	0	0	0	3 (100)
			Mi	0	0	0	0	0	0	1 (100)	0
		Cm	Os	-	-	-	-	-	-	-	-
			Ma	0	0	0	0	0	0	0	0
			Sm	-	-	-	-	-	-	-	-
		So	Dt	0	0	0	0	0	0	0	0
			Sl	-	-	-	-	-	-	-	-
			Dy	0	0	0	0	0	0	0	0

Collection no.	Date	Plant	Area	Oa	Oc	Cp	Cl sp.	Ch sp.	Z sp.	Hv	U
15	16/4/91	Tm	Ww								
			At								
			Mi	-	-	-	-	-	-	-	-
		Rr	Ww	-	-	-	-	-	-	-	-
			Dt	0	0	0	0	0	1 (13)	2 (25)	5 (63)
			Mi	-	-	-	-	-	-	-	-
		Cm	Os	0	0	0	0	0	0	0	0
			Ma	0	0	0	0	0	0	0	1 (100)
			Sm	0	0	0	0	0	2 (25)	2 (25)	4 (50)
		So	Dt	0	0	0	0	0	3 (38)	2 (25)	3 (38)
			Sl	0	0	0	0	0	0	0	1 (100)
			Dy	0	0	0	0	0	0	0	0

Plants : Tm, *Tropaeolum majus* ; Rr, *Rapistrum rugosum* ; Cm, *Cakile maritima* ; So, *Sisymbrium officinale*.

Areas : Ww, Wheal Watkins; At, Athelstone; Mi, Mitcham; Dt, Darlington; Os, O'Sullivan Beach; Ma, Moana Beach; Sm, Semaphore; Sl, Sheoak Log; Dy, Daveyston.

Parasitoid species : Oa, *Opius atricornis* ; Oc, *Opius cinerariae* ; Cp, *Chrysocharis pubicornis* ; Cl sp., *Closterocerus* sp.; Ch sp., *Chrysonotomyia* sp.; Z sp., *Zagrammasoma* sp.; Hv, *Hemiptarsenus varicornis*.

-, no hosts present at site; blank spaces, no host plants present at site.

APPENDIX FOUR

CORRELATION OF HIND TIBIA LENGTH WITH HEAD CAPSULE WIDTH FOR *Opius cinerariae* AND *Hemiptarsenus varicornis*

The hind tibia length (mm) and head capsule width (mm) of parasitoids used for the longevity experiments (Chapter Seven) were measured. These measures were found to be positively correlated for both *O. cinerariae* (Spearman's rank correlation coefficient, r_s = 0.660, P < 0.001; Figure A4.1A) and *H. varicornis* (r_s = 0.895, P < 0.001; Figure A4.1B). Consequently, only hind tibia length was used as a measure of wasp body size. Other studies have found hind tibia length to be a good indicator of body size and weight (Allen 1989).
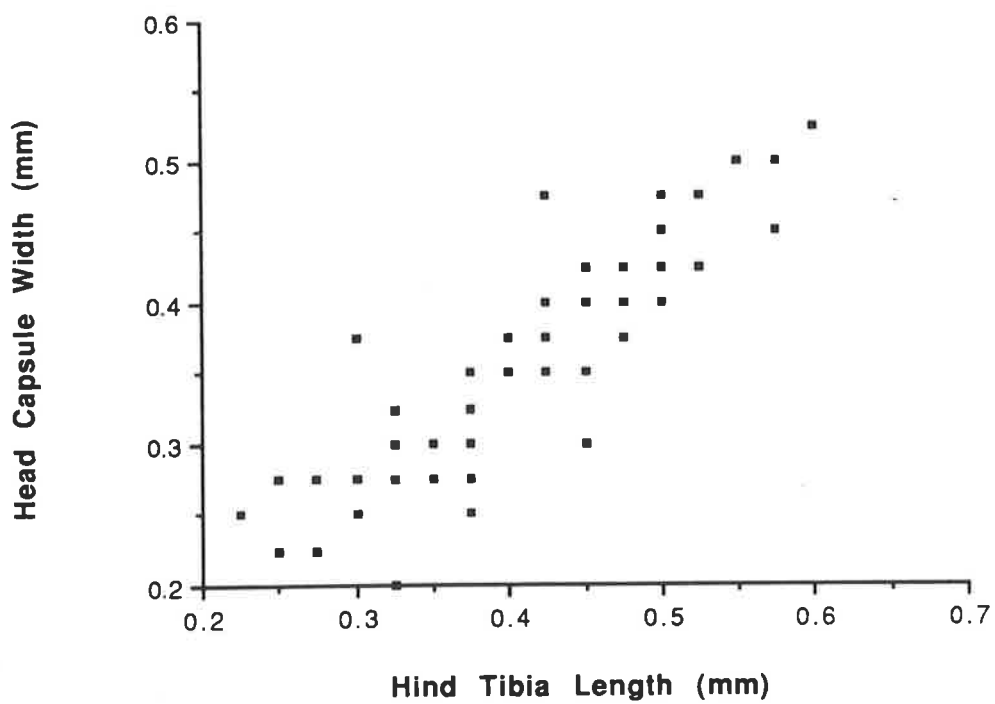
A.
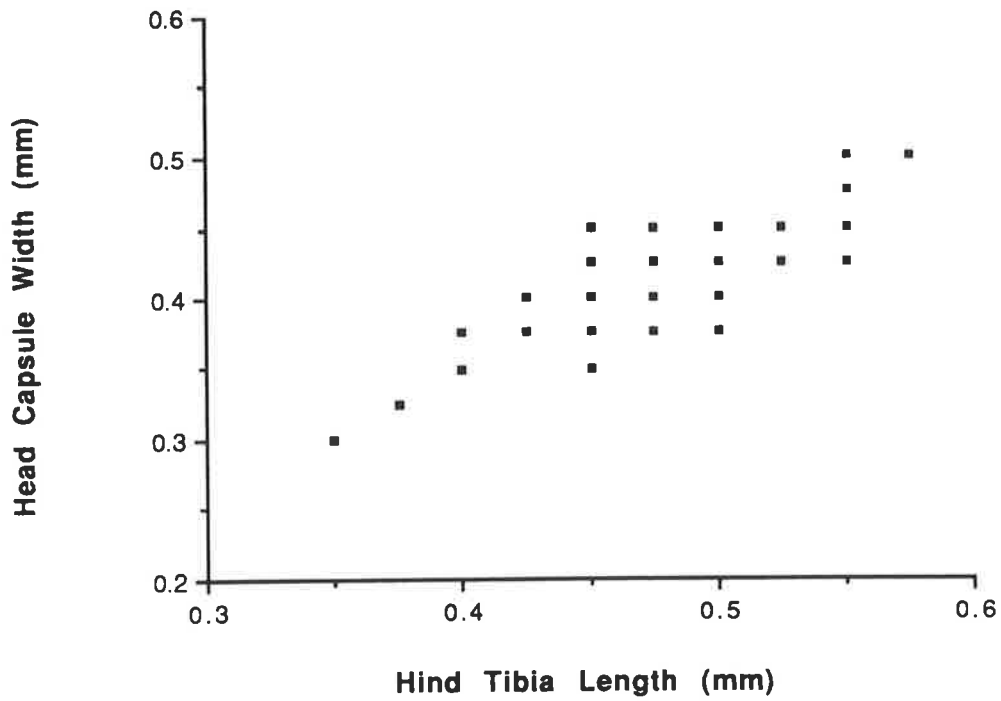
B.

Figure A4.1. Correlation between hind tibia length (mm) and head capsule width (mm) for (A) *O. cinerariae* and (B) *H. varicornis* .

APPENDIX FIVE

RELATIONSHIPS BETWEEN RELATIVE FITNESS COMPONENTS OF *Hemiptarsenus varicornis* AND *Opius cinerariae* FROM DIFFERENT HOST INSTARS

Mean values of relative fitness components measured for progeny of *H. varicornis* and *O. cinerariae* (Chapter Seven) were correlated graphically to examine relationships between the various components and the host instar at oviposition. As longevity, size, egg load and mating success were measured for parasitoids reared at 25°C, only the developmental rate at this temperature was used. Measurements of each fitness component could not be made on the same individuals. Therefore, the mean values (± standard error) were used for all components, except mating success. This was represented by the proportion of males which successfully mated on their first encounter with a female. The relationships presented here may differ from those that would be observed if data from individual insects were examined. For *H. varicornis*, progeny developing from 3rd instars consistently derived greater fitness benefits than those from 2nd instars (Figures A5.1, A5.2). However, relationships between the various fitness components for *O. cinerariae* progeny from each instar were highly variable (Figures A5.3, A5.4).

220

Figure A5.1. Relationships between relative fitness components (mean ± standard error) for female progeny of *H. varicornis* from 2nd and 3rd instar *L. brassicae* larvae.

(A), developmental rate and egg load; (B), developmental rate and size; (C), egg load and size.
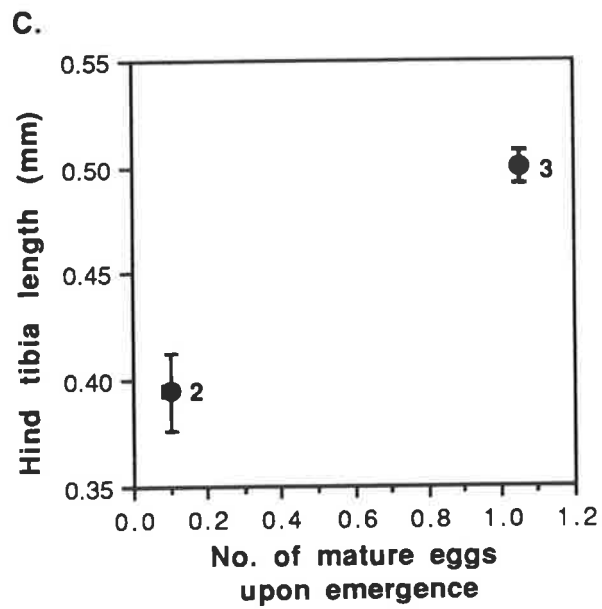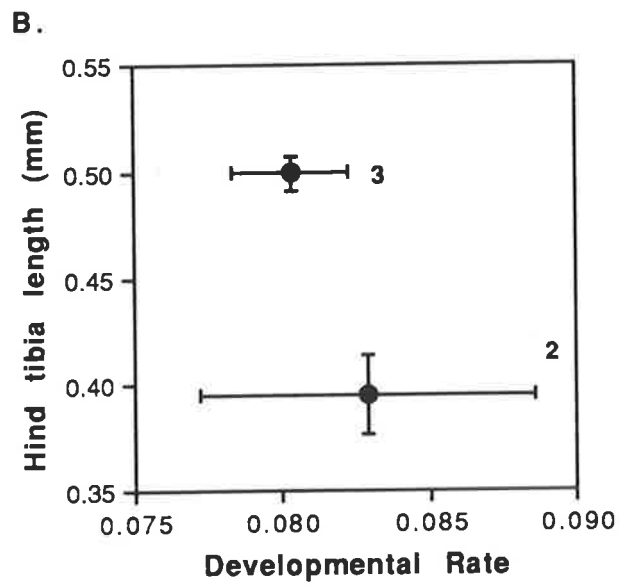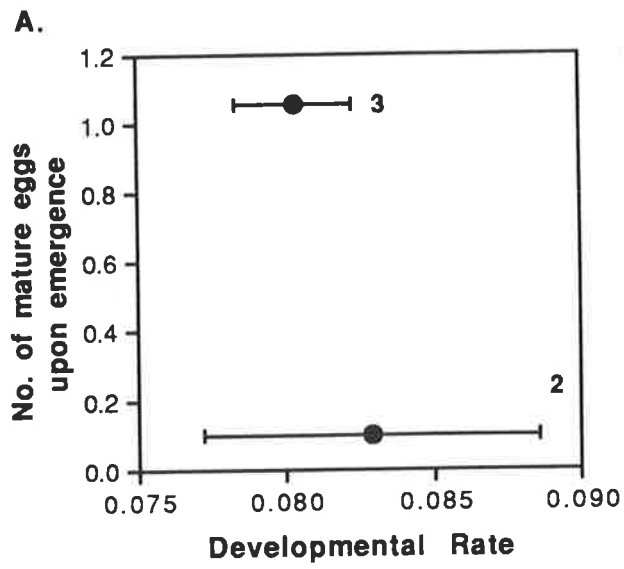
Figure A5.2. Relationships between relative fitness components (mean ± standard error) for male progeny of *H. varicornis* from 2nd and 3rd instar *L. brassicae* larvae. (A), developmental rate and longevity; (B), developmental rate and mating success; (C), developmental rate and size; (D), longevity and mating success; (E), longevity and size; (F), mating success and size.
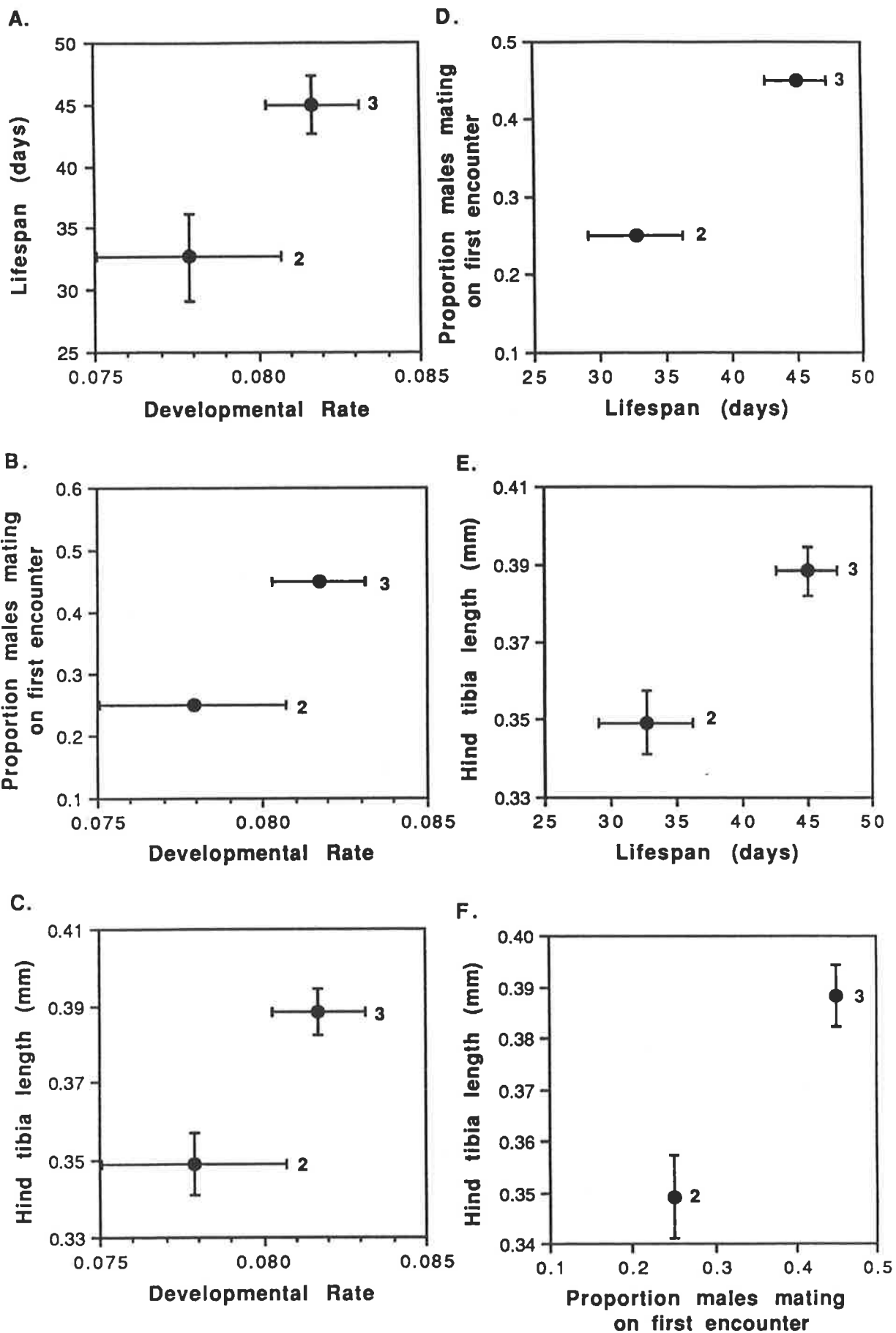
Figure A5.3. Relationships between relative fitness components (mean ± standard error) for female progeny of *O. cinerariae* from each *L. brassicae* instar. (A), developmental rate and longevity; (B), developmental rate and egg load; (C), developmental rate and size; (D), longevity and egg load; (E), longevity and size; (F), egg load and size.
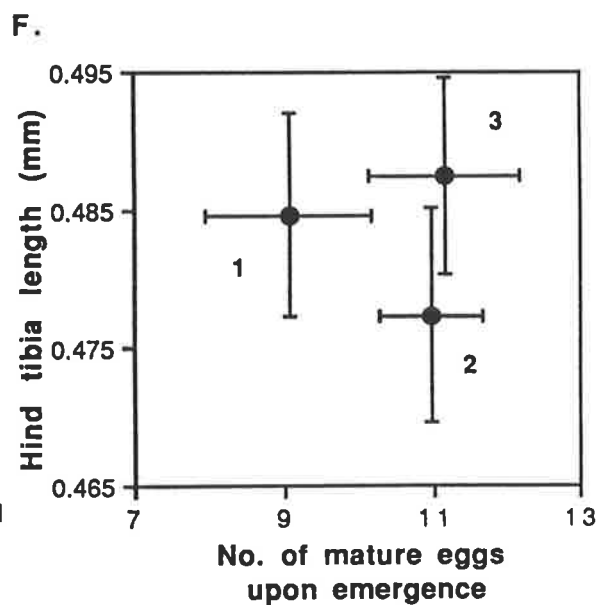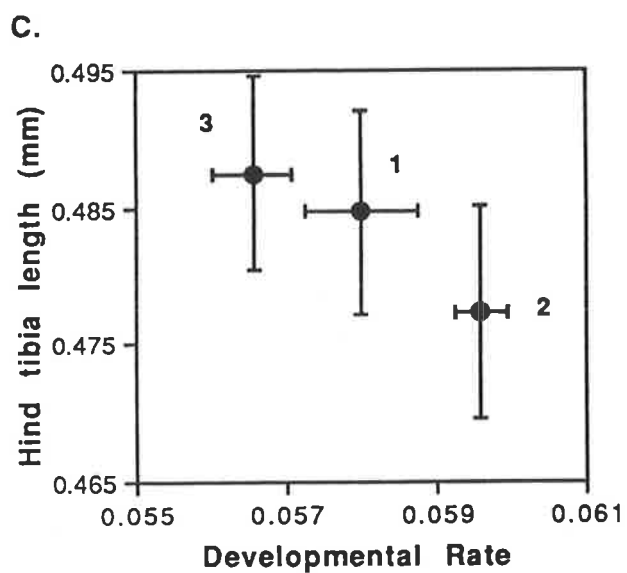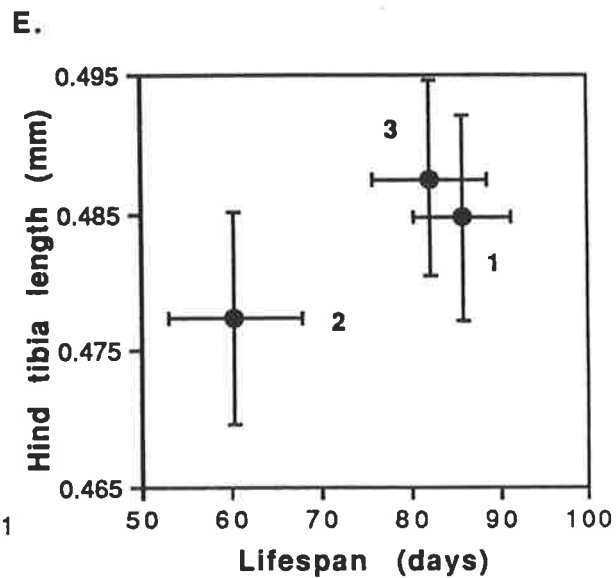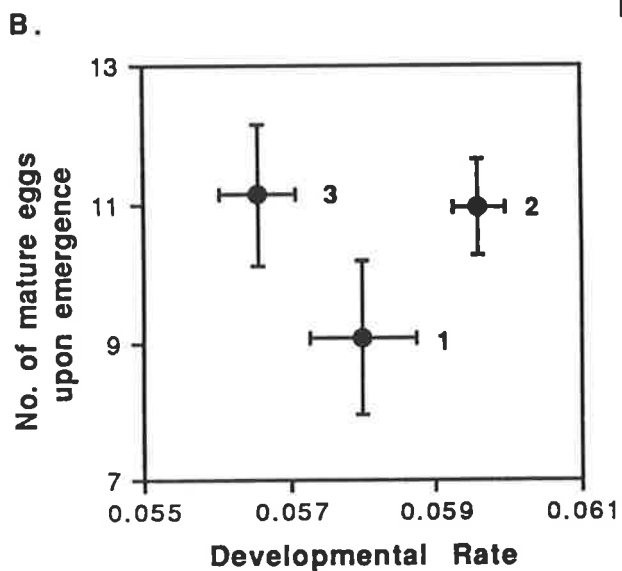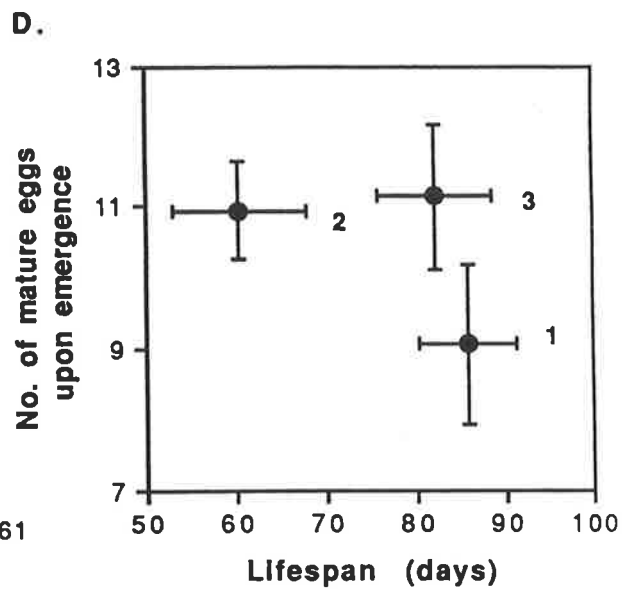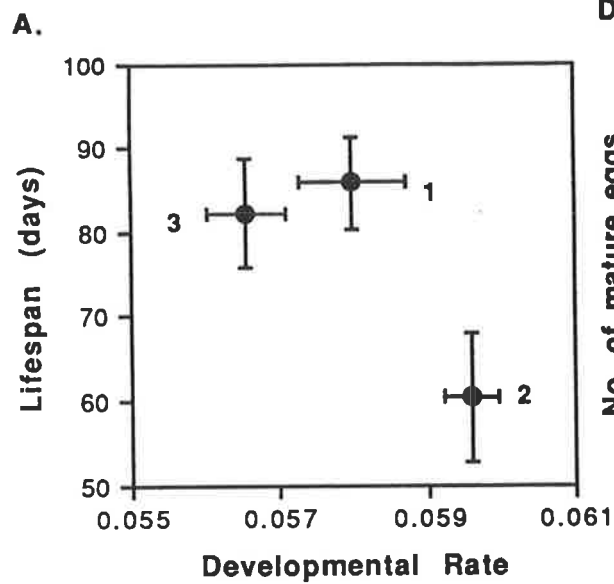
Figure A5.4. Relationships between relative fitness components (mean ± standard error) for male progeny of *O. cinerariae* from each *L. brassicae* instar. (A), developmental rate and longevity; (B), developmental rate and mating success; (C), developmental rate and size; (D), longevity and mating success; (E), longevity and size; (F), mating success and size.
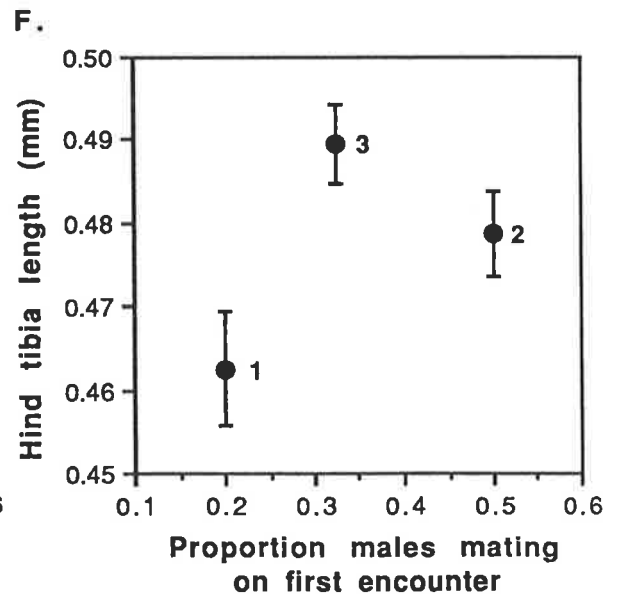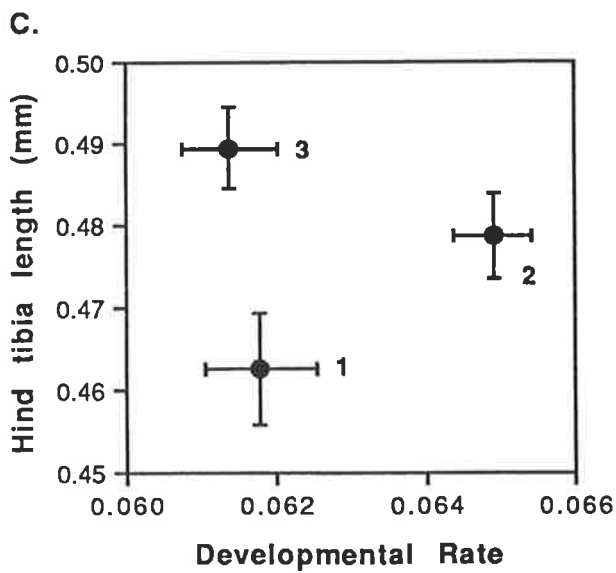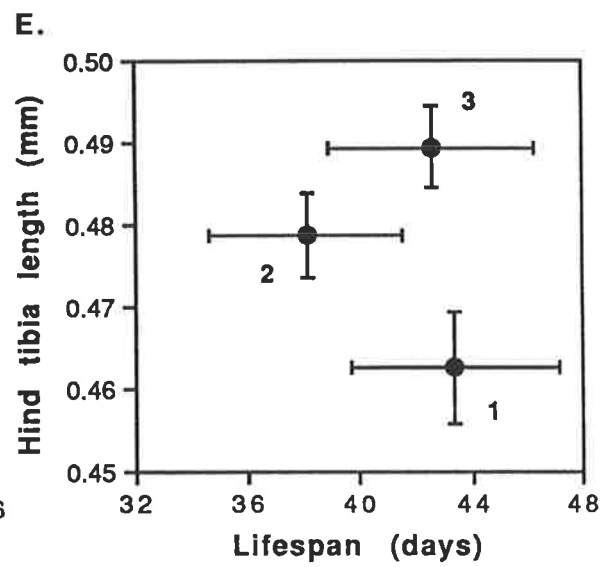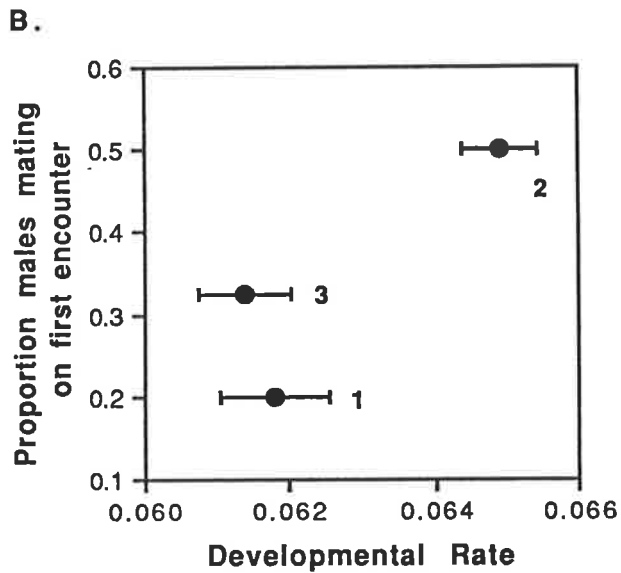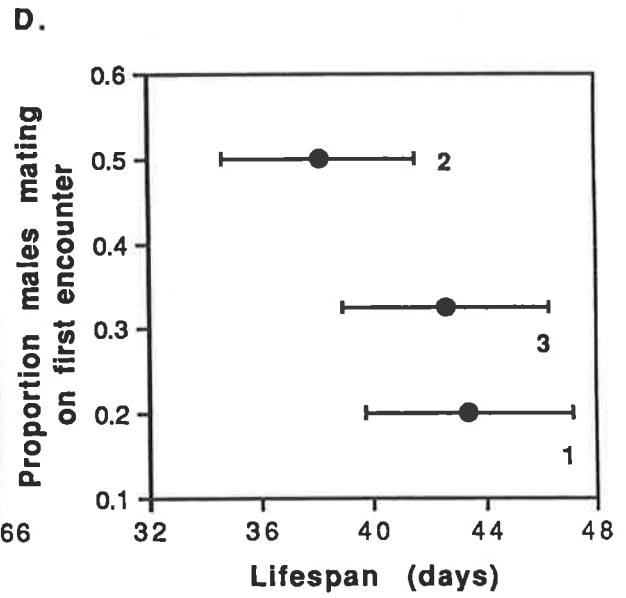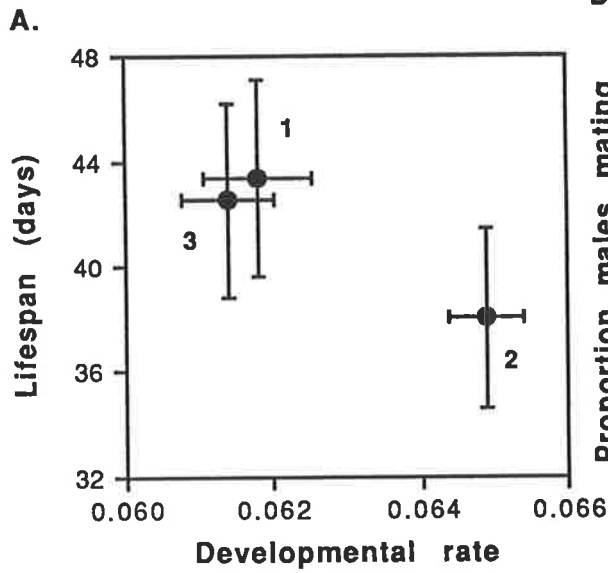
REFERENCES

Abdelrahman, I. (1974a). Growth, development and innate capacity for increase in *Aphytis chrysomphali* Mercet and *A. melinus* DeBach, parasites of California red scale, *Aonidiella aurantii* (Mask.), in relation to temperature. *Australian Journal of Zoology*, **22**, 213-230.

Abdelrahman, I. (1974b). Studies in ovipositional behaviour and control of sex in *Aphytis melinus* DeBach, a parasite of California red scale, *Aonidiella aurantii* (Mask.). *Australian Journal of Zoology*, **22**, 231-247.

Allen, G. R. (1989). Behaviour and ecology of the primary parasitoids *Cotesia urabae* and *Dolichogenidea eucalypti* (Hymenoptera: Braconidae) and their host *Uraba lugens* (Lepidoptera: Noctuidae). Ph. D. thesis, The University of Adelaide.

Allen, G. R. (1990). Influence of host behaviour and host size on the success of oviposition of *Cotesia urabae* and *Dolichogenidea eucalypti* (Hymenoptera: Braconidae). *Journal of Insect Behaviour*, **3**, 733-749.

Allen, J. C. (1976). A modified sine wave method for calculating degree days. *Environmental Entomology*, **5**, 388-396.

Andrewartha, H. G. and Birch, L. C. (1954). *The Distribution and Abundance of Animals*. The University of Chicago Press, Chicago.

Arthur, A. P. and Wylie, H. G. (1959). Effects of host size on sex ratio,

development time and size of *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). *Entomophaga*, **4**, 297-301.

Askew, R. R. (1975). The organisation of chalcid-dominated parasitoid communities centred upon endophytic hosts. *Evolutionary strategies of parasitic insects and mites* (ed. by P.W. Price), pp. 130-153. Plenum Press, New York.

Askew, R. R. and Shaw, M. R. (1986). Parasitoid Communities : their size, structure and development. *Insect Parasitoids, 13th Symposium of the Royal Entomological Society* (ed. by J. Waage and D. Greathead), pp. 225-264. Academic Press Inc., London.

Assem, M. A. H. (1966). Studies of vegetable leaf miners. Ph.D. Thesis, Faculty of Agriculture, Cairo. Cited in *Agromyzidae (Diptera) of Economic Importance* (ed. by K.A. Spencer), (1973). Dr. W. Junk, B.V. Publishers, The Hague.

Austin, A. D. (1985). The function of spider egg sacs in relation to parasitoids and predators, with special reference to the Australian region. *Journal of Natural History*, **19**, 359-379.

Austin, A. D. and Allen, G. R. (1989). Parasitoids of *Uraba lugens* Walker (Lepidoptera: Noctuidae) in South Australia, with descriptions of two new species of Braconidae. *Transactions of the Royal Society of South Australia*, **113**, 169-184.

Avilla, J. and Albajes, R. (1984). The influence of female age and host size on the sex ratio of the parasitoid *Opius concolor*. *Entomologia Experimentalis et Applicata*, **35**, 43-47.

Barrass, R. (1961). A quantitative study of the behaviour of the male *Mormoniella vitripennis* towards two constant stimulus situations.

Bull, J. J. (1981). Sex ratio evolution when fitness varies. *Heredity*, **46**, 9-26.

Burnett, T. (1962). An effect of parasite attack on host mortality as exemplified by *Encarsia formosa* and *Trialeurodes vaporariorum*. *Canadian Entomologist*, **94**, 673-679.

Chandler, L. D. , Gilstrap, F. E. and Browning, H. W. (1988). Evaluation of the within-field mortality of *Liriomyza trifolii* (Diptera: Agromyzidae) on bell pepper. *Journal of Economic Entomology*, **81**, 1089-1096.

Charnov, E. L. (1979). The genetical evolution of patterns of sexuality: Darwinian fitness. *American Naturalist*, **113**, 465-480.

Charnov, E. L. (1982). *The Theory of Sex Allocation*. Princeton University Press.

Charnov, E. L., Los-den Hartogh, R. L., Jones, W. T. and van den Assem, J. (1981). Sex ratio evolution in a variable environment. *Nature*, **289**, 27-33.

Charnov, E. L. and Skinner, S. W. (1984). Evolution of host selection and clutch size in parasitoid wasps. *Florida Entomologist*, **67**, 5-21.

Charnov E. L. and Skinner, S. W. (1985). Complementary approaches to the understanding of parasitoid oviposition decisions. *Environmental Entomology*, **14**, 383-391.

Charnov, E. L. and Stephens, D. W. (1988). On the evolution of host selection in solitary parasitoids. *American Naturalist*, **132**, 707-722.

Clausen, C. P. (1939). The effect of host size upon the sex ratio of hymenopterous parasites and its relation to methods of rearing and colonization. *Journal of the New York Entomological Society*, **47**, 1-9.

Clausen, C. P. (1962). *Entomophagous Insects*. Hafner Publ. Co., New York.

Cloutier, C., McNeil, J. N. and Regnière, J. (1981). Fecundity, longevity, and sex ratio of *Aphidius nigripes* (Hymenoptera: Aphidiidae) parasitizing different stages of its host, *Macrosiphum euphorbiae* (Homoptera: Aphididae). *Canadian Entomologist*, **113**, 193-198.

Cock, M. J. W. (1985). A review of biological control of pests in the Commonwealth Carribean and Bermuda up to 1982. *Technical Communication* **9**, *Commonwealth Institute of Biological Control, Commonwealth Agricultural Bureaux*.

Conover, W. J. (1980). *Practical Nonparametric Statistics, 2nd edition*. John Wiley & Sons, New York, pp. 213-343.

Cornelius, S. J. and Godfray, H. C. J. (1984). Natural parasitism of the chrysanthemum leaf-miner *Chromatomyia syngenesiae* H. (Dipt.: Agromyzidae). *Entomophaga*, **29**, 341-345.

De Bach, P. (1943). The importance of host-feeding by adult parasites in the reduction of host populations. *Journal of Economic Entomology*, **36**, 647-658.

de Jong, P. W. and van Alphen, J. J. M. (1989). Host size selection and sex allocation in *Leptomastix dactylopii*, a parasitoid of *Planococcus citri*. *Entomologia Experimentalis et Applicata*, **50**, 161-169.

Dimetry, N. Z. (1971). Biological studies on a leaf mining diptera, *Liriomyza*

trifolii Burgess attacking beans in Egypt. *Bulletin de la Societe Entomologique d'Egypte*, **55**, 55-69.

Donaldson, J. S. and Walter, G. H. (1984). Sex ratios of *Spalangia endius* (Hymenoptera: Pteromalidae), in relation to current theory. *Ecological Entomology*, **9**, 395-402.

Dorge, S. K. and Dalaya, V. P. (1964). Studies on cabbage leaf miner (*Liriomyza brassicae*, Riley). *Current Science*, **18**, 561-562.

Drea, J. J., Jeandel, D. and Gruber, F. (1982). Parasites of agromyzid leafminers (Diptera: Agromyzidae) on alfalfa in Europe. *Annals of the Entomological Society of America*, **75**, 297-310.

Ehler, L. E. (1990). Introduction strategies in biological control of insects. *Critical Issues in Biological Control* (ed. by M. Mackauer, L. E. Ehler and J. Roland), pp. 111-134. Intercept VCH Publishers, Andover, Hants, U.K.

Fagoonee, I. and Toory, V. (1984). Contribution to the study of the biology and ecology of the leaf-miner *Liriomyza trifolii* and its control by neem. *Insect Science and Its Application*, **5**, 23-30.

Field, S. A. (1990). Courtship and sexual selection in the parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae). Honours thesis, The University of Adelaide.

Fischer, M. (1971). *Index of world Opiinae*. Index of entomophagous Insects. Le François, Paris.

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford.

Flanders, R. V. and Oatman, E. R. (1982). Laboratory studies on the biology of *Orgilus jenniae* (Hymenoptera: Braconidae), a parasitoid of the potato tubeworm, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Hilgardia*, **50**, 1-33.

Flanders, S. E. (1943). The role of mating in the reproduction of parasitic Hymenoptera. *Journal of Economic Entomology*, **36**, 802-803.

Flanders, S. E. (1956). The mechanisms of sex-ratio regulation in the (parasitic) Hymenoptera. *Insectes Sociaux*, **3**, 325-334.

Flanders, S. E. (1965). On the sexuality and sex ratios of hymenopterous populations. *American Naturalist*, **99**, 489-494.

Fox, P. M., Pass, B. C. and Thurston, R. (1967). Laboratory studies on the rearing of *Aphidius smithi* (Hymenoptera: Braconidae) and its parasitism of *Acyrthosiphon pisum* (Homoptera: Aphididae). *Annals of the Entomological Society of America*, **60**, 1083-1087.

Frijters, A. J. M., Minkenberg, O. P. J. M., Woets, J. and Ravensberg, W. J. (1986). *Chrysocharis parksi* in commercial greenhouses for the biological control of leafminers, *Liriomyza bryoniae* and *L. trifolii*, on tomatoes; Case studies and sampling techniques. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent*, **51**, 987-997.

Gauld, I. D. (1986). Taxonomy, its limitations and its role in understanding parasitoid biology. *Insect Parasitoids, 13th Symposium of the Royal Entomological Society* (ed. by J. Waage and D. Greathead), pp.1-21. Academic Press Inc., London.

Genung, W. G. (1957). Some possible cases of insect resistance to insecticides in Florida. *Proceedings of the Florida State*

Horticultural Society, **70**, 148-152.

Genung, W. G. and Janes, M. A. (1975). Host range, wild host significance and in-field spread of *Liriomyza trifolii* and population buildup effects of its parasites in relation to fall and winter celery (Diptera: Agromyzidae). *Belle Glade AREC Research Report EV, Florida University Research Station*, **5**, 1-18.

Gerling, D. and Bar, D. (1971). Reciprocal host-parasite relations as exemplified by *Chrysomphalus aonidum* (Homoptera: Diaspididae) and *Pteroptrix smithi* (Hymenoptera: Aphelinidae). *Entomophaga*, **16**, 37-44.

Godfray, H. C. J. (1986). Models for clutch size and sex ratio with sibling interaction. *Theoretical Population Biology*, **30**, 215-231.

Godfray, H. C. J. (1987). The evolution of clutch size in parasitic wasps. *American Naturalist*, **129**, 221-233.

Grant, B., Burton, S., Contoreggi, C. and Rothstein, M. (1980). Outbreeding via frequency dependent mate selection in the parasitoid wasp, *Nasonia* (=*Mormoniella*) *vitripennis* Walker. *Evolution*, **34**, 983-992.

Greathead, D. J. (1986). Parasitoids in classical biological control. *Insect Parasitoids, 13th Symposium of the Royal Entomological Society* (ed. by J. Waage and D. Greathead), pp.289-318. Academic Press Inc., London.

Green, R. F. (1982). Optimal foraging and sex ratio in parasitic wasps. *Journal of Theoretical Biology*, **95**, 43-48.

Gunasena, G. H., Vinson, S. B. and Williams, H. J. (1989). Interrelationships between growth of *Heliothis virescens*

(Lepidoptera: Noctuidae) and that of its parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Annals of the Entomological Society of America*, **82**, 187-191.

Hamilton, W. D. (1967). Extraordinary sex ratios. *Science*, **156**, 477-488.

Harcourt, D. G., Guppy, J. C. and Ellis, C. R. (1986). Establishment and spread of *Dacnusa dryas* (Hymenoptera: Braconidae), an exotic parasite of the alfalfa blotch leafminer in Ontario. *Proceedings of the Entomological Society of Ontario*, **117**, 29-33.

Harding, J. A. (1965). Parasitism of the leaf miner *Liriomyza munda* in the Winter Garden Area of Texas. *Journal of Economic Entomology*, **58**, 442-443.

Hassell, M. P. and Waage, J. K. (1984). Host-parasitoid population interactions. *Annual Review of Entomology*, **29**, 89-114.

Heinz, K. M. and Parrella, M. P. (1989). Attack behaviour and host size selection by *Diglyphus begini* on *Liriomyza trifolii* in chrysanthemum. *Entomologia Experimentalis et Applicata*, **53**, 147-156.

Hendrickson, R. M. Jr. (1975). Mass rearing of *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) on *Liriomyza trifoliearum* Spencer (Diptera: Agromyzidae). *Journal of the New York Entomological Society*, **83**, 243-244.

Hendrickson, R. M. Jr and Barth, S. E. (1978). Notes on the biology of *Diglyphus intermedius* (Hymenoptera: Eulophidae), a parasite of the alfalfa blotch leafminer, *Agromyza frontella* (Diptera: Agromyzidae). *Proceedings of the Entomological Society of Washington*, **80**, 210-215.

Hendrickson, R. M. Jr and Plummer, J. A. (1983). Biological control of alfalfa blotch leafminer (Diptera: Agromyzidae) in Delaware. *Journal of Economic Entomology*, **76**, 757-761.

Hendrikse, A. (1980). A method for mass rearing two braconid parasites (*Dacnusa sibirica* and *Opius pallipes*) of the tomato leafminer (*Liriomyza bryoniae*). *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent*, **45**, 563-571.

Hendrikse, A., Zucchi , R., van Lenteren, J. C. and Woets, J. (1980). *Dacnusa sibirica* Telenga and *Opius pallipes* Wesmael (Hym., Braconidae) in the control of the tomato leafminer *Liriomyza bryoniae* Kalt. *IOBC/WPRS Bulletin*, **3**, 83-98.

Hills, O. A. and Taylor, E. A. (1951). Parasitization of dipterous leaf miners in cantaloups and lettuce in Salt River Valley, Arizona. *Journal of Economic Entomology*, **44**, 759-762.

Hopper, K. R. (1986). Preference, acceptance and fitness components of *Microplitis croceipes* (Hymenoptera: Braconidae) attacking various instars of *Heliothis virescens* (Lepidoptera: Noctuidae). *Environmental Entomology*, **15**, 274-280.

Howarth, F. G. (1991). Environmental impacts of classical biological control. *Annual Review of Entomology*, **36**, 485-509.

Huffaker, C. B. and Messenger, P. S. (1976). *Theory and Practice of Biological control*. Academic Press, New York.

Hurlbutt, B. (1987). Sexual size dimorphism in parasitoid wasps. *Biological Journal of the Linnean Society*, **30**, 63-89.

Ibrahim, A. G. and Madge, D. S. (1979). Parasitization of the

chrysanthemum leaf-miner *Phytomyza syngenesiae* (Hardy) (Dipt., Agromyzidae), by *Diglyphus isaea* (Walker) (Hym., Eulophidae). *Entomologists Monthly Magazine*, **114**, 71-81.

Iwasa, Y., Suzuki, Y and. Matsuda, H. (1984). Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. *Theoretical Population Biology*, **26**, 205-227.

Iwata, K. (1966). The comparative anatomy of the ovary in hymenoptera. Supplement on Ichneumonidae, *Coccygomimus luctuosa* Smith, *C. parnarae* Viereck and *C. pluto* Ashmead. *Acta Hymenopterologica*, **2**, 133-135.

Jackson, D. J. (1966). Observations on the biology of *Caraphractus cinctus* Walker (Hymenoptera: Mymaridae), a parasitoid of the eggs of Dytiscidae (Coleoptera). III. The adult life and sex ratio. *Transactions of the Royal Entomological Society of London*, **118**, 23-49.

Jervis, M. A. and Kidd, N. A. C. (1986). Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews*, **61**, 395-434.

Johnson, M. W. (1987). Parasitization of *Liriomyza* spp. (Diptera: Agromyzidae) infesting commercial watermelon plantings in Hawaii. *Journal of Economic Entomology*, **80**, 56-61.

Johnson, M. W. and Hara, A. H. (1987). Influence of host crop on parasitoids (Hymenoptera) of *Liriomyza* spp. (Diptera: Agromyzidae). *Environmental Entomology*, **16**, 339-344.

Johnson, M. W., Oatman, E. R. and Wyman, J. A. (1980a). Natural control of *Liriomyza sativae* (Dip.: Agromyzidae) in pole tomatoes in southern California. *Entomophaga*, **25**, 193-198.

Johnson, M. W., Oatman, E. R. and Wyman, J. A. (1980b). Effects of insecticides on populations of the vegetable leafminer and associated parasites on summer pole tomatoes. *Journal of Economic Entomology*, **73**, 61-66.

Johnson, M. W., Oatman, E. R. and Wyman, J. A. (1980c). Effects of insecticides on populations of the vegetable leafminer and associated parasites on fall pole tomatoes. *Journal of Economic Entomology*, **73**, 67-71.

Jones, W. T. (1982). Sex ratio and host size in a parasitoid wasp. *Behavioural Ecology and Sociobiology*, **10**, 207-210.

Jowyk, E. A. and Smilowitz, Z. (1978). A comparison of growth and developmental rates of the parasite *Hyposoter exiguae* reared from two instars of its host, *Trichoplusia ni*. *Annual Review of Entomology*, **71**, 467-472.

Juliano, S. A. (1982). Influence of host age on host acceptability and suitability for a species of *Trichogramma* (Hymenoptera: Trichogrammatidae) attacking aquatic Diptera. *Canadian Entomologist*, **114**, 713-720.

Kato, M. (1984). Mining pattern of the honeysuckle leaf-miner *Phytomyza lonicerae*. *Researches on Population Ecology*, **26**, 84-96.

Kato, M. (1985). Life history and mortality pattern of the honeysuckle leaf-miner, *Phytomyza lonicerae* Robineau-Desboidy (Diptera, Agromyzidae). *Kontyû*, **53**, 604-615.

Keil, C. B., Parrella, M. P. and Morse, J. G. (1985). Method for monitoring and establishing baseline data for resistance to permethrin by

Liriomyza trifolii (Burgess). *Journal of Economic Entomology*, **78**, 419-422.

King, B. H. (1987). Offspring sex ratios in parasitoid wasps. *Quarterly Review of Biology*, **62**, 367-396.

King, B. H. (1988). Sex-ratio manipulation in response to host size by the parasitoid wasp *Spalangia cameroni* : A laboratory study. *Evolution*, **42**, 1190-1198.

King, B. H. (1989). Host-size-dependent sex ratios among parasitoid wasps : does host growth matter ? *Oecologia*, **78**, 420-426.

King, P. E. and Hopkins, C. R. (1963). Length of the life of the sexes in *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae) under conditions of starvation. *Journal of Experimental Biology*, **40**, 751-761.

Kistler, R. A. (1985). Host-age structure and parasitism in a laboratory system of two hymenopterous parasitoids and larvae of *Zabrotes subfasciatus* (Coleoptera: Bruchidae). *Environmental Entomology*, **14**, 507-511.

Kleinschmidt, R. P. (1970). Studies of some Agromyzidae in Queensland. *Queensland Journal of Agricultural and Animal Sciences*, **27**, 341-384.

Lange, W. H., Agosto, G. G., Goh, K. S. and Kishiyama, J. S. (1980). Field effect of insecticides on chrysanthemum leafminer and a primary parasitoid, *Chrysocharis ainsliei* (Crawford), on artichokes in California. *Environmental Entomology*, **9**, 561-562.

Lardner, R. M. and Hales, D. (1990). Histopathology of an aphid, *Schoutedenia lutea*, parasitized by a gall midge, *Pseudendaphis* sp.

Entomophaga, **35**, 557-567.

LaSalle, J. and Parrella, M. P. (1991). The chalcidoid parasites (Hymenoptera, Chalcidoidea) of economically important leaf-mining *Liriomyza* species (Diptera, Agromyzidae) in North America. *Proceedings of the Entomological Society of Washington*, **93**, 571-591.

Lawrence, P. O. (1981). Interference competition and optimal host selection in the parasitic wasp, *Biosteres longicaudatus*. *Annals of the Entomological Society of America*, **74**, 540-544.

Lawrence, P. O., Baranowski, R. M. and Greany, P. D. (1976). Effects of host age on development of *Biosteres (=Opius) longicaudatus*, a parasitoid of the Carribean fruit fly, *Anastrepha suspensa*. *Florida Entomologist*, **59**, 33-39.

Legner, E. F. (1969). Adult emergence interval and reproduction in parasitic Hymenoptera influenced by host size and density. *Annals of the Entomological Society of America*, **62**, 220-226.

Lema, K. and Poe, S. L. (1979). Age specific mortality of *Liriomyza sativae* due to *Chrysonotomyia formosa* and parasitization by *Opius dimidiatus* and *Chrysonotmyia formosa*. *Environmental Entomology*, **8**, 935-937.

Lewis, W. J. and Vinson, S. B. (1971). Suitability of certain *Heliothis* (Lepidoptera: Noctuidae) as hosts for the parasite *Cardiochiles nigriceps*. *Annals of the Entomological Society of America*, **64**, 970-972.

Liebee, G. L. (1984). Influence of temperature on development and fecundity of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) on celery.

Environmental Entomology, **13**, 497-501.

Liu, S-S. (1985). Development, adult size and fecundity of *Aphidius sonchi* reared in two instars of its aphid host, *Hyperomyzus lactucae*. *Entomologia Experimentalis et Applicata*, **37**, 41-48.

Liu, S-S., Morton, R. and Hughes, R. D. (1984). Oviposition preferences of a hymenopterous parasite for certain instars of its aphid host. *Entomologia Experimentalis et Applicata*, **35**, 249-254.

Luck, R. F. (1990). Evaluation of natural enemies for biological control : A behavioural approach. *Trends in Ecology and Evolution*, **5**, 196-199.

Luck, R. F. and Podoler, H. (1985). Competitive exclusion of *Aphytis lingnanensis* by *A. melinus* : potential role of host size. *Ecology*, **66**, 904-913.

Luck, R. F., Podoler, H. and Kfir, R. (1982). Host selection and egg allocation behaviour by *Aphytis melinus* and *A. lingnanensis*: comparison of two facultatively gregarious parasitoids. *Ecological Entomology*, **7**, 397-408.

Lynch, J. A. and Johnson, M. W. (1987). Stratified sampling of *Liriomyza* spp. (Diptera: Agromyzidae) and associated hymenopterous parasites on watermelon. *Journal of Economic Entomology*, **80**, 1254-1261.

Mackauer, M. (1976). An upper boundary for the sex ratio in a haplodiploid insect. *Canadian Entomologist*, **108**, 1399-1402.

Mangel, M. (1989). Evolution of host selection in parasitoids: does the state of the parasitoid matter? *American Naturalist*, **133**, 688-705.

Miles, H. W. and Cohen, M. (1936). The chrysanthemum leaf miner and its

control. *Journal of the Ministry of Agriculture (Great Britain)*, **43**, 256-261.

Miles, L. R. and King, E. G.. (1975). Development of the tachinid parasite, *Lixophaga diatraeae*, on various developmental stages of the sugarcane borer in the laboratory. *Environmental Entomology*, **4**, 811-814.

Minkenberg, O. P. J. M. (1988a). Life history of the agromyzid fly *Liriomyza trifolii* on tomato at different temperatures. *Entomologia Experimentalis et Applicata*, **48**, 73-84.

Minkenberg, O. P. J. M. (1988b). Dispersal of *Liriomyza trifolii*. *Bulletin OEPP/EPPO*, **18**, 173-182.

Minkenberg, O. P. J. M. (1990). On seasonal inoculative biological control. Ph. D. Thesis, Agricultural University, Wageningen.

Minkenberg, O. P. J. M. and van Lenteren, J. C. (1986). The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants : a review. *Agricultural University Wageningen Papers*, **86**, 1-50.

Morrison, G. and Strong, D. R. Jr. (1981). Spatial variations in egg density and the intensity of parasitism in a neotropical chrysomelid (*Cephaloleia consanguinea*). *Ecological Entomology*, **6**, 55-61.

Narasimhan, A. U. (1984). Comparative studies on *Tetrastichus hagenowii* (Ratzeburg) and *T. asthenogmus* (Waterston), two primary parasites of cockroach oothecae, and on their hyperparasite *Tetrastichus* sp. [*T. miser* (Nees) group] (Hymenoptera: Eulophidae). *Bulletin of Entomological Research*, **74**, 175-189.

Nealis, V. G., Jones, R. E. and Wellington, W. G. (1984). Temperature and development in host-parasite relationships. *Oecologia*, **61**, 224-229.

Nechols, J. R. and Tauber, M. J. (1977). Age-specific interaction between the greenhouse whitefly and *Encarsia formosa* : influence of the parasite on host development. *Environmental Entomology*, **6**, 207-210.

Nunney, L. and Luck, R. F. (1988). Factors influencing the optimum sex ratio in a structured population. *Theoretical Population Biology*, **33**, 1-30.

Oatman, E. R. and Kennedy, G. G. (1976). Methomyl induced outbreak of *Liriomyza sativae* on tomato. *Journal of Economic Entomology*, **69**, 667-668.

Oatman, E. R. and Michelbacher, A. E. (1958). The melon leaf miner, *Liriomyza pictella* (Thomson) (Diptera: Agromyzidae). *Annals of the Entomological Society of America*, **51**, 557-566.

Oatman, E. R. and Platner, G. R. (1969). An ecological study of insect populations on cabbage in southern California. *Hilgardia*, **40**, 1-40.

Opp, S. B. and Luck, R. F. (1986). Effects of host size on selected fitness components of *Aphytis melinus* and *A. lingnanensis* (Hymenoptera: Aphelinidae). *Annals of the Entomological Society of America*, **79**, 700-704.

Orzack, S. H. and Parker, E. D. Jr. (1986). Sex-ratio control in a parasitic wasp, *Nasonia vitripennis*. I. Genetic variation in facultative sex-ratio adjustment. *Evolution*, **40**, 331-340.

Pak, G. A., Buis, H. C. E. M., Heck, I. C. C. and Hermans, M. L. G.

(1986). Behavioural variations among strains of *Trichogramma* spp. : Host-age selection. *Entomologia Experimentalis et Applicata*, **40**, 247-258.

Parrella, M. P. (1982). A review of the history and taxonomy of economically important serpentine leafminers (*Liriomyza* spp.) in California (Diptera: Agromyzidae). *Pan-Pacific Entomologist*, **58**, 302-308.

Parrella, M. P. (1984). Effect of temperature on oviposition, feeding and longevity of *Liriomyza trifolii* (Diptera: Agromyzidae). *Canadian Entomologist*, **116**, 85-92.

Parrella, M. P. (1987). Biology of *Liriomyza*. *Annual Review of Entomology*, **32**, 201-224.

Parrella, M. P. and Bethke, J. A. (1984). Biological studies of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on chrysanthemum, aster and pea. *Journal of Economic Entomology*, **77**, 342-345.

Parrella, M. P. and Keil, C. B. (1984). Insect pest management : The lesson of *Liriomyza*. *Bulletin of the Entomological Society of America*, **30**, 22-25.

Parrella, M. P., Robb, K. L. and Bethke, J. A. (1983). Influence of selected host plants on the biology of *Liriomyza trifolii* (Diptera: Agromyzidae). *Annals of the Entomological Society of America*, **76**, 112-115.

Pollard, E. (1979). Population ecology and change in range of the white admiral butterfly *Ladoga camilla* L. in England. *Ecological Entomology*, **4**, 61-74.

Potter, D. A. and Gordon, F. C. (1985). Parasites associated with the native

holly leafminer, *Phytomyza ilicicola* Loew (Diptera: Agromyzidae) on American holly in Kentucky. *Journal of the Kansas Entomological Society*, **58**, 727-730.

Price, P. W. (1972). Parasitoids utilizing the same host : Adaptive nature of differences in size and form. *Ecology*, **53**, 190-195.

Prasad, Y. K., Vet, L. E. M., Minkenberg, O. P. J. M. and van Lenteren, J. C. (1991). Searching behaviour of *Dacnusa sibirica* Telenga on individual tomato plants infested with or without the leafminer *Liriomyza bryoniae* (Kaltenbach). *In preparation*.

Puttler, B. (1961). Biology of *Hyposoter exiguae* (Hymenoptera: Ichneumonidae), a parasite of lepidopterous larvae. *Annals of the Entomological Society of America*, **54**, 25-30.

Quednau, F. W. (1964). An evaluation of fecundity, host-mutilation and longevity on three species of diaspine scales in *Aphytis lingnanensis* Compere (Hymenoptera, Aphelinidae). *South. African Journal of Agricultural Science*, **7**, 521-530.

Ragusa, S. (1974). Influence of temperature on the oviposition rate and longevity of *Opius concolor siculus* (Hymenoptera: Braconidae). *Entomophaga*, **19**, 61-66.

Rao, S. V. and De Bach, P. (1969). Experimental studies on hybridization and sexual isolation between some *Aphytis* species (Hym.: Aphelinidae). *Hilgardia*, **39**, 515-567.

Rathman, R. J., Johnson, M. W., Rosenheim, J. A. and Tabashnik, B. E. (1990). Carbamate and pyrethroid resistance in the leafminer parasitoid *Diglyphus begini* (Hymenoptera: Eulophidae). *Journal of Economic Entomology*, **83**, 2153-2158.

Reeve, J. D. (1987). Foraging behaviour of *Aphytis melinus* : effects of patch density and host size. *Ecology*, **68**, 530-538.

Rotheray, G. E., Barbosa, P. and Martinat, P. (1984). Host influences on life history traits and oviposition behaviour of *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae). *Environmental Entomology*, **13**, 243-247.

Salt, G. (1970). *The Cellular Defense Reactions of Insects*. Cambridge University Press, pp. 31-50.

Samson, P. R. (1984). The biology of *Roptrocerus xylophagorum* (Hym.: Torymidae), with a note on its taxonomic status. *Entomophaga*, **29**, 287-298.

Sandlan, K. (1979). Sex ratio regulation in *Coccygomimus turionella* Linnaeus (Hymenoptera: Ichneumonidae) and its ecological implications. *Ecological Entomology*, **4**, 365-378.

SAS. (1985). *SAS User's Guide : Statistics, Version 5 Edition*. SAS Institute Inc., Cary, N.C.

Schmidt, J. M. and Smith, J. J. B. (1987). The measurement of exposed host volume by the parasitoid wasp *Trichogramma minutum* and the effects of wasp size. *Canadian Journal of Zoology*, **65**, 2837-2845.

Schuster, D. J. and Patel, K. J. (1985). Development of *Liriomyza trifolii* (Diptera: Agromyzidae) larvae on tomato at constant temperatures. *Florida Entomologist*, **68**, 158-161.

Shiga, M. and Nakanishi, A. (1968). Intraspecies competition in a field population of *Gregopimpla himalayensis* (Hymenoptera:

Ichneumonidae) parasitic on *Malacosoma neustria testacea* (Lepidoptera: Lasiocampidae). *Researches on Population Ecology*, **10**, 69-86.

Shorey, H. H. and Hall, I. M. (1963). Toxicity of chemical and microbial insecticides to pest and beneficial insects on poled tomatoes. *Journal of Economic Entomology*, **56**, 813-814.

Simbolotti, G., Putters, F. A. and van den Assem, J. (1987). Rates of attack and control of the offspring sex ratio in the parasitic wasp *Lariophagus distinguendus* in an environment where host quality varies. *Behaviour*, **100**, 1-32.

Skinner, S. W. (1985). Clutch size as an optimal foraging problem for insects. *Behavioural Ecology and Sociobiology*, **17**, 231-238.

Slansky, F. Jr. (1986). Nutritional ecology of endoparasitic insects and their hosts: an overview. *Journal of Insect Physiology*, **32**, 255-261.

Slansky, F. Jr and Scriber, J. M. (1985). Food consumption and utilization. *Comprehensive Insect Physiology, Biochemistry and Pharmacology (Volume 4: Regulation : digestion, nutrition, excretion)* (ed. by G.A. Kerkut and L.I. Gilbert), pp. 87-163. Pergamon Press, Oxford.

Smilowitz, Z. and Iwantsch, G. F. (1973). Relationships between the parasitoid *Hyposoter exiguae* and the cabbage looper *Trichoplusia ni*:: Effects of host age on developmental rate of the parasitoid. *Environmental Entomology*, **2**, 759-763.

Spencer, K. A. (1973). *Agromyzidae (Diptera) of Economic Importance*. Dr. W. Junk, B.V. Publishers, The Hague.

Spencer, K. A. (1977). A revision of the Australian Agromyzidae (Diptera).

Western Australian Museum Special Publication, **8.**

Speyer, E. R. and Parr, W. J. (1948). Tomato Leaf-miner (*Liriomyza solani*
Hering). *Report of the Experimental Research Station Cheshunt,*
34, 43-51.

Stegmaier, C. E. Jr. (1967). Host plants of *Liriomyza brassicae* , with records
of their parasites from South Florida (Diptera: Agromyzidae).
Florida Entomologist, **50,** 257-261.

Stegmaier, C. E. Jr. (1968). A review of recent literature on the host plant
range of the genus *Liriomyza* Mik (Diptera: Agromyzidae) in the
continental United States and Hawaii, excluding Alaska. *Florida
Entomologist,* **51,** 167-182.

Stephens, D. W. (1987). On economically tracking a variable environment.
Theoretical Population Biology, **32,** 15-25.

Stephens, D. W. and Krebs, J. R. (1986). *Foraging theory.* Princeton
University Press, Princeton, N.J.

Strand, M. R. (1986). The physiological interactions of parasitoids with their
hosts and their influence on reproductive strategies. *Insect
Parasitoids, 13th Symposium of the Royal Entomological Society*
(ed. by J. Waage and D. Greathead), pp. 97-137. Academic Press
Inc., London.

Strand, M. R. (1988). Adaptive patterns of progeny and sex allocation by
parasitic Hymenoptera. *Advances in Parasitic Hymenoptera
Research : Proceedings of the II Conference on the Taxonomy and
Biology of Parasitic Hymenoptera* (ed. by V.K. Gupta), pp. 293-
312. E.J. Brill, New York.

Sugimoto, T. (1977). Ecological studies on the relationship between the ranunculus leaf mining fly, *Phytomyza ranunculi* Schrank (Diptera: Agromyzidae) and its parasite, *Kratochviliana* sp. (Hymenoptera: Eulophidae) from the viewpoint of spatial structure. I. Analysis of searching and attacking behaviours of the parasite. *Applied Entomology and Zoology*, **12**, 87-103.

Sugimoto, T. and Ishii, M. (1979). Mortality of larvae of a ranunculus leaf mining fly, *Phytomyza ranunculi* (Diptera: Agromyzidae) due to parasitization and host-feeding by its eulophid parasite, *Chrysocharis pentheus* (Hymenoptera: Eulophidae). *Applied Entomology and Zoology*, **14**, 410-418.

Sugimoto, T., Kawado, K. and Tadera, K. (1988a). Responses to the host mine by two parasitic wasps, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae) and *Chrysocharis pentheus* (Hymenoptera: Eulophidae). *Journal of Ethology*, **6**, 55-58.

Sugimoto, T., Shimono, Y., Hata, Y., Nakai, A. and Yahara, M. (1988b). Foraging for patchily-distributed leaf-miners by the parasitoid, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae). III. Visual and acoustic cues to a close range patch-location. *Applied Entomology and Zoology*, **23**, 113-121.

Sugimoto, T., Uenishi, M. and Machida, F. (1986). Foraging for patchily-distributed leaf-miners by the parasitoid, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae) I. Discrimination of previously searched leaflets. *Applied Entomology and Zoology*, **21**, 500-508.

Takada, H. and Kamijo, K. (1979). Parasite complex of the garden pea leaf-miner, *Phytomyza horticola* Gourea, in Japan. *Kontyû*, **47**, 18-37.

Takagi, M. (1985). The reproductive strategy of the gregarious parasitoid, *Pteromalus puparum* (Hymenoptera: Pteromalidae). 1. Optimal number of eggs in a single host. *Oecologia*, **68**, 1-6.

Taylor, A. D. (1988). Host effects on functional and ovipositional responses of *Bracon hebetor*. *Journal of Animal Ecology*, **57**, 173-184.

Trivers, R. L. and Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, **179**, 90-92.

Trumble, J. T. and Nakakihara, H. (1983). Occurrence, parasitization and sampling of *Liriomyza* species (Diptera: Agromyzidae) infesting celery in California. *Environmental Entomology*, **12**, 810-814.

Turnbull, A. L. and Chant, D. A. (1961). The practice and theory of biological control of insects in Canada. *Canadian Journal of Zoology*, **39**, 697-753.

van Alphen, J. J. M. (1980). Aspects of the foraging behaviour of *Tetrastichus asparagi* Crawford and *Tetrastichus* spec. (Eulophidae), gregarious egg parasitoids of the asparagus beetles *Crioceris asparagi* L. and *C. duodecimpunctata* L. (Chrysomelidae). I. Host-species selection, host-stage selection and host discrimination. *Netherlands Journal of Zoology*, **30**, 307-325.

van Alphen, J. J. M. and Drijver, R. A. B. (1982). Host selection by *Asobara tabida* Nees (Braconidae ; Alysiinae) a larval parasitoid of fruit inhabiting *Drosophila* species. I. Host stage selection with *Drosophila melanogaster* as host. *Netherlands Journal of Zoology*, **32**, 215-231.

van Alphen, J. J. M. and Nell, H. W. (1982). Superparasitism and host discrimination by *Asobara tabida* Nees (Braconidae: Alysiinae), a

larval parasitoid of Drosophilidae. *Netherlands Journal of Zoology*, **32**, 232-260.

van Alphen, J. J. M. and Thunissen, I. (1983). Host selection and sex allocation by *Pachycrepoideus vindemiae* Rondani (Pteromalidae) as a facultative hyperparasitoid of *Asobara tabida* Nees (Braconidae; Alysiinae) and *Leptopilina heterotoma* Thomson (Cynipoideas; Eucoilidae). *Netherlands Journal of Zoology*, **33**, 497-514.

van Alphen, J. J. M., Nell, H. W. and Sevenster-van der Lelie, L. A. (1976). The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). VII. The importance of host feeding as a mortality factor in greenhouse whitefly nymphs. *Bulletin O.I.L.B./S.R.O.P.*, **4**, 165-169.

van den Assem J. (1971). Some experiments on sex ratio and sex regulation in the pteromalid *Lariophagus distinguendus*. *Netherlands Journal of Zoology*, **21**, 373-402.

van den Assem, J. (1976). Male courtship behaviour, female receptivity signal, and size differences between the sexes in Pteromalinae (Hym., Chalcidoidea Pteromalidae), and comparative notes on other chalcidoids. *Netherlands Journal of Zoology*, **26**, 535-548.

van den Assem, J. (1986). Mating behaviour in parasitic wasps. *Insect Parasitoids, 13th Symposium of the Royal Entomological Society* (ed. by J. Waage and D. Greathead), pp. 137-167. Academic Press Inc., London.

van den Assem, J., Putters, F. A. and Prins, Th. C. (1984). Host quality effects on sex ratio of the parasitic wasp *Anisopteromalus calandrae*

(Chalcidoidea, Pteromalidae). *Netherlands Journal of Zoology*, **34**, 33-62.

van den Bosch, R., Messenger, P. S. and Gutierrez, A. P. (1982). *An introduction to biological control*. Plenum Press, New York.

van Lenteren, J. C. (1976). The development of host discrimination and the prevention of super-parasitism in the parasite *Pseudeucoila bochei* Weld (Hym.: Cynipidae). *Netherlands Journal of Zoology*, **26**, 1-83.

van Lenteren, J. C. (1980). Evaluation of control capabilities of natural enemies: does art have to become science? *Netherlands Journal of Zoology*, **30**, 369-381.

Velthuis, H. H., Velthuis-Kluppell, F. M. and Bossink, G. A. (1965). Some aspects of the biology and population dynamics of *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae). *Entomologia Experimentalis et Applicata*, **8**, 205-227.

Vinson, S. B. (1972). Courtship behaviour and evidence for a sex pheromone in the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Environmental Entomology*, **1**, 409-414.

Vinson, S. B. (1976). Host selection by insect parasitoids. *Annual Review of Entomology*, **21**, 109-133.

Vinson, S. B. and Barras, D. J. (1970). Effects of the parasitoid, *Cardiochiles nigriceps*, on the growth, development, and tissues of *Heliothis virescens*. *Journal of Insect Physiology*, **16**, 1329-1338.

Vinson, S. B. and Iwantsch, G. F. (1980a). Host regulation by insect parasitoids. *Quarterly Review of Biology*, **55**, 143-165.

Vinson, S. B. and Iwantsch, G. F. (1980b). Host suitability for insect parasitoids. *Annual Review of Entomology*, **25**, 397-419.

Waage, J. K. (1982a). Sex ratio and population dynamics of natural enemies - some possible interactions. *Annals of Applied Biology*, **101**, 159-164.

Waage, J. K. (1982b). Sib-mating and sex ratio strategies in scelionid wasps. *Ecological Entomology*, **7**, 103-112.

Waage, J. K. (1986). Family planning in parasitoids: adaptive patterns of progeny and sex allocation. *Insect Parasitoids, Symposium of the Royal Entomological Society* (ed. by J. Waage and D. Greathead), pp. 63-95. Academic Press Inc., London.

Waage, J. K. and Godfray, H. C. J. (1985). Reproductive strategies and population ecology of insect parasitoids. *Behavioural ecology : ecological consequences of adaptive behaviour, 25th Symposium of the British Ecological Society* (ed. by R.M. Sibly and R.H. Smith), pp. 449-470. Alder Press, Oxford.

Waage, J. K. and Hassell, M. P. (1982). Parasitoids as biological control agents - a fundamental approach. *Parasitology*, **84**, 241-268.

Waage, J. K. and Lane, J. A. (1984). The reproductive strategy of a parasitic wasp. II. Sex Allocation and local mate competition in *Trichogramma evanescens*. *Journal of Animal Ecology*, **53**, 417-426.

Waage, J. K. and Ng, S. M. (1984). The reproductive strategy of a parasitic wasp. I. Optimal progeny and sex allocation in *Trichogramma evanescens*. *Journal of Animal Ecology*, **53**, 401-416.

Wardlow, L. R. (1985). Leaf-miners and their parasites. *Biological Pest*

Control - The glasshouse experience (ed. by N.W. Hussey and N. Scopes), pp. 62-65. Cornell University Press, New York.

Waterhouse, D. F. and Norris, K. R. (1987). Liriomyza species. *Biological Control Pacific Prospects*, pp.159-176. Inkata Press, Melbourne.

Webb, R. E. and Smith, F. F. (1969). Effect of temperature on resistance in lima bean, tomato and chrysanthemum to *Liriomyza munda*. *Journal of Economic Entomology*, **62**, 458-462.

Wellings, P. W., Morton, R. and Hart, P. J. (1986). Primary sex-ratio and differential progeny survivorship in solitary haplo-diploid parasitoids. *Ecological Entomology*, **11**, 341-348.

Wene, G. P. (1955). Effect of some organic insecticides on the population levels of the serpentine leafminer and its parasites. *Journal of Economic Entomology*, **48**, 596-597.

Werren, J. H. (1980). Sex ratio adaptations to local mate competition in a parasitic wasp. *Science*, **208**, 1157-1160.

Werren, J. H. (1983). Sex ratio evolution under local mate competition in a parasitic wasp. *Evolution*, **37**, 116-124.

Werren, J. H. (1984). A model for sex ratio selection in parasitic wasps: local mate competition and host quality effects. *Netherlands Journal of Zoology*, **34**, 81-96.

Westerman, P. R. and Minkenberg, O. P. J. M. (1986). Evaluation of the effectiveness of the parasitic wasps *Diglyphus isaea* and *Chrysocharis parksi* in experimental greenhouses for the biological control of the leafminer *Liriomyza bryoniae*, on tomatoes. *Mededelingen van de Faculteit Landbouwwetenschappen*

Rijksuniversiteit Gent, **51**, 999-1008.

Wharton, R. A. (1988). Classification of the Braconid subfamily Opiinae (Hymenoptera). *Canadian Entomologist*, **120**, 333-360.

Wharton, R. A. and Gilstrap, F. E. (1983). Key to and status of Opiine Braconid (Hymenoptera) parasitoids used in biological control of *Ceratitis* and *Dacus s. l.* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, **76**, 721-742.

Wiackowski, S. K. (1962). Studies on the biology and ecology of *Aphidius smithi* Sharma & Subba Rao (Hymenoptera, Braconidae), a parasite of the pea aphid, *Acrythosiphon pisum* (Harr.) (Homoptera, Aphididae). *Polskie Pismo Entomologiczne*, 32, 253-310.

Wilson, F. (1961). Adult reproductive behaviour in *Asolcus basalis* (Hymenoptera: Scelionidae). *Australian Journal of Zoology*, **9**, 739-751.

Wilson, F. and Huffaker, C. B. (1976). The philosophy, scope, and importance of biological control. *Theory and Practice of Biological Control* (ed. by C.B. Huffaker and P.S. Messenger), pp. 3-15. Academic Press Inc., New York.

Wolfenbarger, D. O. (1958). Serpentine leafminer : Brief history and a summary of a decade of control measures in South Florida. *Journal of Economic Entomology*, **51**, 357-359.

Wong, T. T. Y., Ramadan, M. M., McInnis, D. O. and Mochizuki, N. (1990). Influence of cohort age and host age on oviposition activity and offspring sex ratio of *Biosteres tryoni* (Hymenoptera: Braconidae), a larval parasitoid of *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Economic Entomology*, **83**, 779-783.

Wylie, H. G. (1966). Some effects of female parasite size on reproduction of *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae). *Canadian Entomologist*, **98**, 196-198.

Zar, J. H. (1984). *Biostatistical Analysis, 2nd edition.* Prentice-Hall International, Inc., New Jersey.

Zitter, T. A. and Tsai, J. H. (1977). Transmission of three polyviruses by the leaf-miner *Liriomyza sativae* (Diptera: Agromyzidae). *Plant Disease Reporter*, **61**, 1025-1029.

Zucchi, R. and van Lenteren, J. C. (1978). Biological characteristics of *Opius pallipes* Wesmael (Hymenoptera: Braconidae), parasite of the tomato leafminer *Liriomyza bryoniae* Kalt. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent*, **43**, 455-462.

Zwölfer, H. (1971). The structure and effect of parasite complexes attacking phytophagous host insects. *Dynamics of Populations* (ed. by P. J. den Boer and G. R. Gradwell), pp. 405-418. Centre for Agricultural Publishing and Documentation, Wageningen.

ADDENDUM TO THESIS

Comparative Host Stage Utilization of Two Parasitoids of *Liriomyza brassicae* (Diptera : Agromyzidae)

Ross Lardner

INTRODUCTION

This addendum includes corrections to my thesis, as recommended by the examiners. Included are i) a partial revision of Chapter Six following the reanalysis of the sex ratio data; ii) discussion of various concerns raised by the examiners; and iii) some shorter notes correcting minor problems in the thesis.

1. RE-ANALYSIS OF SEX ALLOCATION DATA IN CHAPTER SIX

Abstract

Reanalysis of the sex ratio data presented in Chapter Six confirmed the previous interpretation of the results for no choice experiments but indicated a different interpretation of the choice experiments was required.

Sex allocation by *O. cinerariae* was not influenced by the host stage available, within the first 10 and 40 days of reproduction. During the choice experiments, *O. cinerariae* produced a similar proportion of male offspring on either of the host instars offered, indicating sex allocation was not influenced by host stage. In contrast, *H. varicornis* produced a greater proportion of male offspring on 2nd than 3rd instar *L. brassicae* larvae, within both the first 10 and 40 days of oviposition.

Background

In the light of the examiners comments it was decided that some of the analysis of sex allocation in Chapter Six was inappropriate. The use of contingency table analysis required the pooling of results from all females, thereby ignoring the allocation by individual females. This could lead to erroneous conclusions of the sex allocation strategy of a species as aberrant females may unduly influence the results. To overcome this problem, the data were re-analysed by a technique which used the sex ratios produced by individual females.

Materials and Methods

Data were analysed by ANOVA, or t-tests when appropriate, using arcsine transformed values of sex ratios (proportion males) produced by individual females. The comparison of the proportion of males produced on the instars within each choice experiment was done using paired t-tests.

Results

The new analysis revealed no significant difference in sex allocation by *O. cinerariae* on the various instars of *L. brassicae*, regardless of whether ratios produced between days 1 and 10 (proportion males produced on 1st instars = 0.356 ± 0.052, 2nd instars = 0.355 ± 0.025, 3rd instars = 0.376 ± 0.036) or days 1 and 40 (proportion males produced on 1st instars = 0.321 ± 0.061, 2nd instars = 0.399 ± 0.034, 3rd instars = 0.460 ± 0.053) were considered. Inadequate sample sizes prevented analysis of sex ratios produced between days 15 and 40. *H. varicornis* produced a greater proportion of males on 2nd than 3rd instar larvae within both the first 10 (proportion males produced on 2nd instars = 0.964 ± 0.014, 3rd instars = 0.536 ± 0.037) and 40 days (proportion males produced on 2nd instars = 0.996 ± 0.004, 3rd instars = 0.591 ± 0.054) of oviposition ($F_{1,18}$ = 125.4 and $F_{1, 32}$ = 150.3, respectively, $P < 0.01$).

No significant difference was found between the mean proportion of males produced by *O. cinerariae* on either host instar during both choice tests. In choice experiment one, the mean proportions of males produced (± standard error) were 0.491 ± 0.040 (instars pooled), 0.416 ± 0.053 (1st instars) and 0.531 ± 0.042 (2nd instars). During the second choice experiment, the mean proportions of male offspring were 0.440 ± 0.031 (instars pooled), 0.416 ± 0.049 (2nd instars) and 0.478 ± 0.045 (3rd instars). A comparison of sex ratios produced on all instars in each choice test revealed no significant difference between the two tests.

Discussion

In light of this new analysis, some of the conclusions of Chapter 6 need re-evaluation. *O. cinerariae* did not shift sex allocation in response to having a choice of host instars available, suggesting that host quality is not assessed on a relative basis (in contrast to the previous statement on page 144, lines 24-25). Each instar of *L. brassicae* may represent a similar value resource for *O. cinerariae* and, consequently, females have nothing to gain by differential allocation of the sexes to the various host stages (in contrast to an earlier statement on page 143, line 25-26).

2. DISCUSSION OF EXAMINER'S COMMENTS

For the purposes of Table 2.2 (page 19), the different instars of *L. brassicae* were identified by locating and counting the number of mouth-hooks in the mines. At each moult, a new cephalo-pharyngeal skeleton is formed and the previous one is discarded with the moulted exocuticle. If no mouth-hooks were found in the mine, the larva was a 1st instar; one mouth-hook indicated a 2nd instar larva; and two mouth-hooks meant the larva

was a 3rd instar. Only mines which did not overlap with other mines were used in this study. The cephalo-pharyngeal skeleton was dissected from each larva and the length from the anterior edge of the mouth-hook to the posterior end of the arms of the skeleton was measured using an eye-piece micrometre. During the studies of development, it was also necessary to know the stage of development of the *L. brassicae* larvae. This could be achieved by determining the approximate mouth-hook length of each individual. As the leaf surface was translucent, the mouth-hook was readily visible. Although an accurate length of the mouth-hook could not be determined, it was possible to place the structure within one of the different developmental stages by comparing it's approximate length to the ranges listed in Table 2.2.

Time constraints prevented experiments being conducted investigating the encounter rates of *O. cinerariae* and *H. varicornis* with the mines formed by the different instars of *L. brassicae*. However, studies of other parasitoids of agromyzids have shown that the mines of older host stages are more rapidly located (Sugimoto 1977, Hendrikse *et al.* 1980, Minkenberg and van Lenteren 1986) and it is reasonable to assume similar behaviour by parasitoids of *L. brassicae*. If this is shown to be true, then the results presented in Chapter Four suggest that *O. cinerariae* was not attacking all hosts encountered, particularly 3rd instar hosts. This will remain speculation until observational studies are done enabling differential encounter rates to be distinguished from parasitoid choice. Furthermore, the role of differential mortality must be determined by dissection.

The conclusion that naive parasitoids were conserving reproductive potential may be unjustified (Chapter Four). The low numbers of progeny produced need not indicate conservation of reproductive potential, rather inexperience with locating and handling hosts. Other factors may also limit the oviposition activity of naive females, such as insufficient egg loads and the time spent acquiring nutrients essential for the maturation

of eggs. The naive females used during the experiments discussed in Chapter Four were between 1 and 6 days old and had access to food during this period. During the pre-experimental period, these females should have been able to mature many more eggs than were oviposited during the experiment. Egg loads of newly emerged females (as determined in Chapter 7) indicate that females, particularly of *O. cinerariae*, have many more eggs available than they oviposit.

At equilibrium, equal benefits are supposedly gained from each sex and the sex ratio produced should be approximately 1:1. It was suggested in Chapter Six that differential reproductive fitness of male and female offspring may select for the production of female-biased sex ratios by parasitoids attacking highly variable host populations. One examiner suggested that differential variance in the reproductive success of the sexes would not affect optimal sex ratio. However, if one sex provides greater benefits than the other (i.e. passes on more of the parent's genes to the succeeding generation), then selection should cause a shift to favour the production of this sex. In patchily distributed, low density parasitoid populations, females may be more successful at locating hosts than males are at locating virgin females. The greater reproductive success of females means they will pass on more of their parents' genes than sibling males. Therefore, females which produce female-biased broods are more likely to have their genetic material transferred to the F2 generation and, consequently, female-biased sex ratios would be selected. However, a balance must exist so that enough males are produced to successfully locate and mate the females in the population. Strongly female-biased sex ratios may not provide enough males for this task, while slightly female-biased sex ratios may be better suited to patchily distributed, low density parasitoid populations.

In Chapter Seven, egg loads of parasitoids were determined within the first day of emergence. It was suggested by an examiner that the egg loads recorded may

reflect the different times, post-emergence, that the females were killed rather than differences between females from different stages of host development. Females were frozen within the first 12 hours of emergence so there was some variation in the length of life prior to freezing. These females were not fed, slowing the maturation of eggs. It was not known at the start of this experiment whether these species were proovigenic or synovigenic, although the latter was expected. While it is accepted that different age females will carry different egg loads, similar variation in female ages exist for parasitoids from each host developmental stage. Therefore, the ten-fold increase in egg load of female *H. varicornis* from 3rd instar larvae as compared to those from 2nd instars cannot be solely attributed to the different age of females at the time of freezing. However, this difference may be partly a result of the small sample size of females from 2nd instar hosts. As seen from Chapter 5, very few female *H. varicornis* are produced on 2nd instar *L. brassicae* . Repeated efforts were made to increase the sample size of this group to no avail. With the knowledge that this is a synovigenic species, a repeat experiment using females frozen at the same age would be desirable. However, indications are that this difference in egg load between female *H. varicornis* from different host instars is real, especially considering that females from 2nd instar hosts were found to carry a maximum of 1 mature egg compared to 3 for females from 3rd instars.

In Chapter Seven, an attempt was made to compare the relative fitness of male and female parasitoids through as series of laboratory experiments. Quantitative comparison of male and female fitness, as measured in laboratory experiments, is extremely difficult for several reasons. Firstly, it is impossible to measure all fitness components as many of them remain unknown. Therefore, we can only get a partial impression of fitness. Secondly, the relationship between laboratory fitness measures and field performance is largely unknown. Finally, it is not possible to compare directly male and female fitness as different variables must be measured. Until we know how these

variables relate, laboratory investigations of male and female fitness can only indicate qualitative differences.

3.   MINOR CORRECTIONS THROUGHOUT THESIS

Page 42, lines 15-18:   This should read "Many of the plant species which *L. brassicae* was found attacking around Adelaide have previously been recorded as host plants for this species (Stegmaier 1967, 1968; Kleinschmidt 1970; Spencer 1973, 1977; Beri and Chandra 1983)."

Page 60, lines 20-21:   This should refer to Table A3.2.

Page 62, 63:   The taxonomic confusion concerning some of the parasitoid species, particularly *O. atricornis*, *O. cinerariae* and *Chrysonotomyia* sp., means previous host records should be considered with caution.

Page 64, lines 25-26:   Established parasitoid species may be able to out-compete those species which are colonising an area.

Page 81, Tables 4.5 and 4.6:   Inadequate sample sizes were used to determine if female age influenced the number of progeny produced.   Therefore, the conclusion on line 4 of page 81 is not justified.   It is unknown if the age of female *H. varicornis* or *O. cinerariae* when they first encountered hosts influenced the number of progeny they produced.

Page 109:   Some of the differences observed between days and instars for the no-choice and choice experiments may have been significant by chance.

Page 119, lines 6-10:   This should read "Waage (1982a) hypothesized that host-size dependent sex ratios would only occur in parasitoids of non-growing hosts.   His premise was that for parasitoids of growing hosts, host size at oviposition would not be a good indicator of the amount of larval resource.   If this hypothesis is true, then idiobiont parasitoids should display host-size dependent sex ratios while koinobionts should not (King 1989)."

Page 119, lines 16-17:   This should read "Therefore, there is a high probability of inbreeding."

Page 178, lines 1-3 and 5-7:   as a result of the new analysis in Chapter 6, these statements are not justified.

Page 180, lines 24-26:   This should read "This parasitoid displayed host-stage dependent levels of reproduction, but sex allocation was not influenced by the range of host stages available."

Page 192, line 21 onwards: Disregard this section as the new analysis of sex ratio data in Chapter 6 revealed male production was not greater in 2nd than 1st instars during choice experiment 1.