Sex Ratio Strategies in the Facultatively Autoparasitic Wasp, *Encarsia tricolor* Förster.

By

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ABSTRACT

Heteronomous hyperparasitoids are a group of aphelinid parasitoids in which the sexes show different host relations. Females *always* develop as primary endoparasitoids of Homoptera. Male development is different and has been used to classify the group under three headings:

a: obligate autoparasitoids - males develop hyperparasitically only on immature female conspecifics
b: facultative autoparasitoids - males develop as hyperparasitoids of primary endoparasitoids including conspecific females
c: alloparasitoids - males develop only as hyperparasitoids of non-conspecific primary endoparasitoids.

This study set out to explore the adaptive significance of heteronomous hyperparasitism and the effects that such remarkable host relations have on the sex ratio and population dynamics of these parasitoids.

Encarsia tricolor Förster, a facultative autoparasitoid native to the UK, was selected for study. Initially the biology of this species was investigated using a typical primary host, the Cabbage Whitefly, *Aleyrodes proletella*. *E. tricolor* was long-lived, had a low fecundity and could successfully parasitise all primary (female eggs) and secondary (male eggs) host instars. When all primary host stages were offered, larger hosts were preferentially parasitised, but all whitefly stages were used for host feeding.

Factors affecting the wasp's ovipositional decisions were investigated further. *Encarsia inaron* (Walker), a conventional bisexual endoparasitoid, was used throughout the study as a non-conspecific secondary host for the hyperparasitic *E. tricolor* males. Preferential exploitation of *E. inaron* rather than conspecific *E. tricolor*, was shown in laboratory choice experiments. This ovipositional preference was a critical factor in illustrating the selective advantage to heteronomous hyperparasitism, and strongly influenced the sex ratio and the outcome of inter-specific competition between the conventional parasitoid and *Encarsia tricolor*. In practical terms, such preferences should also modify the use of heteronomous hyperparasitoids for biological control.

Field studies supported the lab-findings of preferential hyperparasitism of *E. inaron* over conspecifics. *E. tricolor* and *E. inaron* occurred together in patches significantly more often than expected given random association. *E. tricolor* male production was more common in large parasitised patches of both species than in smaller patches.

In the absence of alternative male hosts, a heteronomous hyperparasitoid must resort to hyperparasitism of conspecifics (autoparasitism), to which she may be related. Evidence is presented to show that when offered patches of different ratios of parasitised and unparasitised hosts for the production of males and females respectively, *E. tricolor* sex ratio was not influenced by the availability of each host type. Prior ovipositional experience however, was found to be important in affecting the sex ratio of these wasps. Four types of experience were offered:

a: none
b: laying male eggs in *E. tricolor* pupae
c: laying female eggs in whitefly nymphs
d: laying male eggs in *E. inaron* pupae

Wasps exposed to the first two treatments produced unbiased mean sex ratios. Wasps given the third treatment laid a significantly female biased sex ratio. After exposure to *E. inaron* pupae (and then offered different ratios of *E. inaron* and whitefly) a significantly male biased sex ratio was produced. The adaptive significance of such sex ratios are discussed.

Caged competition experiments between these two species showed the heteronomous hyperparasitoid to have a remarkable competitive ability, and completely displaced large populations of the normally-reproducing *E. inaron*. *E. inaron* however, could not manage significant levels of reproduction in a culture cage containing an established population of *E. tricolor*.

Finally, modifications to the classification of heteronomous hyperparasitoids are proposed which removed the strict divisions by which the group was previously recognised. In place, a more ecologically realistic viewpoint is recommended under which ovipositional preferences (commonly observed) are distinguished from physiological necessity (rare) and allow the whole group to be identified simply as "heteronomous hyperparasitoids". The evolutionary pathway of other heteronomous parasitoids is also discussed in the light of these findings.

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TABLE OF CONTENTS

		Page
Abstract		2
Acknowledgements		3
Table of Contents		4
List of Figures		7
List of Tables		8
Chapter 1:	**SEX RATIO THEORY**	9
	1.1 When mating is random	9
	1.2 Structured populations	11
	1.2.1 Local mate competition	11
	1.2.2 Sibling interaction	12
	1.2.3 Haystack models	13
	1.3 Changing fitness	13
	1.3.1 Host quality	14
	1.3.2 Seasonality	15
	1.4 Asymmetry of relatedness	15
	1.5 Sex ratio diseases	15
	1.6 Testing the theory	16
	1.7 Summary	19
Chapter 2:	**INTRODUCTION TO HETERONOMOUS HYPERPARASITOIDS**	20
	2.1 What are heteronomous hyperparasitoids?	20
	2.2 Sex ratio and population dynamics in autoparasitoids	24
	2.3 Introduction to *E. tricolor*	28
	2.4 Aims of the study	28
Chapter 3:	**MATERIALS AND METHODS**	29
	3.1 Collection of whitefly and parasitoids	29
	3.2 Whitefly and parasitoid cultures	29
	3.3 Clip cage experiments	30
	3.4 Data collection	31
Chapter 4:	**BIOLOGY OF *ENCARSIA TRICOLOR***	36
	4.1 Introduction	36
	4.2 Biological characteristics of *Encarsia tricolor*	41
	4.2.1 Longevity	41
	4.2.2 Instar acceptability for female eggs and female development time	45
	4.2.3 Instar acceptability for male eggs and male development time	48
	4.2.4 Fecundity	49
	4.2.5 Culture sex ratio	50

4.2.5.1 Sex ratio dynamics on individual leaves as a function of their period of exposure to parasitism 52
4.2.5.2 Sex ratio dynamics in the culture cage as a function of the age of the culture 52
4.2.6 Oviposition time 54
4.3 Summary 60

Chapter 5: SPECIES DISCRIMINATION AND KIN RECOGNITION 62
5.1 Species discrimination 62
5.2 Kin recogition 65
5.3 Summary 68

Chapter 6: HOST AVAILABILITY AND SEX RATIO IN *E. TRICOLOR* 69
6.1 Introduction 69
6.2 Methodology 71
6.3 Statistical analysis 72
6.4 Results 73
6.5 Discussion 77

Chapter 7: INTERSPECIFIC COMPETITION 81
7.1 Introduction 81
7.2 Methodology 82
7.3 Results 83
7.4 Discussion 88
7.4.1 Some classic examples of competition in biological control 89
7.4.2 Competition involving heteronomous hyperparasitoids 90
7.4.3 Parasitoid complexes containing a heteronomous hyperparasitoid 94

Chapter 8: FIELD WORK 101
8.1 Methodology 101
8.1.1 Honeysuckle: 1986-1987 102
8.1.2 Brussels Sprout: 1987 102
8.1.3 Brussels Sprout: 1988 103
8.2 Results 105
8.2.1 Honeysuckle: 1986-1987 105
8.2.2 Brussels Sprout: 1987 105
8.2.3 Brussels Sprout: 1988 112
8.2.3a parasitism on the lab-treated plants 112
8.2.3b parasitism within the Brussels crop 112
8.3 Discussion 115

Chapter 9: **DISCUSSION** 118
9.1 Decision making during oviposition 118
9.2 Host selection and sex ratio 119
9.3 Autoparasitoid classification 122
9.4 Biological control 124
9.5 The evolution of heteronomous hyperparasitism 127
9.6 Conclusions 130

Chapter 10: **SUMMARY** 133

References 136
Appendix 1: Aphelinid fecundities from the literature 152

LIST OF FIGURES

Number	Title	Page
1.1	The observed sex ratio changes for four fig wasp species and *Nasonia vitripennis*	17
2.1	Diagramatic form of heteronomous parasitoid classification	22
	Photographs: Clip cage (side view)	32
	Clip cage (from beneath)	32
	Several clip cages set up on the same plant	33
	Culture cages in the 25°C room	33
	E. tricolor parasitising whitefly nymphs	34
	E. inaron parasitising whitefly nymphs	34
	Parasitised and unparasitised whitefly on Brussels leaf	35
	Emergence cases of whitefly and *E. tricolor*	35
4.1-4.2	Regression of parasitoid longevity on size for both sexes of *E. tricolor*	43
4.3	Pattern of mortality for both sexes of *E. tricolor* over the duration of the longevity experiment	44
4.4	Pattern of emergence of female *E. tricolor* from each host instar	47
4.5	Fecundity of *E. tricolor* when laying female eggs in unparasitised hosts over the lifetime of the wasp	51
4.6-4.9	Emergence of both sexes of *E. tricolor* from samples taken from plants exposed to parasitism for 14-35 days	53
4.10	Mean percentage parasitism of whitefly on individual leaves after different periods of exposure to parasitism	55
4.11	Overall sex ratio of *E. tricolor* emerging from leaves sampled following different periods of exposure to parasitism	55
4.12	Predicted flucuations in culture sex ratio over the period of the formal sampling regime	56
6.1-6.4	Scatter plots of sex ratios laid following four types of prior ovipositional experience	74-75
6.5	Mean sex ratio (with 95% CL) laid following four types of ovipositional experience	76
6.6	Mean number of hosts parasitised (with 95% CL) following each type of ovipositional experience	76
7.1a-7.4a	Mean percentage parasitism in each sample from competition experiment: cages 1-4	84-87
7.1b-7.4b	Changes in the number of *E. inaron* in each sample from competition experiment: cages 1-4	84-87
7.1c-7.4c	Changes in the number of *E. tricolor* in each sample from competition experiment: cages 1-4	84-87
7.5	Competitive exclusion of *Amitus hesperidium* and *Encarsia smithi* by *Encarsia opulenta* during Citrus Blackfly biocontrol program: **a:** Blackfly population **b:** Estimated percentage parasitism by each species **c:** Parasitoid populations	93
8.1-8.3	Flucuations in the number of *Aleyrodes lonicerae* nymphal stages and number of parasitised scales over the 1986-1987 honeysuckle sampling program: sites 1-3	106-108
8.4	Regression of proportion primary parasitism by *E. tricolor* on number of whitefly per leaf	114

LIST OF TABLES

Number	Title	Page
4.1	Primary host records of *Encarsia tricolor*	37
4.2	Longevity of *E. tricolor* in relation to size	42
4.3	Instar acceptability and development time of females	45
4.4	Instar acceptability and development time for males	49
4.5	Emergence of parasitoids at different intervals after introduction to the culture	54
4.6	Observed mean oviposition times for male and female eggs in conspecific pupae and late instar whitefly nymphs respecively	57
4.7	Female ovipositional preferences when all primary host instars were offered to previously unexperienced females	58
4.8	Dissection data showing the frequencies of encapsulation of male amd female eggs	59
5.1	Species discrimination by *E. tricolor*	63
5.2	Effect of host species on size of male *E. tricolor*	64
5.3	Kin recognition in *E. tricolor*	67
6.1	GLIM analysis of the effect of host availability on sex ratio	73
6.2	GLIM analysis of the effect of experience on sex ratio	77
6.3	Effect of experience on mean sex ratio and mean number of eggs laid	78
7.1	Natural communities: a) facultative autoparasitoid dominant	95
	b) primary parasitoid dominant	98
7.2	Biocontrol parasitoid complexes: a) facultative autoparasitoid dominant	99
	b) primary parasitoid dominant	100
8.1	Diagnostic features of pupae/pupal cases used in field studies	103
8.2	Percentage parasitism of *A. lonicerae* in honeysuckle samples	105
8.3	Number and types of patch recorded in Brussels crop, 1987	109
8.4	Patch size, percentage parasitism and sex ratio for mono-specific and mixed patches in Brussels crop, 1987	110
8.5	The relationship between *E. tricolor* sex ratio and size of patch	111
8.6	The relationship between *E. tricolor* sex ratio and number of *E. inaron* present in each mixed species patch	112
8.7	*E. tricolor* sex ratio in early and late part of 1987 field season	112
8.8	Summary of results from lab-parasitised Brussels plants	113
8.9	GLIM model: Regression of percentage parasitism by *E. tricolor* on whitefly density	115
9.1	Species of suspected obligate autoparasitoids for which the host relations are uncorroborated or ambiguous	123

CHAPTER 1

SEX RATIO THEORY

1.1 When mating is random

An evolutionarily stable strategy (ESS) when adopted by a population, cannot be invaded by the spread of a rare mutant playing an alternative strategy (Maynard Smith 1972). For the majority of animal species, the production of an unbiased sex ratio is an ESS. This is because individuals of dioecious species are equally related to each of their parents; exactly half the genome of each offspring comes from the paternal male and half from the maternal female. Each sex makes an equal genetic contribution to the production of progeny. Thus, overrepresentation of one sex in the population leads to greater reproductive success for the rarer sex. Fisher (1930) argued that selection will act in favour of mothers biasing the sex ratio of their progeny towards the underrepresented sex. This frequency dependent natural selection will result in a balance at which reproductive gains through sons equal those through daughters.

When the reproductive gains through each sex are equal, a parent with a fixed amount of reproductive effort should invest equally in the production of each sex. For an organism in which the cost of producing each sex is identical the optimal primary sex ratio is 1:1.

The costs of the sexes may differ however, due to energy requirements in rearing, the probability of mortality in the period of parental care, or the parental mortality which one sex may inflict with a greater probability than the other. The optimal sex ratio (r) in this situation will be dependent on the relative costs of sons and daughters, such that:

$$sex \quad ratio, \quad r = \frac{cost \quad of \quad a \quad daughter}{cost \quad of \quad a \quad son}$$

Fisher's theory is remarkably robust withstanding many kinds of life-histories, differential mortality and migration (Kirkpatrick and Bull 1987).

MacArthur (1965) formalised the ESS approach to sex allocation by showing that a female in a population showing discrete generations should maximise the product of

average number of sons *(m)* and daughters *(f)* surviving to adulthood. MacArthur derived a general equation [from one originally produced by Shaw and Mohler (1953)] to show the conditions under which a rare mutant sex ratio could invade a population:

$$W_t \propto \frac{\bar{m}}{m} + \frac{\bar{f}}{f} \quad \ldots\ldots\ldots\ldots (1)$$

Where $W_t =$ total maternal fitness

$\bar{m} =$ surviving sons from a mutant female

$m =$ surviving sons from a normal female

$\bar{f} =$ surviving daughters from a mutant female

$f =$ surviving daughters from a normal female

The parameters m and f can equally be considered as differences in fitness gains through male and female function. A mutant will only be selected for if the right hand side of the equation exceeds 2, i.e. if a mutant has greater fitness than the normal-type female. Charnov (1982) points out that when production of extra individuals of one sex does not affect the reproductive success of other individuals of that sex, the ESS strategy is to maximise the product of fitness gains through male and female function through control of brood size, resource allocation and sex ratio:

$$maximise(m.f)$$

Although as previously mentioned, m and f are fitness gains accrued through individuals which survive to reproduce, the survivorship values cancel out when deriving equation (1). Thus in this situation, differential mortality of the sexes does not affect the primary sex ratio (at conception). The predicted ESS sex ratio will depend on the tradeoff between male and female production as shown graphically by Charnov (1982). Where the tradeoff is linear, producing a daughter instead of a son (or vice versa) requires no change in parental investment. In this case the product theorem equates to Fisher's theory.

One of the fundamental assumptions of these theories is that of random mating. As Leigh, Herre and Fischer (1985) point out however, mating is never truly random. In most populations an individual will probably mate with a neighbour. When the population is spatially structured, an individual will find itself in an increasingly restricted circle of available mates.

1.2 Structured populations

1.2.1 Local mate competition

Hamilton (1967) considered a population structure that was particularly applicable to parasitoid wasps. Females search for isolated patches or hosts. Each patch is discovered by a variable number (n) of diploid foundress females. These females produce a discrete generation of sons and daughters which develop and mate at random within the patch. Males cannot disperse but inseminated females emigrate to find new patches where the process is repeated.

Following the method of Charnov (1982) and Karlin and Lessard (1986) let the common brood sex ratio be r and then consider a rare mutant who produces a proportion $\hat{r}$ sons. The mutant will find itself in a group of $(n-1)$ foundress females who each produce b offspring. A mutant female will accumulate fitness (W_t) via her daughters and via those females fertilised by her sons:

$$W_t = No.\ mutant\ females + \frac{No.\ mutant\ males}{Total\quad males}(No.\ of\ mutant\ females + No.\ of\ normal\ females)$$

$$W_t = b(1-\hat{r}) + \left(\frac{\hat{r}b}{\hat{r}b + rb(n-1)}\right)(b(1-\hat{r}) + b(1-r)(n-1)).....(2)$$

The ESS sex ratio (r^*) is that at which a mutant cannot do better by setting $\hat{r}$ different from r, i.e. $\partial W_t/\partial \hat{r}\,|_{r=\hat{r}} = 0$. At this point:

$$r^* = \frac{n-1}{2n}.....(3)$$

When the number of foundress females is large, the ESS sex ratio tends to 0.5. When n=1 there is only sibmating and a female should only produce enough sons to mate all her daughters. Equation (3) has been derived in a slightly altered form under a number of different assumptions:

a. if males and females are laid in different patches (Maynard Smith 1985),

b. if males form leks whereas females disperse and mate after dispersal (Werren 1983; Bulmer 1986),

c. if sibmating is prohibited within the patch [given that $n \geq 2$] (Charnov 1982),

d. if the organism shows haplodiploid sex determination (Hamilton 1979; Taylor and Bulmer 1980).

In all cases a female biased sex ratio of varying degrees is predicted.

Actually, two factors have been identified as advantageous to individuals producing a female biased sex ratio in response to local mate competition (LMC):

1. diminishing returns associated with producing each additional son

2. females can increase the reproductive success of their sons by providing more daughters - given high probability of sibmating.

When sibling males no longer compete locally but the structure of the population is retained, the optimum sex ratio returns to 0.5 (Maynard Smith 1985).

1.2.2 Sibling Interaction

If one sex has a deleterious effect on the other, such that a change in the sex ratio changes the sex-specific mortality or fitness in a brood, then a bias in favour of the less detrimental sex will result. In parasitoids, males tend to survive better than females. Females also tend to suffer a greater decriment in fitness when developing with brothers rather than with sisters (Waage and Godfray 1985).

Godfray (1986) has developed models to elucidate the effect of sibling interaction on clutch size and sex ratio applicable to parasitoids developing gregariously. He paid particular attention to LMC situations when the sexes were affected differently by the sexual composition of their competitors. When progeny only competed with siblings for food, but competed with all the offspring in the patch for mates, Godfray showed that an increase in the fitness of sons could be achieved by a change in brood composition. When the progeny competed amongst related and unrelated members of the patch equally for food and for mates, then a change in brood sex ratio was shown to benefit all the

males in the patch equally. Such a strategy does not preferentially benefit the sons of an individual foundress female and so fails as an ESS. In this situation, sex ratio or clutch size adjustment will only be influenced by food competition between females.

1.2.3 Haystack models

A few papers have shown that there are other pressures acting to produce female biased sex ratios in structured populations. Bulmer and Taylor (1980) considered the sex ratio when the population divides and reproduces in discrete groups, such as that of a hypothetical mouse inhabiting haystacks.

At the beginning of each season a stack is colonised by a number of fertilised female mice whose offspring breed there until the following year when new haystacks become available. Females mate before dispersal to new stacks. The "success" of each stack depends on the number of females that disperse from it. Selection *within* stacks favours a Fisherian sex ratio of 0.5. Stacks containing individuals which produce more daughters are, however, more productive compared to completely Fisherian stacks. Thus selection *between* stacks favours a female bias.

Colwell (1981), Wilson and Colwell (1981) consider an almost identical situation (without the mice) in which the population is made up of Fisher-type females producing an unbiased sex ratio and Hamilton-type females who produce a sex ratio <0.5. Again the population is subdivided into locally mating groups (demes). Wilson and Colwell clearly demonstrate that the greater productivity of demes containing (by sampling error) more Hamiltonian females allows this trait to be maintained despite selection for unbiased sex ratio at the level of the individual. When the Hamiltonian females produce the optimal proportion of males $\frac{n-1}{2n}$, Hamiltonian genes actually increase in frequency globally (Colwell 1981). They attribute this phenomenon purely to selection at the level of the group rather than LMC. As previously mentioned however, Maynard Smith (1985) showed that group structure without LMC leads to an unbiased sex ratio. Thus LMC by itself must be able to select for the female bias (Harvey 1985).

1.3 Changing fitness

The previous section on population structure assumed that when the whole population produces a 1:1 sex ratio, the sexes have equal fitness (Taylor 1981). There are cases however, when the fitness of one sex changes to a proportionally greater extent than the other. Here I will consider two factors which have been shown to effect progeny fitness and which have relevance to parasitoids: host quality and seasonality.

1.3.1 Host quality

Trivers and Willard (1973) introduced the idea that progeny fitness may vary with sex in response to specific environmental conditions. If a mother can produce a daughter of average fitness or a son of above average fitness, depending on her own condition, then selection favours production of a son (given a roughly equal population sex ratio).

Charnov and Bull (1977) suggested that if there are benefits to adjusting the sex ratio then selection may favour a genetic system which permits this. They considered in particular, environmental sex determination. For parasitoids, haplodiploidy has the same benefits with maternal rather than zygotic control of the sex ratio.

The effect of host size on parasitoid sex ratio has been observed repeatedly (Clausen 1939; Arthur and Wylie 1959; Kotchetova 1977 [and references therein]; Charnov, Los-den Hartogh, Jones and van den Assem 1981; Cloutier, McNeil and Regnière 1981; Charnov 1982 [and references therein]; Avilla and Abajes 1984; Luck and Podoler 1985; Huldén 1986; Reeve 1987; Löhr, Neuenschwander, Varela and Santos 1988). The observed tendency is for smaller hosts to be used for the production of males and larger host for females.

To appreciate the adaptive reasons for this, consider a solitary parasitoid in which the size of the developing wasp is limited by the size of the host; larger hosts tend to produce larger parasitoids. If the reproductive consequences of being a particular size differ according to sex, then selection will act to favour larger hosts being used for production of the sex that gains most from being large. It is usually assumed that the rate of fitness gain against size is higher for females than for males. Thus, the bigger or better quality hosts tend to be allocated for the development of females, whereas males tend to develop in hosts of a lower grade.

Models of sex ratio and host quality (Charnov 1979; Charnov et. al. 1981; Bull 1981) make two general predictions:

1. Host size is relative. Large and small are relative terms dependent only on the distribution of host sizes available

2. The sex ratio produced in each size of host depends on the frequency of that host size. When small hosts are scarce, they should be used for producing mostly males.

When small hosts are abundant, they should be used for producing a greater proportion of females to balance the over-production of males from small hosts (Werren 1987).

All the models assume that parasitoids can perceive the population distribution of host sizes.

1.3.2 Seasonality

Werren and Charnov (1978) considered the optimal sex ratio in the absence of a stable age distribution. Assuming sexes of equal cost, no sex dependent mortality and random mating, the single ESS sex ratio remains 0.5. If however, the sexes have generations which overlap asymmetrically such that at certain periods one sex suffers greater reproductive competition from individuals remaining from an earlier generation, and if females can *adjust* their sex ratio according to the prospective fitness expected of a son or daughter born at one particular time, or season in the case of the Werren and Charnov model, then the ESS sex ratio will show seasonal shifts in favour of the sex with the greater lifetime reproductive success (Charnov 1982).

1.4 Asymmetry of relatedness

In arrhenotokous parasitoid wasps unfertilised (haploid) eggs become males whereas fertilised (diploid) eggs become females. This haplodiploid system of sex determination in Hymenoptera means that there are asymmetries of relatedness between parents and their offspring and between siblings of different sexes. A female wasp is equally related to her sons and her daughters - by a factor of 1/2. Paternal males however, are only related to their daughters. Despite this the optimum sex ratio in an outbreeding population remains 0.5. This is because although females produce more offspring than males, all the genes carried by a male are passed to his progeny, whereas only half the genes carried by a female are passed to her offspring. This was shown explicitly by Hartl and Brown (1970) and Green (1980).

Among siblings, daughters share on average, 3/4 of their sisters genes but only 1/4 of their brothers genes. Thus daughters favour three times as much investment in sisters as in brothers. This will only be important in gregarious species where siblings interact and compete for resources.

1.5 Sex ratio diseases

Selection usually favours a balance between male and female function because autosomal genes are transmitted equally through both sexes. Cytoplasmic genes in

contrast, are nearly always transmitted via the egg cytoplasm. Sperm have almost no cytoplasm surrounding the nucleus, so transmission of cytoplasmic factors is virtually impossible through male function, although there is one example of a paternally transmitted cytoplasmic sex ratio disorder so far recorded (Werren, Skinner and Charnov 1981).

Since usually only females transmit these factors there is strong selective pressure for cytoplasmic genes that can bias the sex ratio in favour of female production. The conflict of interest between cytoplasmic and autosomal genes means that any autosomal modifier gene that can suppress the cytoplasmic induced distortion will spread because it assures a greater representation of its own copies in future generations (Leigh, Herre and Fischer 1985).

These cytoplasmic agents are usually parasitic microorganisms such as viruses, microsporidia, and rickettsia and are found infecting numerous plants and animals (Werren,Skinner and Huger 1986).

Meiotic drive only affects the sex ratio of animals with chromosomal sex determination. Meiotic drive occurs when one chromosome is represented more than would be expected given simple Mendelian segregation e.g. genes on one chromosome segregate that chromosome to the egg cytoplasm rather than the polar body during meiosis. Meiotic drive has been recorded for both sex chromosomes (X and Y) with obvious consequences for the balance of the sex ratio. As with cytoplasmic factors, the driving genes work against the interests of the genome as a whole. Drive-suppressing genes at autosomal loci should evolve and spread quickly to counteract the asymmetric segregation and restore the sex ratio to parity (Leigh, Herre and Fischer 1985, Werren 1987).

1.6 Testing the theory

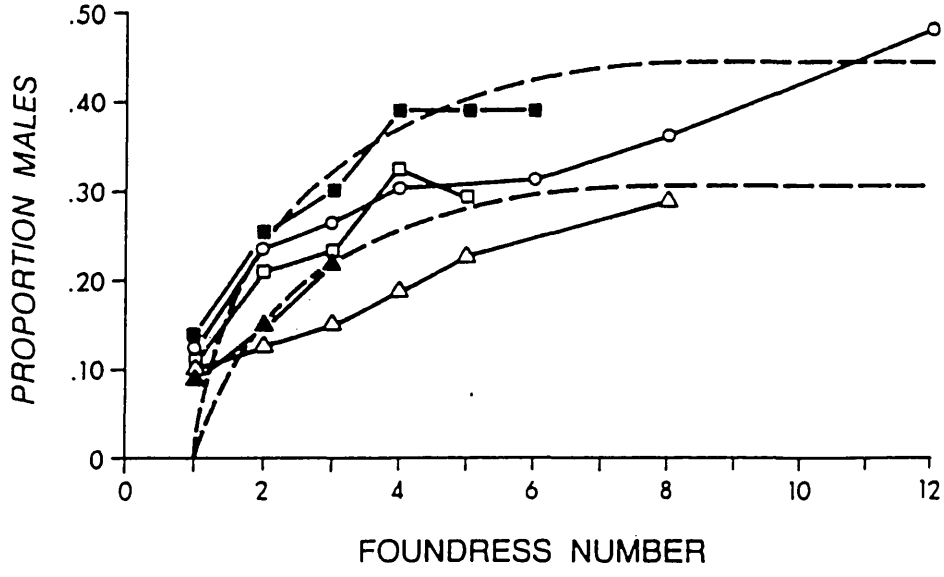
Considering the wealth of theoretical papers and ideas which variable sex ratios have generated, empirical tests of the theory remain relatively few. Some of the best studies consider how females adjust the size and sex ratio of their clutch in response to local mate competition (LMC).

Two systems have been particularly well described. First is the small pteromalid *Nasonia vitripennis* which lays up to 50 eggs in the pupae of corpse-infesting blowflies. Second are the fig wasps which enter, pollinate and lay eggs in fig tissue. The offspring

feed from the fig, mature and mate amongst themselves. Females cut their way out of the fruit and disperse to repeat the cycle. In both systems the patches are discrete and males are wingless and can only mate in the vicinity of the birth site.

Work by Werren (1983), Frank (1985) and Herre (1985) was summarised by Werren (1987) to show that for *Nasonia* and 4 fig wasp species, brood sex ratios increase with foundress number (figure 1.1). With one exception, those species with higher levels of inbreeding also had lower overall sex ratios. Thus, all these species are able to assess the number of foundress females in a patch and alter the brood sex ratio in the expected direction.

Figure1.1: The observed sex ratio changes for 4 fig wasp spp. (△ ▲ □ ■) and *Nasonia vitripennis* (○). The dashed lines show the calculated optimal sex ratio in an outbred species (upper curve) and inbred (lower curve) haplodiploid species. From Frank (1987).



Quite how females assess the number of foundresses in the patch is uncertain. It has been shown that *Nasonia* can detect and respond to previously parasitised hosts. This wasp can also detect the presence of other females in close proximity (Wylie 1965; Werren 1983 and references therein).

If a female superparasitises a host such that the *n* number of foundress females in the patch increases by one, then a more male biased sex ratio is predicted for the brood of the second female. This was confirmed experimentally for *Nasonia* (Wylie 1966;

Holmes 1972; Werren 1980, 1983) although this understanding of adaptive sex ratios has recently been challenged following the discovery of genetic strains of *Nasonia* that violate LMC predictions (Orzak 1986; Orzak and Parker 1986).

The variation in the data suggest that there is little selection for precise sex ratios in these species. There may be several reasons for this. The wasp may not be able to precisely control the sex ratio, accurately assess the number of foundress rivals or may be responding to factors not controlled by the experimenter such as host quality. Precise sex ratios in which the variance in the number of males in a brood is less than binomial ***have*** been described from other parasitoids however (Green, Gordh and Hawkins 1982; Waage 1982; Putters and van den Assem 1985). For the broods of such species there is not a fixed probability of ***any*** egg in an oviposition sequence being unfertilised, but rather the female produces a precise number of unfertilised eggs usually at set intervals during an egg laying sequence.

With regard to sexual asymmetries, Pickering (1980) investigated 70 broods of the ichneumonid *Pachysomoides stupidus*, a parasitoid of *Polistes* wasp larvae. Many parasitoids can develop on a single host. During feeding, the larval parasitoids eat their way down the host's larval cell excluding some of their siblings as they grow. Larvae displaced from the feeding site suffer reduced weight gain compared to their more voracious sibs. Pickering observed that in any given brood, males were generally smaller and pupated at the end of the host furthest from the host remains, while the opposite was true for females. He attributed this sexually asymmetric competition to asymmetries of relatedness between brothers and sisters. As stated before, mothers are equally related to each sex whereas sisters are related to each other by 3/4 and to brothers by 1/4. Selfish action on the part of the females is probably the reason why males are deprived of food and suffer greater mortality and reduced growth as a result. Altruistic behaviour on the part of the males would have the same effect and could not be completely ruled out however.

An obvious mother/daughter conflict arises in the sex ratio; the daughters preferring a female biased sex ratio whereas the mother favours equal production of the sexes. Once the brood has been laid the mother can play no role in regulating progeny feeding so competition is purely among the sibs.

To overcome this problem, it appears that females produce extremely variable brood sex ratios. This increases the mean coefficient of relatedness within broods, and

decreases the competition between asymmetrically related siblings. It is the male that gains most from this strategy for it is the male that would suffer most if all broods were uniform in their sex ratio. In this way the overall sex ratio produced is maintained at 0.5.

1.7 Summary

As Werren (1987) points out "One unifying principle that applies to all sex allocation systems is that of asymmetric transmission. Whenever genes have a higher transmission through one sex than the other...selection will favour genes that increase the production of that sex".

This applies to all the situations I have discussed. Whether due to the age or spatial structure of the population, the effect of host quality, conflicts in gene transmission or as a result of differential relatedness between brothers and sisters, asymmetries of reproductive success lead to asymmetrical sex ratios.

Symmetrical inheritance of genes from each parent originally led Fisher (1930) to assert the reasons why sex ratios of 1:1 were so ubiquitous. We will return again to his arguments several times in the thesis to consider how they apply to heteronomous hyperparasitoids - a group of parasitoid wasps with a remarkable method of sex allocation.

CHAPTER 2

INTRODUCTION TO HETERONOMOUS HYPERPARASITOIDS

2.1 What are heteronomous hyperparasitoids?

The Aphelinidae is a family of minute hymenopteran parasitoids. Over 1000 species from some 50 genera have so far been described, although many species and genera remain undescribed. They are rarely more than 1mm long. However, their role in biocontrol of homopterous pests such as aphids, whiteflies, and scale insects, makes them of marked economic importance.

Most aphelinids are primary parasitoids of these sternorrhynchous Homoptera. Within the Aphelinidae there are a small number of genera in which the sexes differ in their host relationships. Females always develop as primary ENDOparasitoids of Aleyrodidae or Coccididae, whereas the male develops as a primary ECTOparasitoids of an Homopteron, as a hyperparasitoid of a hymenopteron, or as a primary endoparasitoid of lepidopterous eggs.

These differences have been described previously as "differential development/relations of the sexes" (Flanders 1936a, 1936b), "deviant or divergent male ontogeny" (Flanders 1967), "sex linked hyperparasitism" (Williams 1977) and "sexual ditrophicity" (Yasnosh 1980).

After discovering the phenomenon in 1936, Flanders first attempted a classification in 1959 using female ovipositional behaviour. This was of little use taxonomically. It also proved to be based on invalid changes in female behaviour post-mating. Zinna (1961,1962) then proposed a more detailed classification based on male development, but which made no distinction between direct/indirect hyperparasitism (see definition later in chapter) or endo/ecto- hyperparasitic development. The terminology Zinna proposed was overtly complicated. Ferriere (1965) modified Zinna's classification but failed to improve substantially on the precision or the terminology.

Walter (1983a) has reviewed and simplified the classification of this group using the term "heteronomy", meaning "subject to different laws or modes of growth". Heteronomous parasitism has been unequivocally demonstrated for 8 aphelinid genera:

Aneristus
Coccophagoides
Coccophagus
*Encarsia**
Euxanthelus
Lounsburyia
Physcus
Prococcophagus

* The genera *Aspidiotiphagus* and *Prospaltella* have been synonymised with *Encarsia* by Viggiani and Mazzone (1979)

Certain species of *Pteroptrix* (formally *Casca*), *Archenomus* and *Azotus* may also be heteronomous (Walter 1983a and references therein).

The terminology proposed by Walter (1983a) depends purely on the divergent nature of male development in heteronomous parasitoids. This allows the group to be classified under three major headings:

1. Diphagous parasitoids - males develop as primary ECTO-parasitoids of coccid or aleyrodid hosts,

2. Heteronomous hyperparasitoids - males develop as secondary parasitoids of their own or a related species,

3. Heterotrophic parasitoids - males are primary endoparasitoids of lepidopterous eggs.

Heteronomous hyperparasitoids can be further classified into:

a. Obligate autoparasitoids - males only hyperparasitic on conspecifics

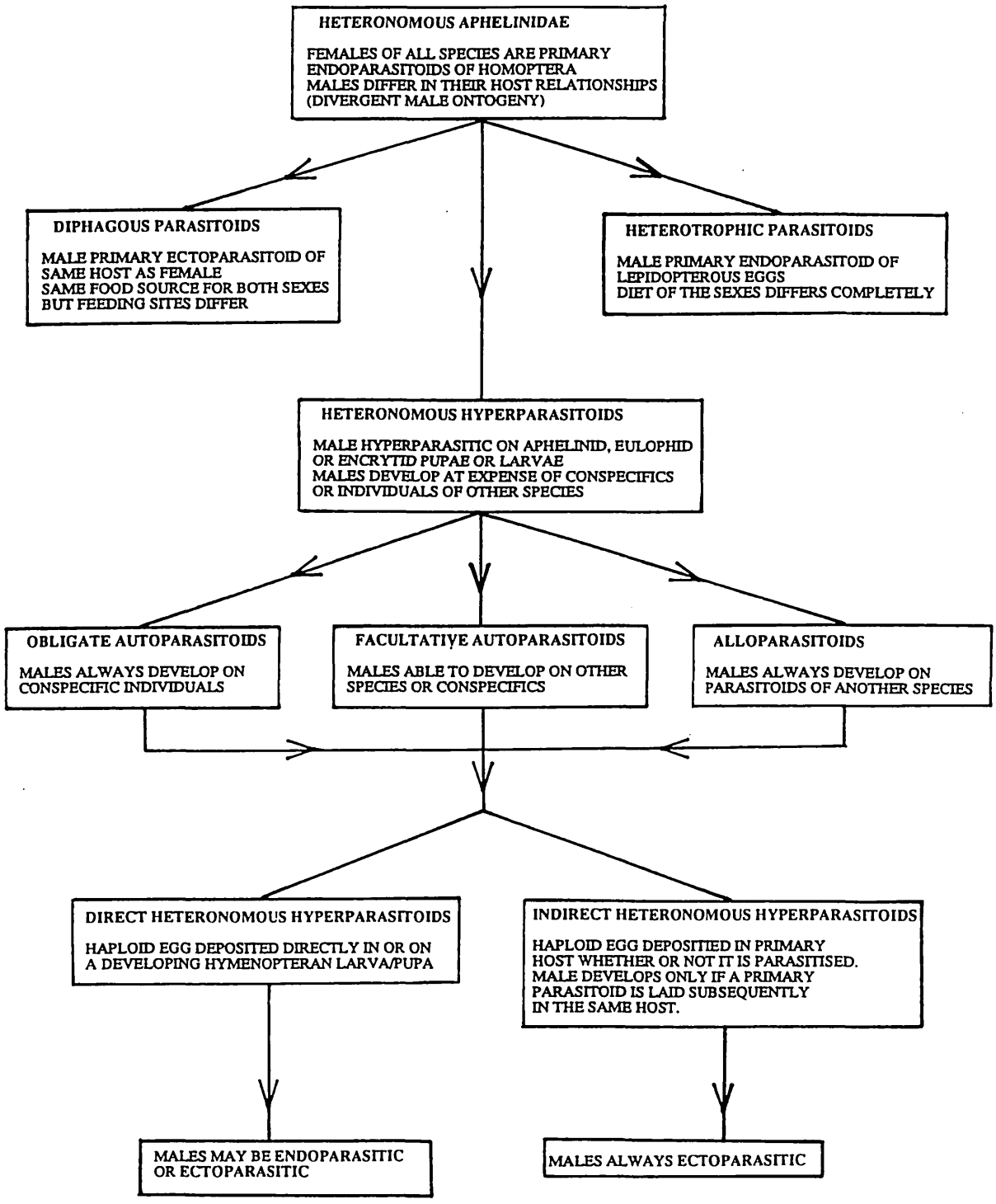
b. Facultative autoparasitoids - males hyperparasitic on conspecifics or individuals of another species

c. Alloparasitoids - males hyperparasitic only on other species

This system is presented diagrammatically in figure 2.1.

figure 2.1:

DIAGRAMATIC FORM OF HETERONOMOUS PARASITOID CLASSIFICATION



ADAPTED FROM WALTER (1983a)

In his review of heteronomous parasitoid literature, Walter (1983a) lists 11 known species of obligate autoparasitoids, 23 species of facultative autoparasitoids, 5 species of alloparasitoids, and 22 species of heteronomous hyperparasitoid for which the exact host relationships of the male are unknown. Since 1983 an additional 14 species from the 8 genera showing heteronomy have been described. Of these, 3 have been shown to be facultative autoparasitoids (Gerling, Spivak and Vinson 1987, Lopez-Avila 1987). Ten of the remaining 11 species are *Encarsia* and *Coccophagus*; the genera richest in heteronomous hyperparasitoids.

Of those species with hyperparasitic males investigated to date, half develop endoparasitically and half ectoparasitically. Hyperparasitism may also be direct or indirect. In the former, the haploid egg is deposited directly in or on a suitable hymenopteran larva or pupa. In the latter, the egg is deposited in the body fluids of a healthy or parasitised coccid host. The male embryo develops for a few days and then enters a period of quiescence. Hatching of the male is inhibited until a primary parasitoid larva deposited in the same host consumes the host body fluids. This stimulates the male to complete its development at the expense of the primary larva. All indirect heteronomous hyperparasitoids develop ectoparasitically (Walter 1983b).

Mention should be made of those non-heteronomous species showing occasional autoparasitic development as a result of incidental use of conspecifics for production of the same or a different sex. An example cited by Walter (1983a) is that of *Marietta* in which both sexes are hyperparasitic. When competition for hosts particularly strong, males may be found developing as tertiary parasitoids of conspecific females.

Huldén (1986) describes sexual differentiation in host relations of the Eulophid *Eretmocerus secreta* a solitary endoparasitoid of *Aleurochiton aceris*. Females mainly develop in female whitefly pupae and males in male whitefly pupae of the same species. This cannot be classified as true heterotrophic parasitism however, as the host relations are not obligate but reflect the fitness gains associated with laying the sexes in hosts of different sizes. Female whitefly pupae tend to be larger than their male conspecifics and therefore produce larger (fitter) parasitoids (see chapter 1).

From an evolutionary perspective the most interesting of the heteronomous groups are the autoparasitoids, for they appear to hinder the growth of their own populations by destroying potentially fecund females in the production of males.

2.2 Sex ratio and population dynamics in autoparasitoids

Explanations of sex ratio in autoparasitoids have focused on proximate causation; the role of physiology and the environment in adjusting parasitoid oviposition behaviour. These proximate explanations fall into two categories.

1. The first states that female ovipositional behaviour is changed irrevocably by mating. Newly emerged females seek and oviposit in parasitised hosts, whereas once mated, oviposition behaviour switches (completely) to primary parasitism. Sex ratio therefore depends solely on the number of hosts encountered prior to mating relative to unparasitised female hosts encountered subsequently. This was a misconception by Flanders (1942). However it was reiterated by himself (Flanders 1943, 1956, 1967) and others (Valentine 1964; Gerling 1966a; Yasnosh 1980) to such an extent that it was not until 1967 that the fallacy began to be recognised (Flanders 1967). Now it is accepted that heteronomous aphelinids, just as other arrhenotokous hymenoptera, can regulate their sex ratio by selective fertilisation following mating (Williams 1972, 1977; Viggiani 1981, 1984; Walter 1983a, 1983b; Hunter in press)

2. Flanders (1942, 1967), Zinna (1961, 1962) and Williams (1977) have all stated that the relative abundance of male and female hosts determines the sex ratio of heteronomous hyperparasitoids. As a result of this, the greater the percentage parasitism, the higher the population sex ratio. No experimental or field data beyond these observations were available when the statements were made.

Such unsupported statements are without doubt far too simplistic, in that they do not consider a possible selective advantage to individual females from laying eggs according to host availability. Only in completely exploited patches (100% parasitism) or completely undiscovered patches (0% parasitism) will the choice of sex ratio be removed from the ovipositing female. Partially exploited patches offer a range of sex ratio possibilities.

Two studies have subsequently given support to the host abundance hypothesis. Donaldson (1984) who studied the effect of encounter with different host types on individual sex ratios in *Coccophagus atratus*. Eggs of both sexes were deposited in a random sequence. Field sex ratios fluctuated between 0-80% male. This was correlated with changes in host availability. The host scale insect showed completely discrete generations.

Kuenzel (1975) found that field sex ratios in another facultative autoparasitoid *Encarsia pergandiella* oscillated around 50% and then increased rapidly to 73%. This was concurrent with a rise in the proportion of secondary hosts following an exponential increase in primary hosts some 250 degree-days earlier.

Colgan and Taylor (1981) applied Fisher's (1930) principle of equal investment in the sexes to autoparasitoids. Their calculations were as follows. Let N be the total egg production of a female, a proportion r of which are male. The costs in time and energy for laying a male egg is C_1 and for a female egg is C_2. P_1andP_2 are the probabilities of larval development occurring for males and females respectively. The probability that a female parasitizes her own daughters is Q.

Investment in male offspring is direct from laying haploid eggs and indirect in supplying some female larvae to serve as male hosts. Thus:

$$\text{Total male investment} = rNC_1 + \frac{QP_2rNC_2}{P_1}$$

Investment in females is the cost of laying diploid eggs less the value of the females that are used for production of male siblings...

$$\text{Total female investment} = (1-r)NrC_1 - \frac{QP_2rNC_2}{P_1}$$

With equal investment the equilibrium sex ratio reduces to:

$$r = \frac{1}{C + 2PQ + 1}$$

$$\text{Where } C = \frac{C_2}{C_1} \text{ and } P = \frac{P_2}{P_1}$$

If a female very rarely parasitizes her own daughters (Q≈0), the sex ratio moves in favour of males. The observation that field sex ratios are often female biased (Flanders 1967; Donaldson 1984) suggests that either Q is large (males often develop

at the expense of their sisters), or that the cost of male eggs (C_1) is much larger than female eggs (C_2). The former of these two points is discussed in chapter 6 and the latter in chapter 5.

Most recently Godfray and Waage (in prep.) have considered optimal heteronomous sex ratios when the sexes have equal reproductive success and yield equal fitness returns to the mother i.e. there is no LMC and little chance of males developing at the expense of their siblings. As described in chapter 1, when the cost of producing each sex is equal then the population sex ratio should show no bias. However, there may be differences in the time or risks involved with locating or parasitising hosts for one of the sexes. That sex will consequently be more costly to the mother who should proportionally bias her sex ratio in favour of the cheaper sex.

Godfray and Waage argued that for a direct heteronomous hyperparasitoid whose reproduction is limited by the ability to locate suitable hosts, the optimal strategy will be to invest equal time in searching for each host type. Such behaviour will result in parasitism of each host type in direct proportion to the relative encounter rates with parasitised and unparasitised hosts. The population sex ratio will thus evolve to reflect the degree of exploitation of the primary host. Under these assumptions, the female biased field sex ratios frequently recorded (references above) should result from a large search-time investment needed for male eggs and a consequent bias in favour of females.

Godfray and Waage also made predictions as to the sex ratio of other groups of heteronomous parasitoids. These are described later in the text where appropriate.

Hassell, Waage and May (1983) considered the effect obligate autoparasitism had on population dynamics by producing a model based on the Nicholson and Bailey (1935) difference equations, in which P female parasitoids are assumed to search at random for the N available hosts. Parasitoid searching efficiency is described by a constant and characteristic "area of discovery" (a). The number of encounters with hosts in each discrete generation follows a Poisson distribution and occurs in direct proportion to host density. The basal term of the Poisson series gives the number of hosts that survive attack (N)...

$$N = N_t e^{-aP_t}$$

Assuming that parasitoid larvae are solitary and suffer no mortality, that adults have an unrestrictive functional response and are not affected by parasitoid density. To complete the model, let hosts reproduce at a fixed rate . This gives the classical equations for which the number of hosts in the next generation (N_t) is...

$$N_t = \lambda N_t e^{-aP_t}$$

and for parasitoids...

$$P_t = N_t - N$$

$$= N_t\left(1 - e^{-aP_t}\right)$$

Hassell et al. incorporate the role of sex ratio by assuming that for an autoparasitoid, a female will emerge from a host parasitised once, whereas a male will emerge from a host parasitised more often. Under such a regime, the number of female parasitoids will be a function of the number of hosts which receive only ***one*** attack. This is described by the first term of the Poisson series rather than the basal term as before, giving...

$$P_{t+1} = cN_t aP_t e^{-aP_t}$$

The term c denotes the mean number of parasitoids emerging per host attacked. For heteronomous hyperparasitoids this will approach unity.

At equilibrium...

$$P^* = \frac{\ln \lambda}{a}$$

$$N^* = \frac{\lambda}{a.c}$$

Which is the same as the Nicholson Bailey equilibrium for parasitoids. Host equilibria however, appear much simpler and more sensitive to the host rate of increase λ. The dynamics of the model are such that the initial host and parasitoid population sizes determine the amplitude of the neutrally stable cycles which it generates. Hassell et al.

show that the addition of any stabilising factors such as a negative binomial distribution of parasitoid attacks (such as would be seen in response to a patchy distribution of hosts) rather than a Poisson distribution, quickly dampens the initial oscillations producing an extremely stable interaction for even the slightest degree of patchiness.

The interaction between autoparasitoid population dynamics and their density dependent sex ratios may be the reason why these aphelinids have been remarkably successful agents of biological control.

2.3 Introduction to *Encarsia tricolor*

Encarsia tricolor was first described by Förster (1878) from the Cabbage Whitefly *Aleyrodes proletella* (=*A. brassicae*). Although it has been placed in as many as six genera by various authors (Hayat 1983), since Mercet (1930) it has been accepted as the type species of the genus.

E. tricolor is the only heteronomous hyperparasitoid so far recorded in Britain. More specifically, it is a facultative autoparasitoid. The heteronomous nature of its host relations were first published by Mazzone (1976) who observed that *E. tricolor* males could develop at the expense of both *E. partenopea* and conspecific females in Italy. The parasitoid occurs frequently but usually in low numbers on four species of native whitefly.

The annual crop of Brussels sprouts that are planted in Silwood Park are always host to populations of the Cabbage Whitefly *A. proletella*. This provides a small but viable complex of parasitoids, one of which is *E. tricolor*.

2.4 The aims of the study

The questions that I attempt to answer in this thesis are as follows:

1. In what situations does being a heteronomous hyperparasitoid constitute a selective advantage over parasitoids that reproduce in the orthodox manner?

2. What factors are most important in determining heteronomous hyperparasitoid sex ratios?

3. What are the implications of 1 and 2 for population dynamics of systems containing a heteronomous hyperparasitoid?

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of whitefly and parasitoids

Overwintering pupae of *Aleyrodes proletella* were collected from the crop of brussels sprouts (var. winter harvest) during January and February 1986. The leaves were placed in a muslin-walled cage in a constant temperature room at 15°C (16:8h L:D) and those whitefly emerging were allowed to infest young potted brussels plants. These plants were then used to start the whitefly culture. The whitefly were confirmed as *Aleyrodes proletella* (Linnaeus 1758) by Dr. J.H. Martin(B.M.N.H.).

Parasitoids were collected by placing brussels sprout plants heavily infested by *A. proletella* under viburnum bushes and amongst wild honeysuckle (*Lonicera pericylemum*) in Nash's Copse, Silwood Park. Viburnum and honeysuckle are host to the whiteflies *Aleurotrachelus jelinekii* and *Aleyrodes lonicerae* respectively. Both of these whitefly are parasitized at very low levels by *E. tricolor* (Southwood and Reader 1988, pers. obs.). A total of 147 female and 26 male *E. tricolor* were so obtained. Additional males were produced by confining female *E. tricolor* with *E. formosa* pupae on tomato leaves from a Silwood glasshouse; the later species acting as secondary hosts for the former.

3.2 Whitefly and parasitoid cultures

Young potted Brussels sprout plants (var. winter harvest) 20-30cm tall with approximately 10 leaves were grown year round in a peat based compost in a glasshouse at Silwood Park. Plants were cleaned thoroughly using a small nylon bristled paintbrush to remove any unwanted insects (particularly aphids), before being used. All cultures were maintained in a constant temperature room at 25±1°C, 55-70%(ambient) RH, 16:8h L:D. Whitefly were found extremely simple to culture. Rectangular muslin-walled cages 40x40x50cm with perspex tops were used to contain between 2-5 brussels plants. Whitefly would readily infest and oviposit on any clean fresh plants added to the cage. A minimum of three such cages were maintained throughout the study period. Infested plants were taken for experiments or used to maintain the parasitoid culture when needed and replaced by clean fresh plants immediately.

The parasitoid culture was kept in a single cage under the same conditions as above. The *E. tricolor* culture was started over a 26 day period by addition of a total of 138 females and 90 males at 2-8 day intervals using the field collected material and the

males reared from *E. formosa*. Following mating in gelatin capsules, these parasitoids were released into the culture cage containing brussels plants heavily infested with all whitefly instars. Whitefly infested plants were added periodically and remained in the culture until death. It was necessary to regularly remove infested leaves and collect the parasitoids that emerged from them for use in experiments and to give a back-up supply of mated females should numbers fall low in the culture cage. Additions to both whitefly and *Encarsia* cultures were made using field collected material when available. *Encarsia tricolor* was identified by Dr. A. Polaszeck of the Commonwealth Inst. of Entomology (CAB International).

3.3 Clip cage experiments

All clip cage experiments lasted 24h at 25±1°C, 16:8 L:D (ambient humidity). Clip cages of two designs were used to confine parasitoids to a leaf area containing known numbers of parasitized and/or unparasitized hosts. The clip cages were constructed of one half of a Petri dish 35mm diameter, 5mm deep, sealed to the leaf by white foam draught excluder around the lip of the dish and held in place on the leaf by an elastic band. Clip cages used in the kin recognition experiment were basically the same although 48mm diameter, 12mm deep, and divided in half internally by " Spontex" cellulose cloth glued across the diameter when creating the experimental arena, or not divided at all when offering the entire arena to a single parasitoid.

Use of clip cages varied according to the experiment. Generally, cages were used to confine several young mated female parasitoids on patches containing an abundance of whitefly of all instars when it was necessary to generate patches with mixtures of parasitised and unparasitised hosts, which later were offered in identical cages for clues as to ovipositional decision making in *E. tricolor*. All such choices of oviposition were made by individual young mated females confined in a clip cage with a single young male.

Those females destined for use in experiments emerged individually in gelatin capsules and were always less than 24hrs old when initially chosen. An active male was introduced to the capsule and observed to mate with the female. Females which showed no interest were discarded.

For several experiments females were "experienced" by exposure to hosts of different types prior to being placed on the decision making patch. When such treatments

were necessary, individual females were allowed to mate and transferred with a male to clip cages containing the required host type for 24hrs at 25°C. After this time male and female were both transferred to the experimental arena.

Following exposure to the leaf area the parasitoids were returned to the culture. Parasitoid pupae (male hosts) were removed from the experimental arena and placed individually in gelatin capsules leaving the parasitised whitefly (female hosts) to develop until pupation whereupon they too were transferred individually to gelatin capsules. All such operations were carried out with the aid of Kyowa SDZ-PL binocular microscope (x7-x45) illuminated by an Intralux 5000 fibre optic unit. The experimental leaf remained attached to the plant until all parasitoid pupae had been removed or, if the leaf became detached from the plant, it was kept alive by placing its cut stalk in a vial of tap water. The plant was kept in a separate cage in the 25°C room.

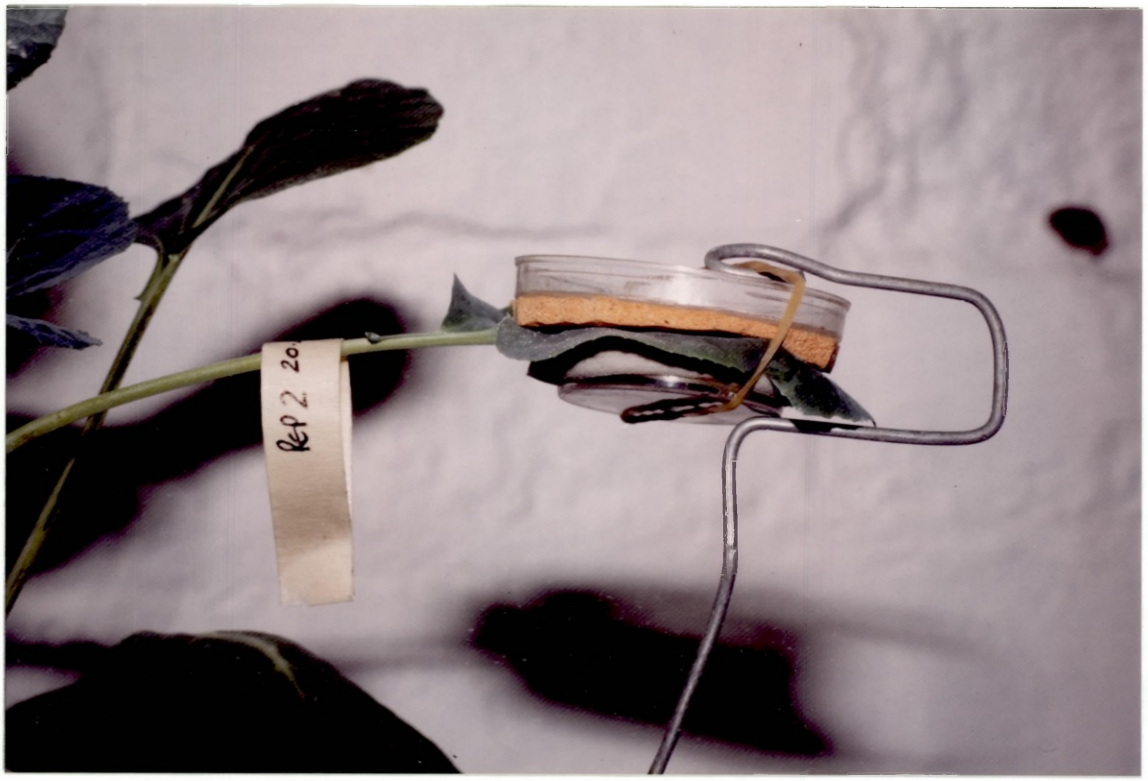
3.4 Data collection

Leaves from the culture were kept singly in plastic dishes with moist "Spontex" cellulose cloth for humidity and a muslin lid for ventilation. Parasitoids which emerged over the previous 24hrs were collected, counted and sexed at room temperature, which took 15-30 minutes. When necessary, small numbers of wasps were used in experiments before being returned to the culture cage. Daily emergence data of experimental pupae held in gelatin capsules were obtained by examination and sexing of parasitoids in individual capsules. Sexing of *E. tricolor* was achieved by the following diagnostic features:

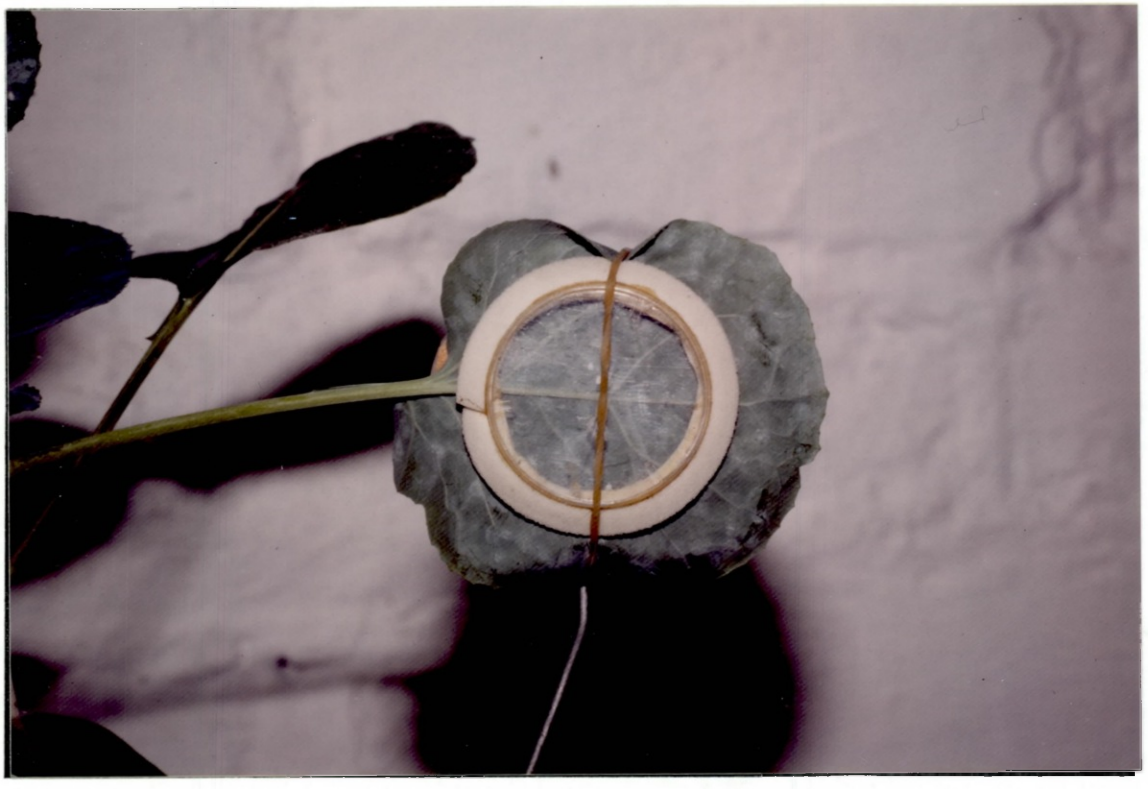
Characteristic	Male	Female
Eye colour	Black	Yellow
Antennae	5 segs (long)	6 segs (shorter)
Ovipositor	Absent	Present

Experimental pupae that failed to emerge and pupae in the species discrimination experiment were dissected for sexing or to discover the presence of hyperparasitic male larvae. Such dissections were either carried out dry, or under Ringer's saline solution using fine entomological needles mounted in the ends of matchsticks.

This chapter describes the materials and methods used generally. Specific details varied among experiments and are described in the relevant sections following.



A Clip Cage in use (side view)



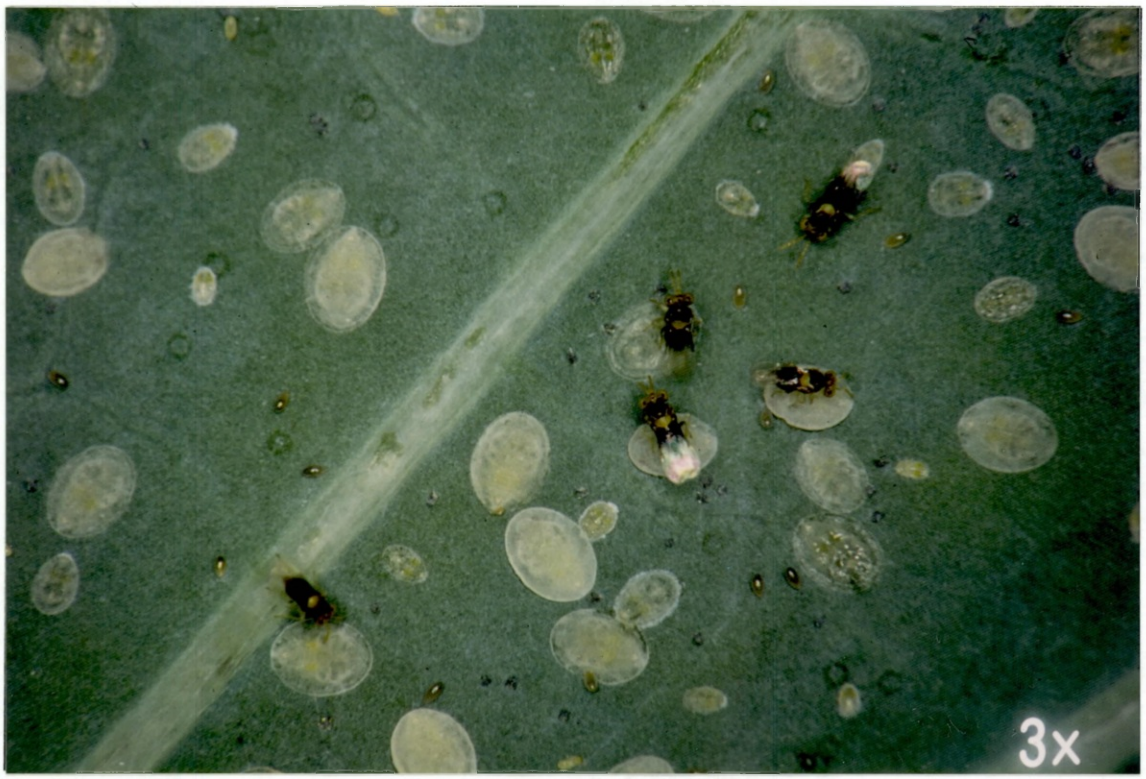
Clip Cage (from beneath)



Several Clip Cages set up on the same plant

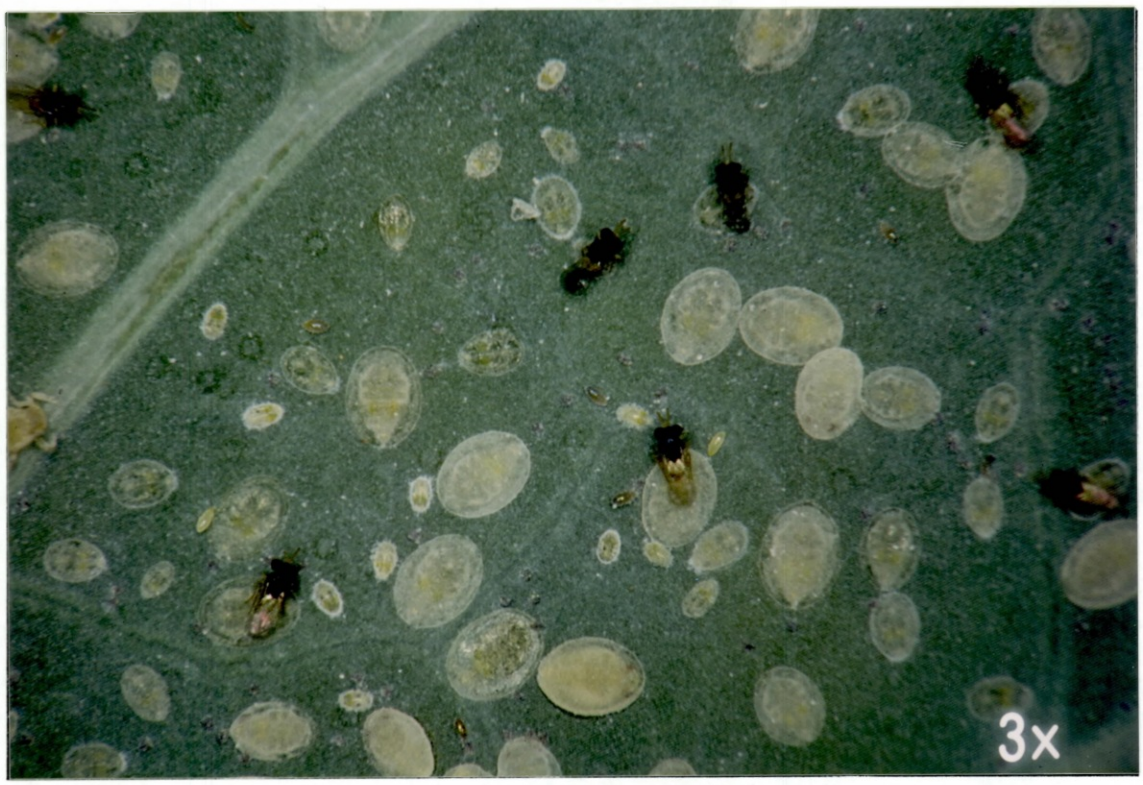


Culture Cages containing Brussels Sprout plants in the 25°C room

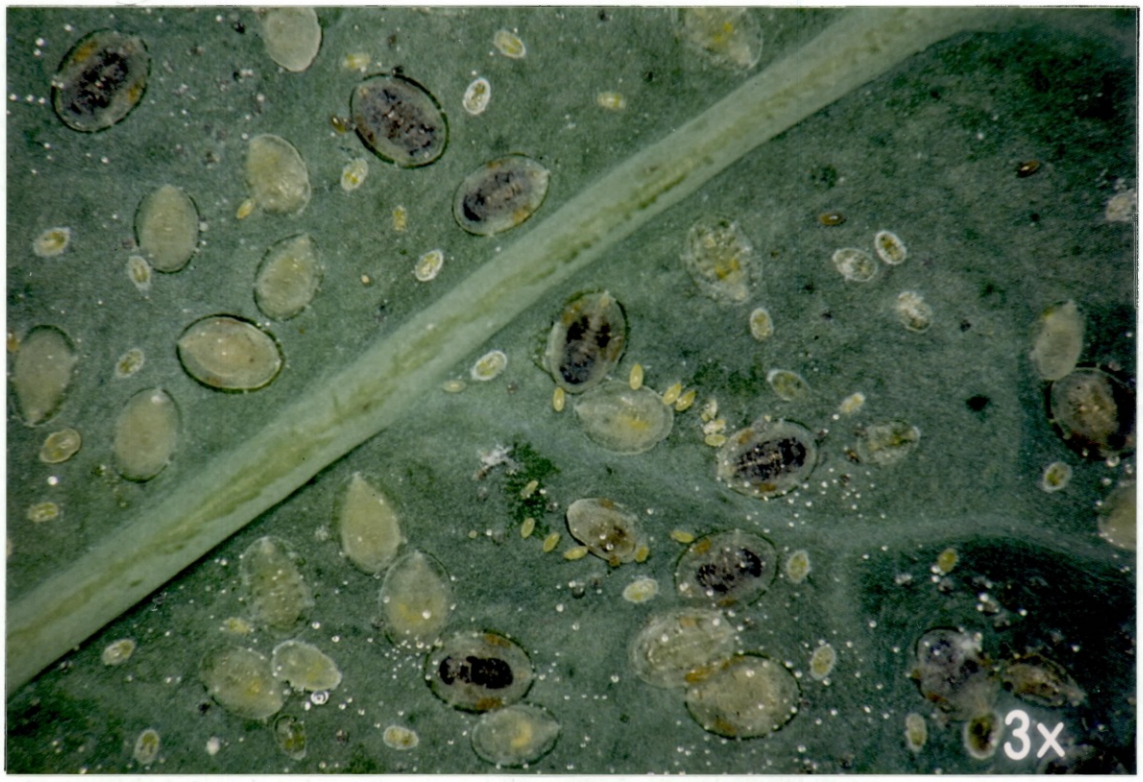


Mated Female *E. tricolor* parasitising late instar whitefly hosts (laying female eggs)

1mm
scale

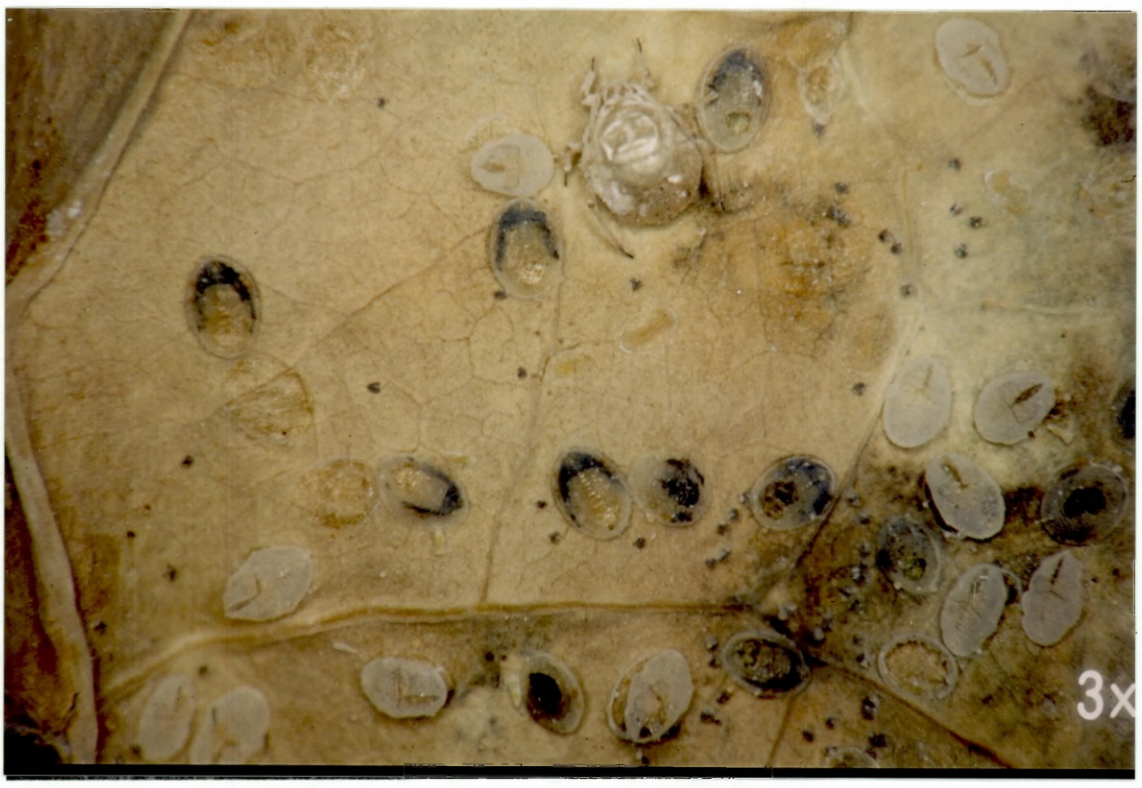


Mated Female *E. inaron* ovipositing in late instar whitefly nymphs (laying both sexes as primary endoparasitoids)



Parasitised (darkened) and Unparasitised (pale) Whitefly Nymphs on the underside of a Brussels Sprout leaf

1mm
scale



Emergence cases of Whitefly (opaque white) and Female *E. tricolor* (transparent). Note the exit hole and the remains of the pupa which are typical of previous occupation by a female parasitoid of this species.

CHAPTER 4

BIOLOGY OF *ENCARSIA TRICOLOR*

4.1 Introduction

Considering the large number of heteronomous hyperparasitoid species and the potentially valuable role they may play in biocontrol of scale insect and whitefly populations, there have been very few investigations of the basic biology of species within the group. The immediately obvious reasons for this are size and sex allocation. Problems as basic as classification and ethology caused by their minute size have deterred detailed investigations until very recently. The deviant male ontogeny complicates studies of fecundity, host preference, development and oviposition behaviour.

The first work on the biology of *E. tricolor* (host: *A. proletella*) was by Stuben (1949) who described larval development, mating behaviour, oviposition, host-feeding and hyperparasitic development. He stated the sex ratio to be 1:1. However, it is obvious that he was unaware of sexual differences in the mode of development and the ability of virgins to produce only males.

Castresana et. al. (1979) and Avilla and Copland (1987) have studied in detail the development of this species in *Trialeurodes vaporariorum* and are cited in the chapter where relevant.

Most of the remaining studies have focused on the potential of *E. tricolor* in biocontrol of *T. vaporariorum* which is a field pest of economic importance during the southern European summers (Arzone 1976; Isart 1977; Bordas et. al. 1981). Arzone (1977) reported *E. tricolor* to be remarkably well synchronised with the primary host life cycle. The parasitoid emerged in time to parasitise the 4th instar nymph, developed while healthy whitefly were adults, eggs or newly hatched nymphs, and pupated during the 2nd instar stage of the host population. Adults then emerged and mated during the life of the 3rd instar nymph, ready to continue the cycle again. This occurred for up to 5 generations at sea level in the Italian Riviera and resulted in high percentage parasitism where insecticides were not in use (Arzone 1976).

E. tricolor is a facultative autoparasitoid under the classification proposed by Walter (1983a). The number of host records of *E. tricolor* show it to be non-specific in its ability to develop successfully in a range of whitefly species (table 4.1). *Encarsia tricolor* males have been reared as hyperparasitoids of 6 species of *Encarsia* (Vet and

van Lenteren 1981; Viggiani 1984; Avilla and Copland 1987; pers. obs.) and one species of *Euderomphale* (Williams in press). Less definite male host records include *E. partenopea* (Butler 1936; Carden 1972) and even as a hyperparasitoid of the coccid *Pulvinaria regalis* (Polaszek pers. comm.).

Table 4.1: Primary host records of *Encarsia tricolor*

Whitefly species	Country	Parasitoid species from the same material	Reference
Aleyrodes fragariae	USSR	none given	Nikolskaja and Jasnosh (1968)
Aleyrodes lonicerae	USSR	none given	Nikolskaja and Jasnosh (1968)
	England	*Euderomphale chelidonii*	Pers. Obs.
Aleyrodes proletella	England	*Encarsia partenopea* (*)	Butler (1936) Thompson(1950) Carden (1972)
	England	*Encarsia inaron*	Pers. Obs.
	Italy	*Encarsia partenopea*	Silvestri (1915)
	Spain	none given	Gomez-Menor (1943)
Aleurotrachelus jelinekii	Italy	*Amitus aleurotubae* *Encarsia aleurotubae* *Encarsia margaritiventris* *Eretmocerus longicornis*	Laudonia and Viggiani (1984)
	England	none	Southwood and Reader (1988)
Trialeurodes vaporariorum	Spain	none given	Isart (1977) Albajes et al (1980) Bordas et al (1981)
	Italy	none given	Arzone (1976, 1977)
		Encarsia partenopea	Mazzone (1976)
	England	*Encarsia formosa* (**)	Pers. Obs.

Note: (*) these records probably *Encarsia inaron* (Polaszek, pers. comm.).
(**) from natural glasshouse populations at Silwood.

Female larvae are endoparasitic, hymenopteriform, and go through three larval instars before pupation (Arzone 1976). When an egg was deposited in the 1st, 2nd, or 3rd instar nymph, Avilla and Copland (1987) found hatching occurred in the following instar. Female development was shortest at 28°C. However, higher temperatures produced wasps significantly smaller than those developing between 16 and 24°C (Artigues et. al. 1987; Avilla and Copland in press). Females had 6 ovarioles (3 per ovary) regardless of host stage or temperature.

Male larvae are initially caudate and develop endoparasitically in hymenopteran hosts. The male has three larval instars, the final instar being hymenopteriform. At this point, most of the primary parasitoid host has been consumed and feeding is functionally ectoparasitic. Pupation and emergence follow as normal (pers. obs.). The production of a cocoon prior to pupation has been recorded in a number of aphelinids (Walter 1983b) but this feature is not seen in *E. tricolor*.

Like many aphelinid species (Flanders 1950; Jervis and Kidd 1986), adult *E. tricolor* are synovigenic and emerge with very few mature oocytes, typically 0.8-2.6. Females must host-feed before full egg maturation can occur. No relationship between the number of mature oocytes at emergence and parasitoid size or subsequent fecundity has been found in *E. tricolor* (Avilla and Copland 1987) although a strongly positive correlation has been reported for fecundity in *Encarsia formosa* (van Lenteren, van Vianen, Gast and Kortenhoff 1987).

Females of *E. tricolor* are arrhentokous. Unmated females can deposit unfertilised haploid eggs for production of males, but female production requires mating and fertilisation of eggs. By selective fertilisation, the mated female can control the sex of each egg laid.

Female receptivity in parasitoids is usually signalled initially by the release of pheromones. These may originate from various parts of the female body. In the case of the aphelinid *Aphytis linganensis*, the area of thorax at the base of the wings has been found to emit a volatile factor attractive to males. For the ichneumonid *Campoletis sonorensis* all parts of the female body elicited male courtship behaviour, suggesting pheromone secretion through cuticular glands (Matthews 1974 and references therein). In *E. tricolor*, the male pays particular attention to the dorsal thorax of the female by drumming it with his antennae before mounting (pers. obs.).

Male wing beating behaviour appears to be almost universal in hymenopteran courtship. This draws air over the male body from front to back, allowing orientation to the odour source (Vinson 1972). Wings also play a role in the actual courtship ceremony, although they need not be essential. When both sexes of *Aphytis* sp. were de-alated, insemination was 100% successful (Rao and DeBach 1969).

Far more important are the antennae. Chemo-tactile stimulation of the antennae seems esential for both sexes. In male *Encarsia* antennal sensilla have been observed. These structures seem particularly important during pre-coital courtship when segments abundant in the sensilla are rubbed over the female antennal club (Viggiani 1980; Lopez-Avila 1987). The antennae of both sexes are involved in the pre-coital mating sequence of *E. tricolor* (see diagram). The readiness or non-receptivity of the female is commonly signalled by stereotyped antennal positions (Matthews 1974 and references therein).

Courtship behaviour can be divided into three distinct phases once the sexes come together in close proximity. The first phase is pre-coital courtship in which the male assesses the receptivity of the female, mounts her, and induces her to copulate. Of those aphelinid species studied to date, this is a very brief period, typically between 3 and 15 seconds (Tower 1914; Gordh and DeBach 1978).

The second phase is copulation. For aphelinids, this is the briefest phase lasting 2-5 seconds (Bar and Gerling 1971; Donaldson 1984)

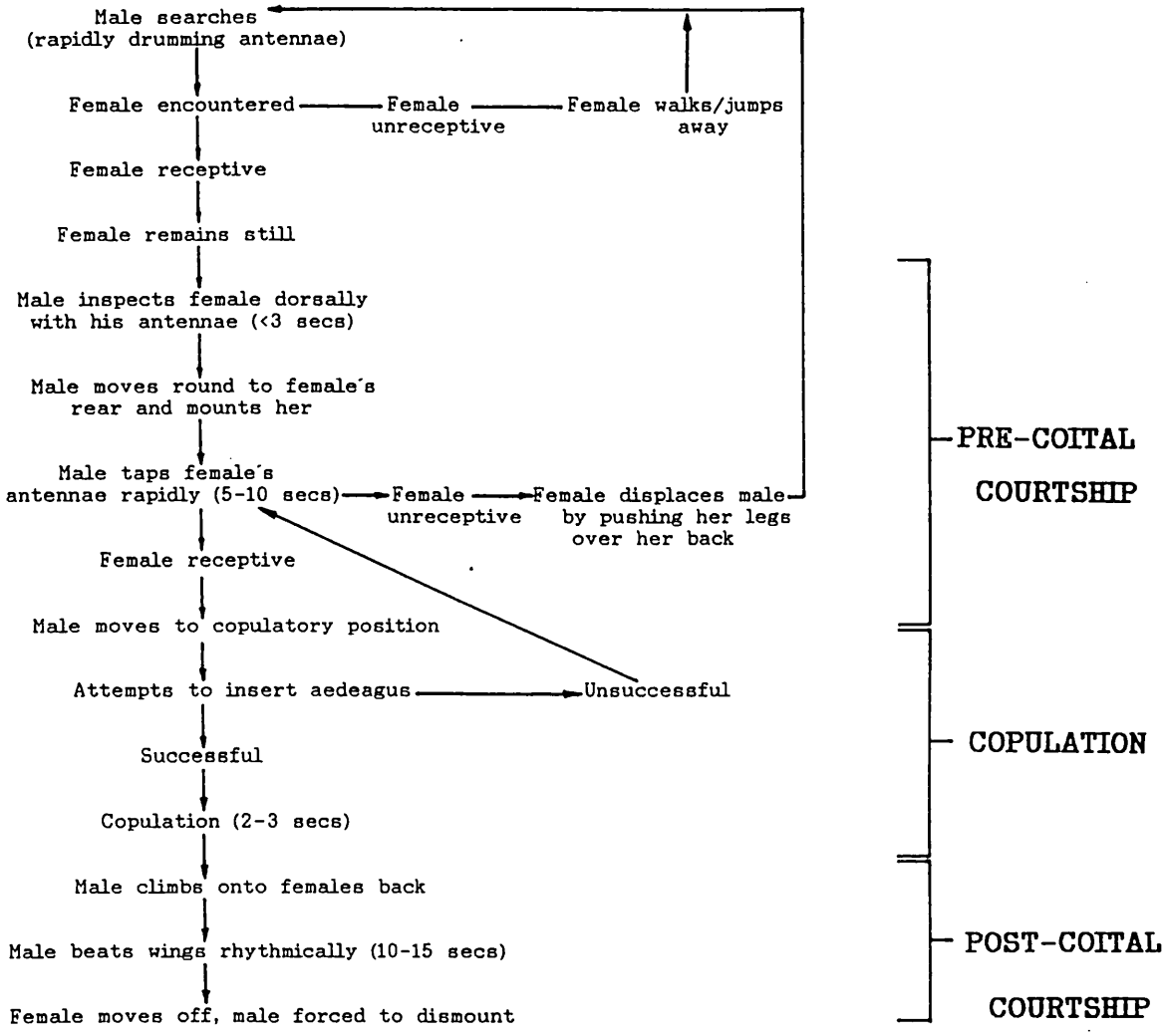
The third phase is post-coital courtship and is of two types in the Aphelinidae:

1. there are those species which leave the female as soon as insemination has been achieved (Broodryk and Doutt 1966 for *Coccophagoides utilis*, Cendana 1937; Zinna 1961; Donaldson 1984; Walter 1984 for *Coccophagus* spp., Stuben 1949; Viggiani and Mazzone 1978 for *Encarsia* spp.).

2. those species showing quite prolonged contact with the female following copulation (Gordh and DeBach 1978 for *Aphytis linganensis*, Walter 1984 for *Coccophagus* spp., and Tower 1914 for *Encarsia (Prospaltella) perniciosi*).

E. tricolor falls in the second of these two categories (see diagram overleaf).

MATING SEQUENCE IN ENCARSIA TRICOLOR



Such mate-guarding (Parker 1978) behaviour suggests that the female remains responsive to further mating attempts from competing males for a short period after the initial mating. By indulging in post-coital activity with the female, the first male protects his genetic investment (spermatozoa) from being displaced by an opportunistic rival.

In the field, *Encarsia* males have been observed to wait beside female pupae ready to mate the instant the female emerges (pers. obs. for *E. inaron* and *E. tricolor*). Reference to mating upon emergence among aphelinids is quite common (Kuwana 1934; Cendana 1937; Zinna 1962; Chumakova and Goyunova 1963; Gerling 1966a).

Generally, for the aphelinids, pheromones and tactile stimuli from antennae are the most important signals in the pre-coital ritual, whereas wings and legs are used more post-coitally.

When confined in a tube, up to 5 or 6 male *E. tricolor* will simultaneously attempt to court a single receptive female. Males pay little attention to unreceptive females (pers. obs.).

Stuben (1949) measured pre-coital courtship at 3-4 minutes, copulation at less than a second and zero post-coital courting for *E. tricolor*. His description of the sequence strongly suggests the females used had mated previously, for the female repeatedly dislodged the male from her back.

4.2 BIOLOGICAL CHARACTERISTICS OF *ENCARSIA TRICOLOR*

In this chapter I look at longevity of the sexes, female fecundity, the ability of the sexes to develop in different host instars, oviposition time for male and female eggs, and preferred whitefly instar for the development of females.

4.2.1 *Longevity*

Methodology

Parasitoid pupae from the culture were allowed to emerge in dishes at 25±1°C and collected every 24hrs. Males and females came into contact with the opposite sex during collection and so females were assumed to be virtually all mated. Males and females were separated and placed individually in glass vials with honey. These tubes were kept at 25±1°C (16:8 L:D) and examined daily at room temperature for approximately 15

mins. Fresh honey was added as required. Upon death, the head capsule was measured at its widest point to an accuracy of 0.01mm. This was used as an indicator of adult parasitoid size.

Results

Results are shown in table 4.2 below and are plotted as figures 4.1,4.2 and 4.3 overleaf. The correlation between longevity and size is linear, positive and highly significant for both sexes. The regression equation for males is $y = 0.79x - 2.57$ and for females, $y = 0.93x - 5.77$.

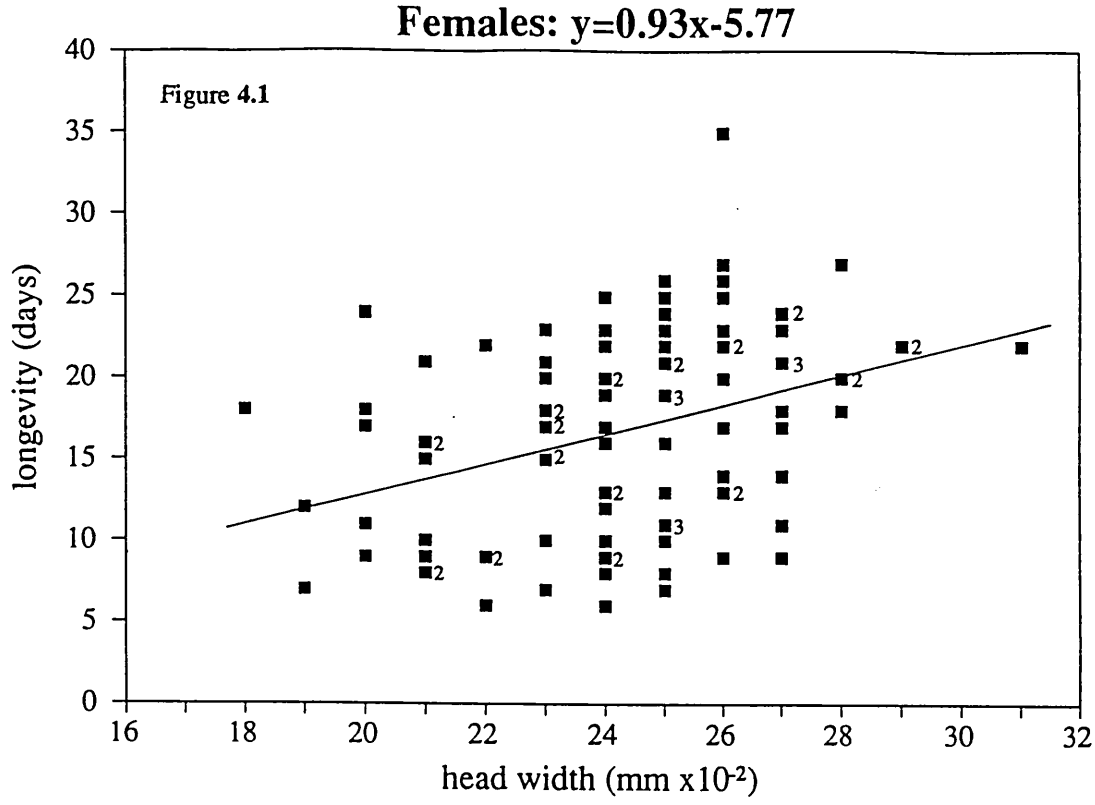
Table 4.2: Longevity of *E. tricolor* in relation to size

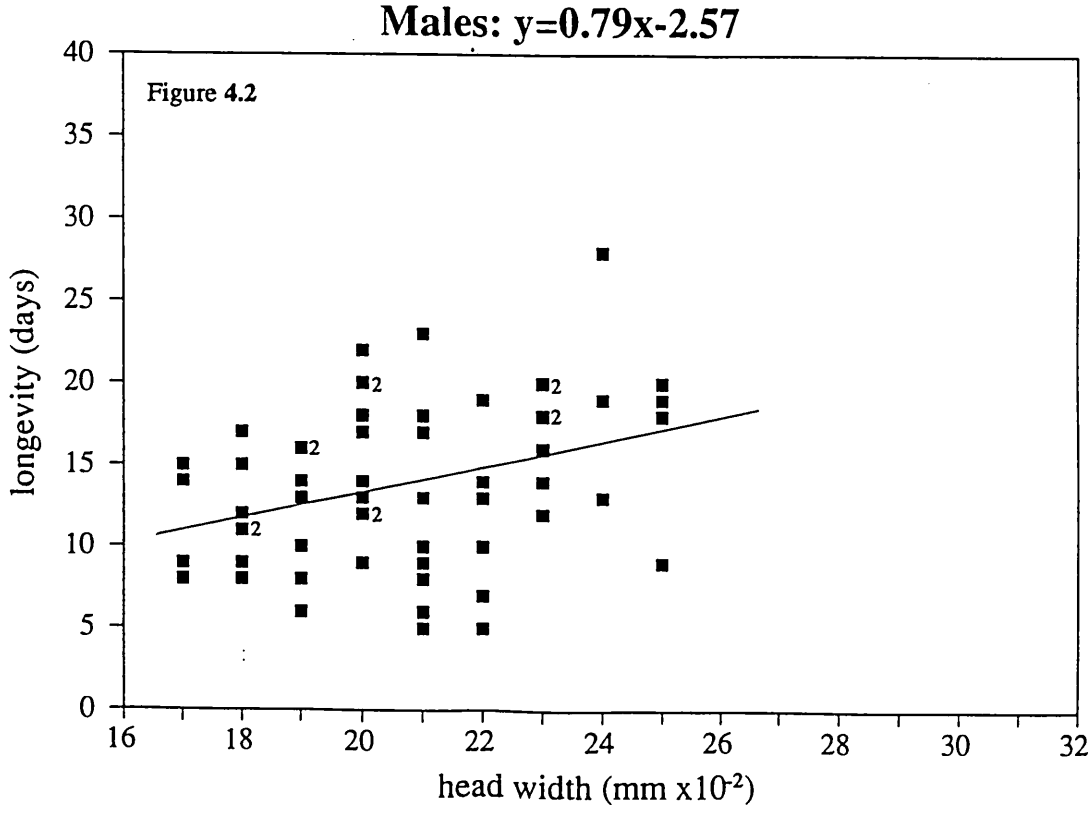
	mean	SD	n	r^2	F value	P
Female head size(mm)	0.243	0.025	96	0.139	15.19	P<0.001
Female longevity (days)	16.9	6.1				
Male head size(mm)	0.207	0.023	58	0.123	7.85	P<0.001
Male longevity (days)	13.8	5.0				

Lopez-Avila (1988) reported comparable mean longevities from a similar experiment with *Encarsia cibciensis* (male 9.3d; female 17.4d), *Encarsia adrianae* (male 17.1d; female 23.5d) and *Encarsia deserti* (male 18.3d; female 22.6d) from *Bemisia tabaci* at 25°C.

Van Lenteren, van Vianen, Gast and Kortenhoff (1987) investigated the effect of diets such as water, glucose, honey, whitefly nymphs and whitefly honeydew on longevity and fecundity in *Encarsia formosa*. Wasps lived longest on a diet of honey. Host plant material also appeared to have marked effects on the longevity of honey-fed parasitoids with cucumber being superior to tomato which in turn was superior to tobacco. They found no correlation however, between parasitoid size and longevity.

Figures 4.1,4.2: Regression of Parasitoid Longevity on Size (head width) for both sexes of *E. tricolor*. Numbers by points indicate multiple points.





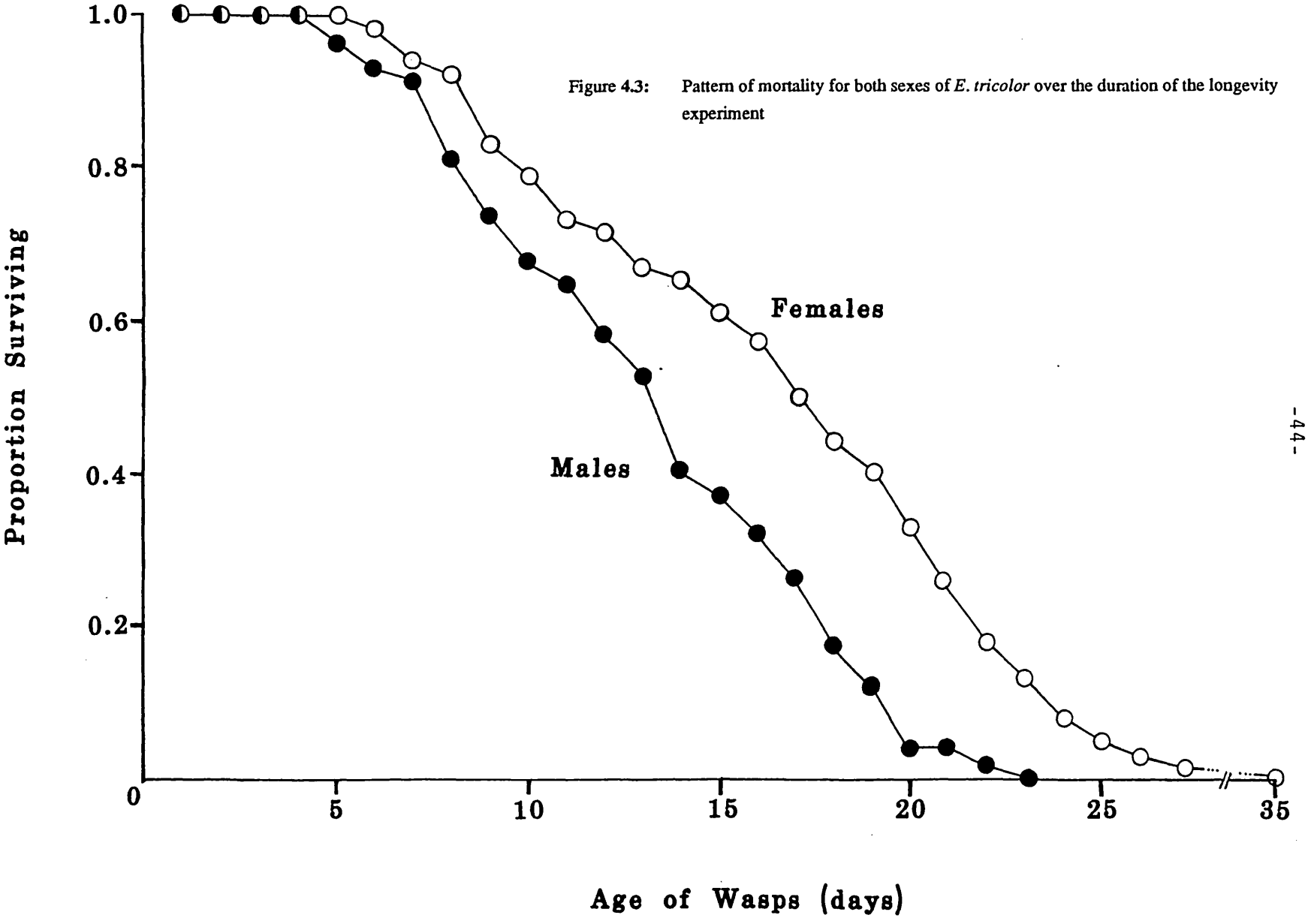


Figure 4.3: Pattern of mortality for both sexes of *E. tricolor* over the duration of the longevity experiment

4.2.2 *Instar acceptability for female eggs and female development time.*

Methodology

Females having emerged individually into gelatin capsules at 25°C in the previous 24-48hrs, were allowed to mate with young males at room temperature and then introduced to a leaf area containing an abundance of *A. proletella* (50-80 nymphs/arena) of a particular instar. The female was confined using a clip cage (described previously) for 24hrs at 25°C, after which time she was removed and the parasitised scales left to develop. When these scales appeared black, the leaf was cut from the plant and placed in a plastic dish with muslin lid and moist "Spontex" cloth. Dishes were checked daily at room temperature for parasitoid emergence.

Results

Data on instar acceptability and development time for females are presented in table 4.3. The third instar nymph suffered more parasitism on average although the result was quite variable in all replicates. Female parasitoid development was shortest in the fourth and longest in the first instar. A total of 33 replicates failed to produce any result either due to death of the leaf before parasitised hosts could develop sufficiently to allow parasitoid pupation, or because the female failed to mate sucessfully, or because the parasitoid became stuck in the honeydew secreted by the whitefly nymphs.

Table 4.3: Instar acceptability and development time of females

Host	No. reps.	Mean no. eggs developed /rep. ±SE	First pupae seen (days)	Mean development time (days)±SE	Range of development times(days)
Instar 1	10	4.9±0.69	12	22.3±0.34	18-28
Instar 2	10	5.6±0.56	11	19.6±0.20	17-24
Instar 3	10	6.6±1.08	10	19.1±0.24	16-24
Instar 4	13	5.5±0.68	9	18.7±0.25	14-26

Three factors have relevancy to this experiment:

1. mortality of eggs is assumed to be equal in all host instars. Eggs laid in earlier instars may be subject to host mortality and are exposed to host defence systems for longer than conspecifics laid in late instars, which themselves may be able to muster a more aggressive initial immune response.

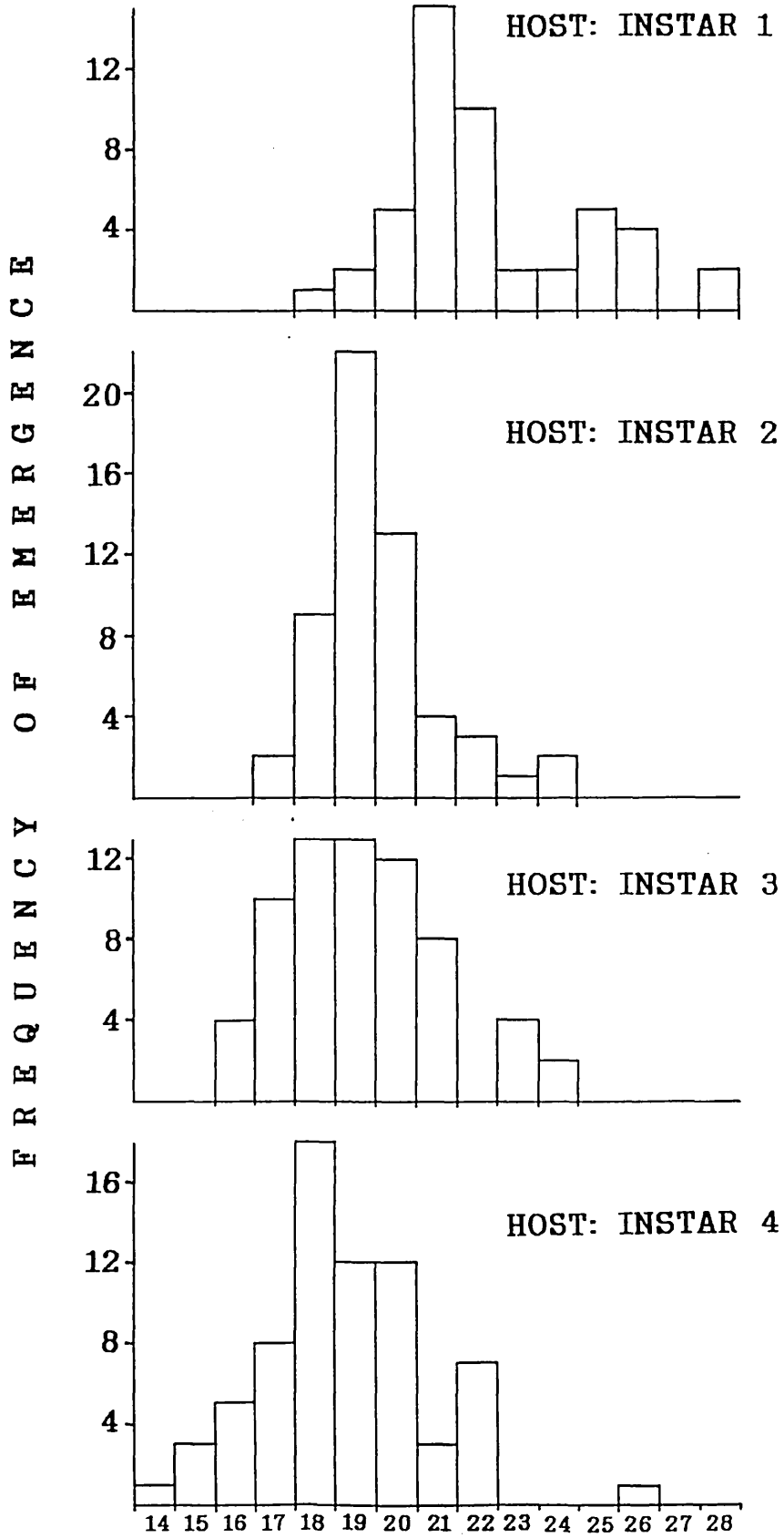
2. females had no experience of the range of hosts available to them. If offered late instar hosts before the experiment, they may have refrained from ovipositing in the early instars during the test period. However, such exposure would have reduced the number of ripe eggs in the ovarioles by an uncertain amount and increased handling would have increased mortality.

3. individual females also had no choice of instars within a particular replicate. Data on female preference for later instars are given from behavioural observations in the section on oviposition time.

The results are in close agreement with those of Avilla and Copland (1987) who used the glasshouse whitefly *Trialeurodes vaporariorum* on tomato plants. Mean female development time(±SE) ranged from 22.3±0.1 days in the 1st instar to 18.0±0.1 days in 3rd instar nymphs at 24°*C*. Variation in female development time was also similar given that their sample size was 2-3 times larger than the present study. When eggs were laid in the 1st, 2nd, or 3rd instar, hatching occurred in the following instar. Third instar nymphs yielded the largest female parasitoids. Castresana et al.(1979) reported similar development times and variation following the same pattern of longer developmental times in younger instars.

Variable female development times have been recorded for a number of aphelinid species (Flanders 1939a; Gerling and Bar 1971; Donaldson 1984; Gerling 1987). Broodryk and Doutt (1966) recorded an initial peak of female emergence 35 days after parasitism in the autoparasitoid *Coccophagoides utilis*. Emergence peaked again some 25 days later (concurrent with emergence of males following hyperparasitism by virgin females at 35 days). Small numbers of females were still emerging up to 45 days after the initial peak. Broodryk and Doutt believed this to be a consequence of the intrinsic ability of some females to lay slow-developing diploid eggs. As they increased the number of foundress females on a patch, so the frequency of simultaneous emergence

Figure 4.4: Patterns of emergence of female *E. tricolor* from each host instar



of both sexes also increased, i.e. a fraction of the female population possessed a slow-development trait; as their sample size increased so did the chances of inclusion of females expressing this trait.

Encarsia tricolor failed to show overtly variable female development times either in this study, or that of Castresana et al.(1979) or Avilla and Copland (1987) mentioned above. This was confirmed by examination of figure 4.4, which show the frequency of female developmental times for each of the four host instars. None of the four histograms show bimodality, skew in either direction or pronounced platykurtosis.

4.2.3 *Instar acceptability for male eggs and male development time.*

Methodology

Within 24hrs of emergence, individual virgin females were confined on a leaf using the clip cage described earlier. Whitefly on the leaf had previously been exposed to approximately ten mated females from the culture to give a high ratio of parasitised to unparasitised hosts of uniform age. These female larvae were allowed to develop until early larval (2-3 days old), mature larval (6-7 days old), or early pupal (10-11) stages were present . The leaf was then offered to the virgin female for 24hrs at 25°*C*. Each virgin could utilise the unparasitised hosts for host-feeding but could lay unfertilised eggs only in the parasitised hosts.

All replicates were allowed to develop until the primary parasitoids were 12-13 days old, when they were individually transferred to gelatin capsules, maintained at 25°*C*, and checked daily for emergence. Parasitised scales which failed to emerge were dissected to discover the fate of their contents.

Results

The results are presented in table 4.4. The variable number of parasitised hosts offered per replicate means that it was not possible to detect any preference for a particular host instar. Male development was possible in all parasitoid stages from early larval to early pupal. Significant differences in the development times of males utilising different primary host stages show that male development is extended when younger stages are attacked. This suggests that the developing male waits for the female host to grow to a late larval or pupal size (a matter of up to 6 days) before hyperparasitic development proceeds. Such delayed development has been seen in *Encarsia (Prospaltella) perniciosi* (Chumakova and Goryunova 1963) but is especially common in indirect heteronomous hyperparasitoids (Cendana 1937; Flanders 1936a,1936b,1952,1959,1969; Walter 1983).

Table 4.4: Instar acceptability and development time for males

	Age of female host parasitoid		
	2-3 day	6-7 day	10-11 day
No. successful reps	6	5	6
No. males emerging	41	27	36
mean dev. time ± SE(days)	19.87±0.22	16.48±0.26	14.28±0.14
Range of dev. times(days)	18-22	15-20	12-16

These results differ from those published by Avilla and Copland (1987) who found no significant differences in the development rates of *E. tricolor* males on young larval, mature larval or pupal female conspecifics (mean range 16-17.5 days at 24°C). The ability of *E. tricolor* to use alternative species for male production was confirmed as males developed fastest (14.8± 0.2 days mean± SE estimated from graph) in mature *E. formosa* larvae ($P<0.01$ Newman-Keuls).

Differences in the study described by Avilla and Copland may be attributable to interspecific variation in their host plant, whitefly, and origin of their *E. tricolor* (a glasshouse culture in Catalonia, N. Spain).

4.2.4 *Fecundity*

The problem of assessing heteronomous hyperparasitoid fecundity is complicated by sex ratio. The logistic problems of supplying a female sequentially with patches identical in host stage and ratio of parasitised to unparasitised hosts make experiments of this kind impracticable. Where such experiments have been described in the literature, the degree of control over the ratio of host types and the developmental stage (particularly of the secondary host) has been minimal or non-existent. For this reason, the vast majority of studies have neglected fecundity involving haploid eggs and supplied parasitoids solely with primary hosts for laying fertilised (female) eggs.

Methodology

As in previous experiments virgin females were collected and allowed to mate. Single females were confined on an infested leaf, with a male, in a clip cage, for the first two days of the experiment, after which the male was removed. Females were offered fresh leaf patches with an abundance of third instar hosts at 48 hourly intervals until death. Parasitised scales were allowed to develop and emerge as previously. Individuals

which pupated but failed to emerge were included in the fecundity data. No data were collected on developmental mortality from egg to pupa. Only female eggs were laid during the experimental period. The experiment was run at 25°C and replicated 12 times.

Results

Results are presented graphically in figure 4.5. Mean lifetime egg production was 85.36 ±13.85 (mean ±SE) at a mean of 7.31 ±0.27 eggs per female per day. When data from the 12 replicates were pooled, an increase in fecundity was seen on days 3-4 of the experiments, but this rise was not significant ($P>0.05$ paired t-test). Walter (1984) observed such a rise on day 2 of the life of *Coccophagus bartletti* females. This may reflect a physiological response by the female to earlier host feeding allowing increased protein anabolism and a consequent rise in egg production. Such responses to host feeding are common in synovigenic species and have repeatedly been shown to enhance the lifetime fecundity and longevity of parasitoids from many genera (Jervis and Kidd 1986)

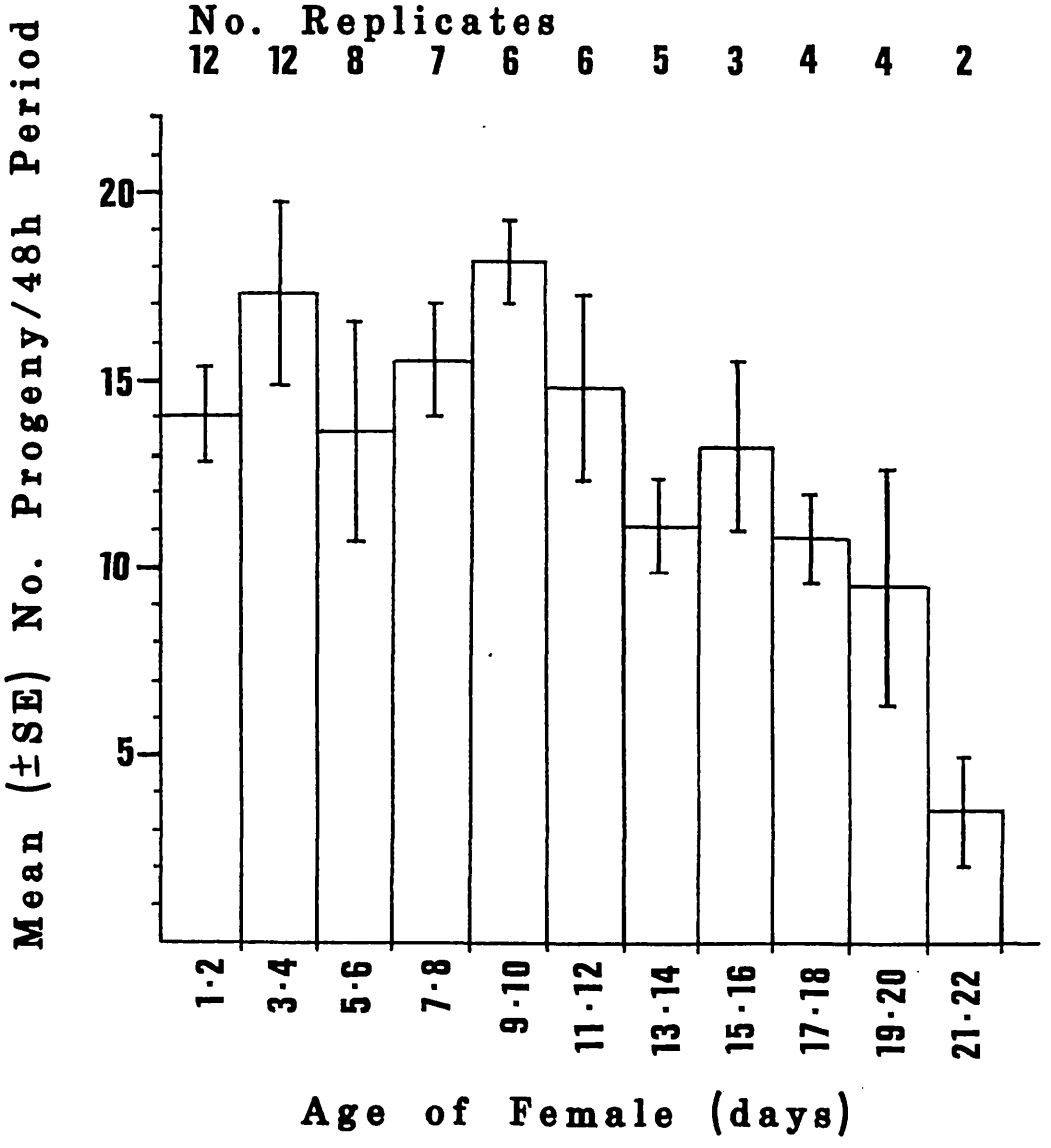
Total mean egg production peaked at 9-10 days and gradually declined thereafter. No post-ovipositional period was apparent. The fecundities observed in this experiment are comparable to those found in other aphelinids, as shown in the appendix which is a survey of the available literature on aphelinid fecundities. The range of fecundities shows that without exception, aphelinids have very low fecundities with maximum egg production under optimal conditions of up to 28 eggs per day for *Eretmocerus serius*, but more typically between 4 and 10 eggs per day for the vast majority of species.

4.2.5 *Culture sex ratio*

In maintaining the culture it was found necessary to periodically remove infested leaves and collect the parasitoids that emerged from them. These wasps were used for the propagation of experiments or were returned to the culture cage. Clues as to changes in sex ratio on individual leaves and in the culture cage were also obtained.

Plants infested with all whitefly stages were placed in the culture cage. Initially leaves were removed on an ad-lib basis between 9 and 62 days after being placed in the cage. The number and sex ratio of parasitoids emerging from each leaf was recorded daily and the parasitoids returned to culture. Each leaf was monitored in this way for approximately two weeks after parasitoid emergence had ceased whereupon the leaf was discarded. Later however, the sampling routine was formalised to removal of two leaves per plant per week with an additional plant added to the culture cage every fortnight. The data presented below are solely from the period of formal sampling. Samples were also taken as estimates of percentage parasitism (of whitefly) at weekly intervals. In this

Figure 4.5: Fecundity of *E. tricolor* when laying female eggs in unparasitised hosts at 48 hourly intervals over the lifetime of the wasp



way patterns of emergence of both sexes from individual leaves were typified according to the length of time they had been exposed to parasitism. This is described in the first section below.

From knowing the number of plants in the culture cage, the number of leaves on those plants and their age on any particular week, together with the estimates of emergence of the sexes from individual leaves, it was possible to calculate the probable changes in sex ratio occuring within the culture cage over the period of formal management. These predictions are described in the second section below.

Results

When sampling under the formal regime, the overall sex ratio was significantly male biased at 0.58 male ($\chi^2_1 = 1576 \quad P < 0.001 \quad n = 10174$). When sampling on a completely ad. lib. basis with irregular additions to, and samples taken from the culture cage, as was practised initially over 45 weeks, the overall sex ratio was significantly female biased at 0.46 male ($\chi^2_1 = 39.8 \quad P < 0.001 \quad n = 7711$).

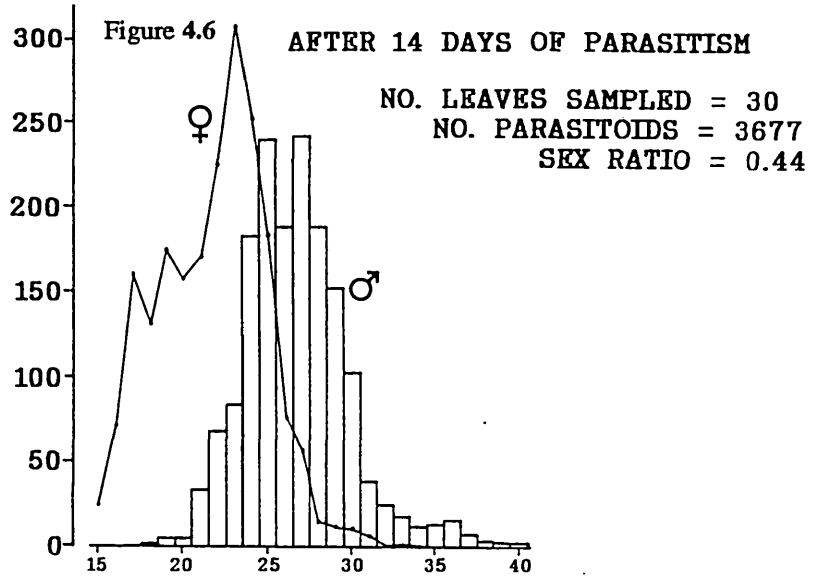
4.2.5.1 *Sex ratio dynamics on individual leaves as a function of their period of exposure to parasitism*

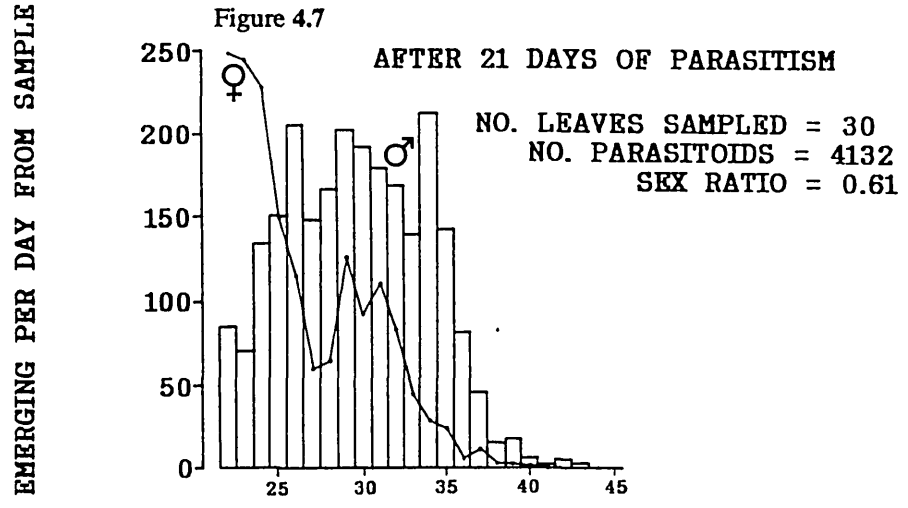
Sampling leaves at 2,3 and 4 weeks yielded a progressively more male biased sex ratio. The distribution of emergence of the sexes also shifted from distinct peaks of females followed by males at 2 weeks (0.44 male), to a tail-end decline of female emergence and broadened peak of male emergence at 4 weeks (0.76 male) (figures 4.6-4.8). Percentage parasitism increased from 23-87% during this period. The sampling regime was such that most plants were defoliated by 4 weeks. This accounts for the reduced sample at 5 weeks (figure 4.9). Figures 4.10 and 4.11 show the relationship between percentage parasitism (primary and secondary combined) and emergent sex ratio from 2 to 5 weeks. The two graphs show that the sex ratio emerging from each leaf is a reflection of the relative availability of hosts for both sexes.

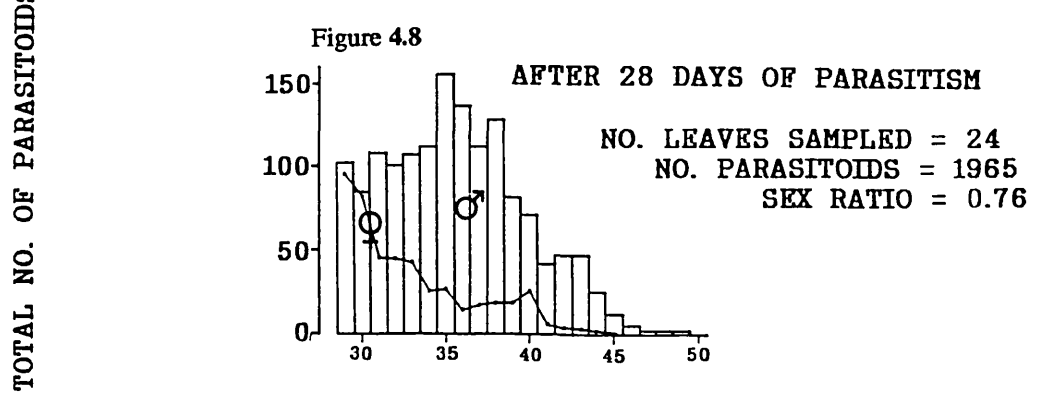
4.2.5.2 *Sex ratio dynamics in the culture cage as a function of the age of the culture*

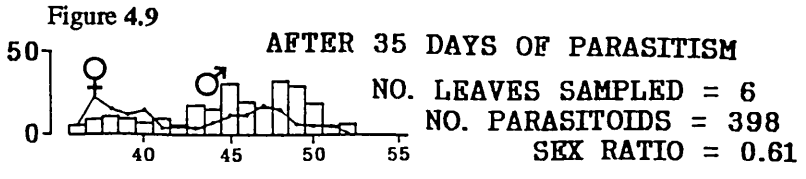
The emergence data were used to estimate changes in culture numbers and sex ratio over the 30 weeks of in which formal sampling occurred. Emergence from 0-21 days, 22-28 days, and 29-35 days were calculated as mean numbers of parasitoids of both sexes per leaf, using the relevant parts of figures 4.6, 4.7 and 4.8 respectively (see table 4.5). A small number of leaves, from the leafiest plants, were exposed to culture for more than 35 days. Emergence from these leaves was estimated from the latter part of figure 4.8. The latter part of figure 4.9 was used to estimate emergence beyond 42 days.

Figures 4.6-4.9: Emergence of both sexes of *E. tricolor* from samples taken from plants exposed to parasitism for 14-35 days









TOTAL NO. OF PARASITOIDS EMERGING PER DAY FROM SAMPLE

Estimates beyond 42 days accounted for less than 7% of the total. These estimates were then applied to the conditions of the culture by considering the number of leaves present and their age on any particular week.

When the data of table 4.5 were combined with the continuous records of leaf numbers and age in the culture cage, regular cycles of fluctuations in sex ratio were predicted (figure 4.12). These estimated sex ratio fluctuations ranged from 0.17 to 0.66 about a mean of 0.41. This value is obviously markedly more female biased than the overall sex ratio sampled from individual leaves, showing that early female emergence accounts for a large part of the dynamics of the culture. The fact that the cycles were always multiples of 2 shows the role of fortnightly addition of fresh whitefly material in permitting further primary parasitism. Less frequent additions of fresh material would undoubtedly drive the culture to a more male biased state. The significance of these data in the mass-rearing of autoparasitoids for biological control releases is discussed later.

Table 4.5: Emergence of parasitoids at different intervals after introduction to the culture. Data from figures 4.6-4.9 of the culture sampling program.

Days since initial exposure to parasitism	No. leaves	Total estimated emergence in this period		Mean No. Wasps Emerging/Leaf	
		males	females	males	females
0-21	30	45	895	1.5	29.8
22-28	30	959	1097	32.0	36.6
29-35	24	774	366	25.8	12.2
35-42	24	620	104	20.7	3.5
43-49	6	186	83	31.0	13.8

4.2.6 *Oviposition time*

The aim of this section was to determine if there were significant differences in the investment females allocated to laying males and females, by measuring the ovipositional time necessary to lay eggs of each sex. Data were also collected showing female preference for oviposition in late instar whitefly nymphs.

Figure 4.10: Mean percentage parasitism of whitefly on individual leaves after different periods of exposure to parasitism

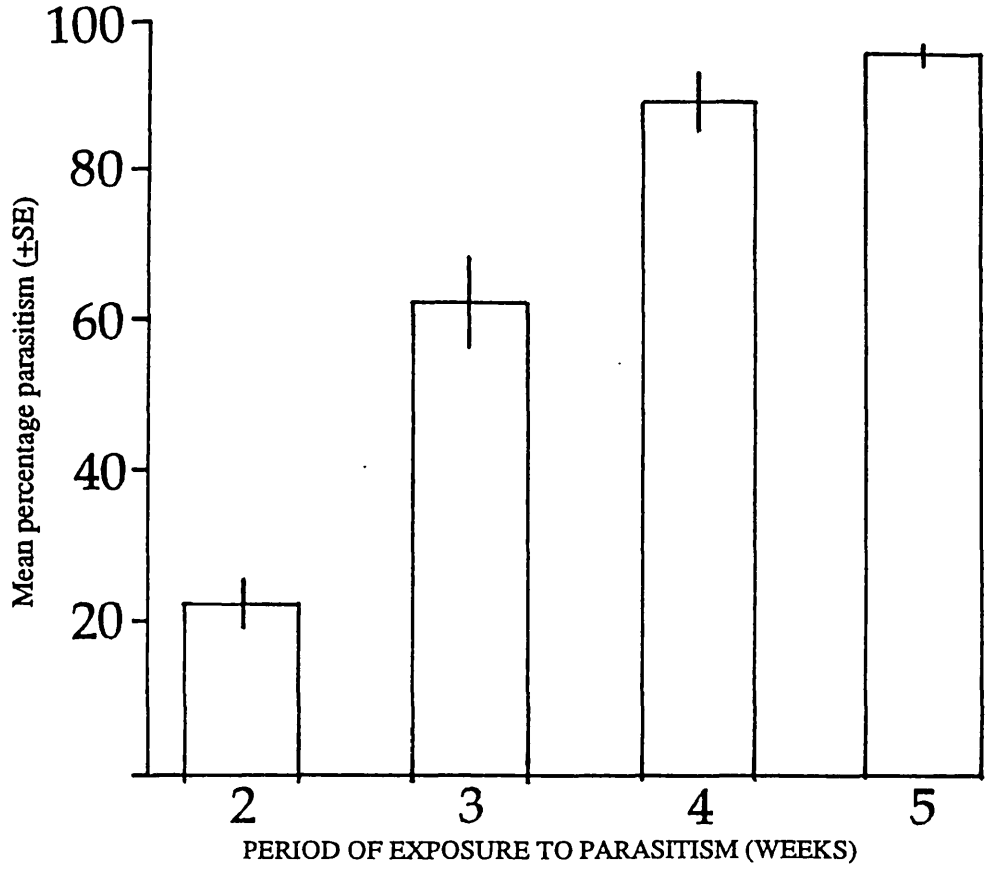


Figure 4.11: Overall sex ratio of *E. tricolor* emerging from leaves sampled following different periods of exposure to parasitism (data from figures 4.4-4.9)

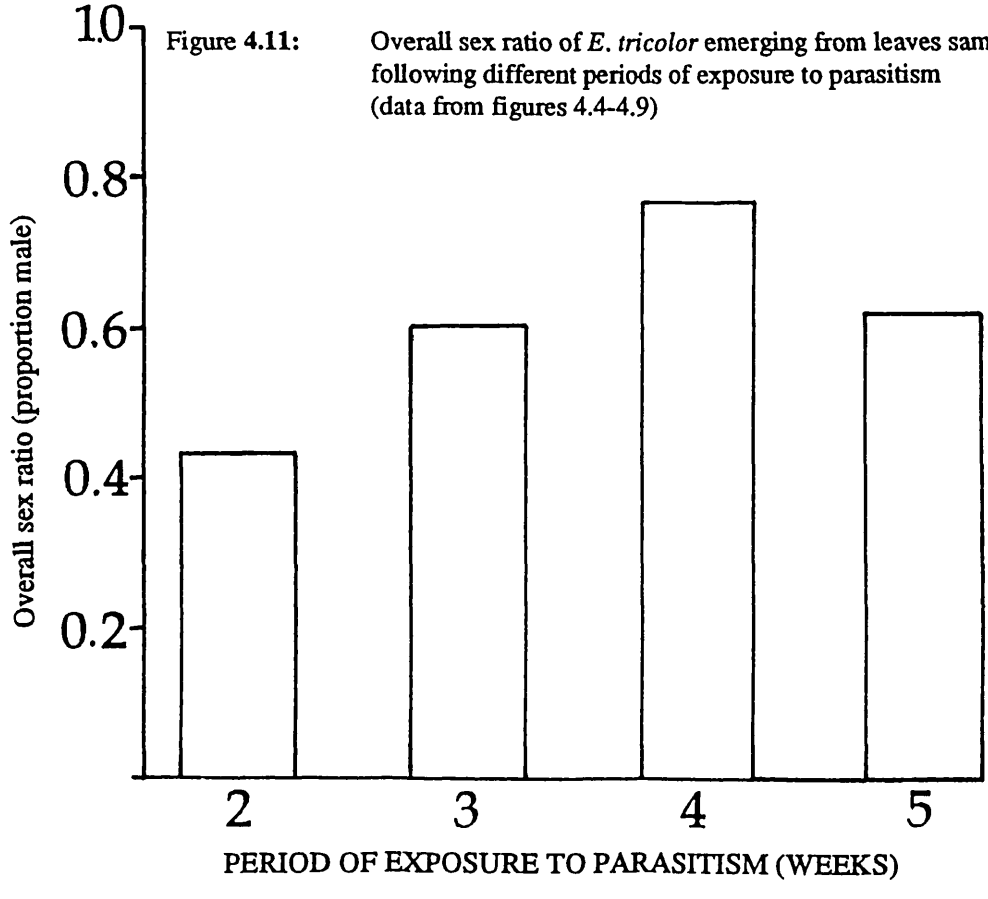
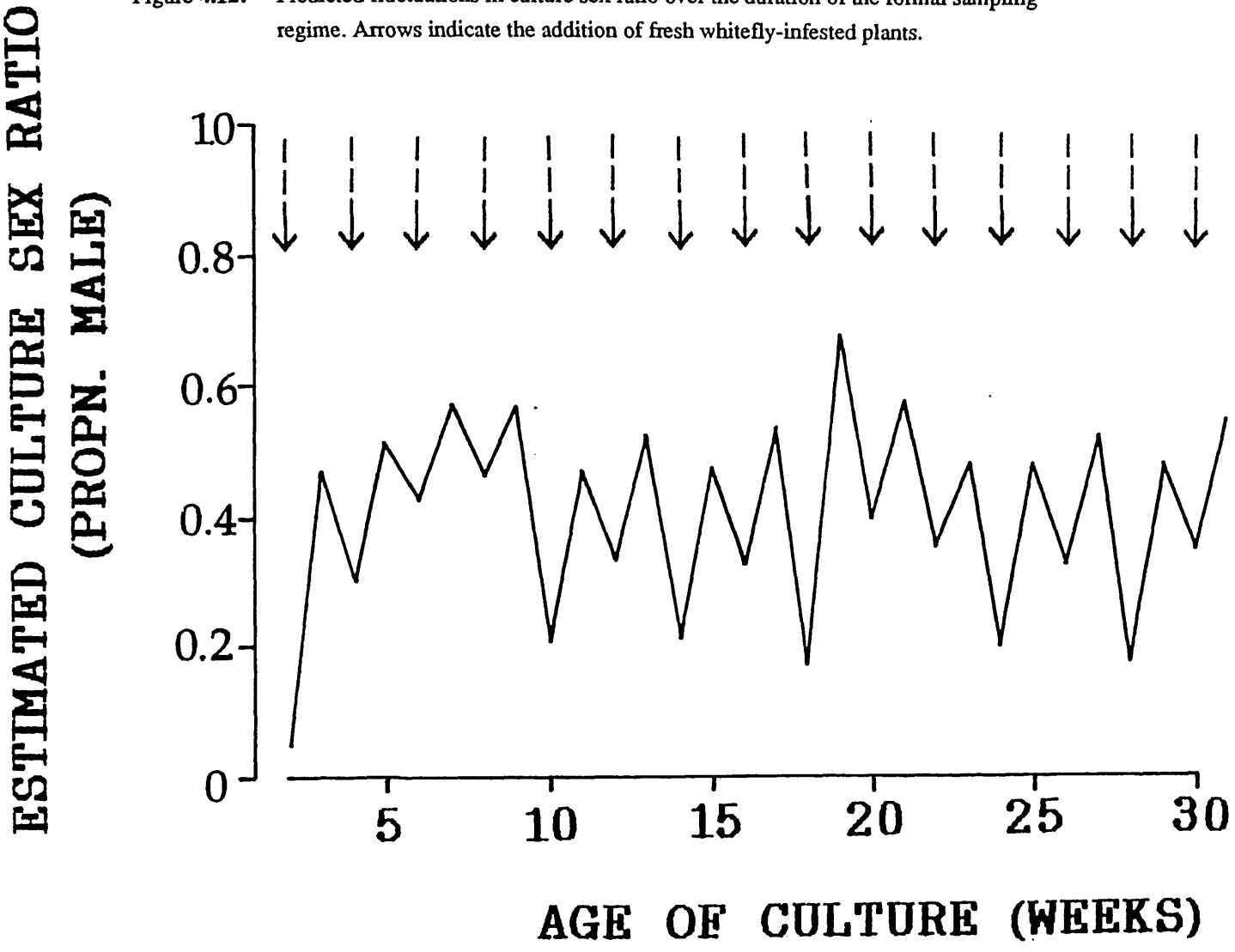


Figure 4.12: Predicted fluctuations in culture sex ratio over the duration of the formal sampling regime. Arrows indicate the addition of fresh whitefly-infested plants.



Methodology

Young mated females (24-48hrs old), with no ovipositional experience were placed in pairs on a leaf area containing all whitefly instars in varying proportions. Each pair were observed for 1h at 25°C and ovipositional times recorded to an accuracy of 1 second using a digital stopwatch.

The methodology differed slightly for each sex. For diploid (female) eggs, a plan was made of the nymphs on each leaf area which allowed those parasitized to be distinguished later from those which had been probed and rejected. The sequence in which each female encountered each nymph was also recorded. Female preference for late instar whitefly was determined by comparison of the proportion of nymphal instars accepted for oviposition against those encountered but not parasitised. Parasitism was determined by dissection 3-5 days after the observations were made.

To determine oviposition times for haploid eggs, pairs of females were offered leaf areas containing only pre-pupal and pupal stage *E. tricolor* females as hosts for males. All pupae which had been probed during the observational period were placed individually in labelled gelatin capsules and held at 25°C. Pupae were disected 3-5 days later to detect the presence of hyperparasitic male larvae laid during the experimental period.

Results

The mean oviposition times recorded for late whitefly nymphs (female eggs) and conspecific pupae (male eggs) are given in table 4.6 below. The combined drilling and oviposition times were significantly longer for 4th instar compared to 3rd instar nymphs, but longest of all for conspecific pupae.

Table 4.6: Observed mean oviposition times for male and female eggs in conspecific pupae and late instar whitefly nymphs respectively

Type of host	Sex of egg	No. of observations	Mean (±SE) oviposition time (seconds)	t-test
3rd instar nymph	Female	16	210.18±43.07	
				P<0.01
4th instar nymph	Female	24	277.42±78.29	
				P<0.001
E. tricolor pupa	Male	14	669.00±84.11	

Table 4.7: Female ovipositional preferences when all primary host instars were offered to previously unexperienced females

	WHITEFLY INSTAR			
	1st	2nd	3rd	4th
Total number encountered	95	35	80	98
No. parasitised	0	0	16	24
No. drilled but rejected	0	1	23	33
No. used for host-feeding	3	5	5	7

Previous work on this system gave drilling times of 340 seconds for 4th instar *A. proletella* nymphs (female eggs), and 512 seconds for late larval conspecific *E. tricolor* (male eggs) at 20°*C* (O. Alomar, pers. comm.). Drilling times for 3 species of *Encarsia* on 3rd and 4th instar *Bemisia tabaci* were also in line with the present study. Mean oviposition time was shortest in the smallest species *E. deserti* 241±17 secs (mean±SE), intermediate in *E. cibciensis* 275±14 secs and longest in the largest species *E. adrianae* 327±41 secs at 25°*C* (Lopez-Avila 1988). This pattern continued in oviposition time of male eggs into conspecific pupae, although mean hyperparasitism times and the differences between primary and secondary parasitism ovipositional times, were greater than those found for *E. tricolor.*

As aphelinids have a low fecundity (see appendix), differences in the ovipositional period required for each sex are probably not important factors of differential sexual investment. However, if oviposition in different hosts carries with it a mortality risk, then the "cost" of each sex may differ. The effect of ovipositional mortality risk has been recognised by Weis, Price and Lynch 1983, Isawa, Suzuki and Matsuda 1984, and Charnov and Skinner 1985. All these models indicate that it acts to reduce the frequency of ovipositions and increase the number of progeny allocated to each host. Isawa et. al. (1984) reasoned that this factor is probably not important in the field as handling time is usually negligible compared to searching time (Hassell 1978) and the ovipositional mortality risk must be very large to induce any change in preference for a less "risky" host.

There was a marked preference of ovipositing females to parasitise late instar whitefly nymphs (table 4.7). When all host instars were offered simultaneously, 3rd and 4th instar nymphs were attacked to a similar extent whereas 1st and 2nd instars were used only for host feeding.

There are three advantages to laying female eggs in later instar hosts:

1. the female is able to assess the host resource exactly with regard to quality, size and suitability at the moment of oviposition,

2. eggs laid in early instars will suffer the mortality risks associated with the age of the host - leaf death, disease, moulting etc.,

3. shorter developmental times in late instars reduce the period for which the female is susceptible to hyperparasitism.

The oviposition observations and subsequent work described in chapters 5 and 6 gave data on the rate of encapsulation of haploid and diploid eggs in both types of host. Table 4.8 gives the results of the dissections and shows that encapsulation of eggs occurred at a very low rate in parasitised and unparasitised hosts. Mechanical damage during the transfer of parasitised scales into gelatin capsules was a more common cause of immature parasitoid mortality, but was easy to identify and investigate.

Table 4.8: Dissection data from the ovipositional observations and subsequent work on species recognition and sex ratio, showing the frequencies of encapsulation of male and female eggs.

	3rd+4th instar Whitefly	*E. tricolor* pupae	*E. inaron* pupae	*E. tricolor* pupae[a]
No. scales dissected	97	170	213	46
No. scales parasitised	40	24	82	236
No. eggs encapsulated	1	0	1[b]	1

Notes: **a** - data from sex ratio experiment in which 46 out of 236 hyperparasitised scales failed to emerge and were dissected. Most of the mortality was attributable to manipulative damage.
b - 2 dead male larvae also appeared to have been encapsulated.

4.3 Summary

Those biological features of *E. tricolor* most significant to its ecology and sex ratio are summarised below.

1. *E. tricolor* is a facultative autoparasitoid (Walter 1983a). Males and females develop endoparasitically on immature primary parasitoids and whitefly nymphs respectively. Neither sex is highly specific in its host relations: females have been recorded from 5 species of whitefly and males from 7 parasitoid species, including conspecifics.

2. *E. tricolor*, as other parasitic hymenoptera, uses haplodiploid sex determination. Females are arrhenotokous. This permits deposition of unfertilised male eggs by virgins and facultative control of sex ratio following mating. Mating behaviour was typically brief but showed pre and post-coital ritual to be necessary.

3.The correlation between size and longevity was linear and positive for both sexes.

4.Females can develop in all whitefly stages. When a range of instars were available parasitism occurred only in late instars; all stages were used for host feeding.

5. Male development times were extended when young larval conspecifics were attacked. This indicates that development does not proceed until the primary parasitoid has attained a certain size or age.

6. Fecundity peaked 2-3 days after exposure to hosts, probably as a result of host feeding. As with other aphelinids, *E. tricolor* is egg limited laying some 7 eggs per day.

7. Under a formal sampling regime, sex ratio dynamics were determined both in relation to the length of time each leaf was exposed to parasitism and in relation to the changes in sex ratio within the culture cage. The emergent sex ratio from individual leaves shifted from 0.44 at 2 weeks to 0.76 after 4 weeks in the culture. The overall emergent sex ratio from these samples was 0.58. These data were used in conjunction with records of leaf and plant numbers in the culture cage to predict the actual sex ratio fluctuations occurring in the culture over time. The predicted sex ratios cycled aroud a mean of 0.41 showing that early female emergence contributed in a large part to the dynamics of the culture sex ratio. The periodicity of these cycles was driven by the addition of fresh whitefly hosts.

8. Finally, oviposition of male eggs was found to take approximately three times longer than for female eggs. This represents a greater investment in males over females, and therefore would be expected to cause a shift in the sex ratio in favour of females under Fisher's principle of equal investment. As aphelinid oviposition times are probably small compared to the time necessary to search for, locate and assess hosts of both types, such inequalities are unlikely to be significant factors in selecting for a biased sex ratio.

CHAPTER 5

SPECIES DISCRIMINATION AND KIN RECOGNITION

5.1 Species discrimination

Historically, explanations of heteronomous hyperparasitism and the role of competing species in acting as male hosts, have suffered greatly from being phrased in terms of group selection. Perhaps the most relevant idea to this section in the early literature was also the most misguided. Zinna 1962 (in Flanders 1967) believed that autoparasitism was an adaptation in which the sacrifice of a percentage of the female population was necessary to maintain equilibrium with the host. To Zinna, heteronomy amongst the Aphelinidae represented an increasing series of adaptations "..having the purpose of reducing the trophic pressure and competition on host populations".

In the natural environment, a heteronomous hyperparasitoid will almost always find itself in competition with a complex of other parasitoids: Aphelinidae, Encyrtidae, Platygasteridae, Pteromalidae, Eulophidae (Clausen 1978) attacking whitefly and scale insect populations. Heteronomous hyperparasitism depends on interspecific discrimination. The ability of obligate autoparasitoids to reject other species, of alloparasitoids to reject conspecifics, and of facultative autoparasitoids to choose between acceptance and rejection, is what defines the classification of each group.

For facultative autoparasitoids given a choice of competing species or conspecifics for male production, it is intuitively obvious that hyperparasitism of competing species would ensure increased local mating opportunities among conspecifics. If patches containing more than one species of primary parasitoid are regularly encountered during the searching life of a female autoparasitoid, selection should act for preferential attack of competitors (male production) and primary parasitism to ensure mates for sons.

In this experiment interspecific discrimination was tested using *E. tricolor* already experienced with conspecific pupae.

Methodology

Between one and four mated females of both *E. tricolor* and *E. inaron* were offered a patch of third and fourth instar whitefly simultaneously for 24hrs at 25°C to generate areas containing various ratios of both species. Both sexes of *E. inaron* develop as primary parasitoids. Parasitised scales were allowed to develop until the early pupal stage (approximately 10 days), whereupon the leaf was cleared of unparasitised nymphs.

Individual mated *E. tricolor* females were allowed access to conspecific larvae and pupae for 24hrs, then transferred to the leaf patch and confined in a clip cage for 24hrs. During this period each female had a choice of laying male eggs in either species of *Encarsia*.

After the experimental period pupae were transferred individually to gelatin capsules. Hyperparasitic male *E. tricolor* were allowed to develop for 2-3 days at 25°C. Scales were then dissected to identify host species (primary parasitoid) and to discover the presence of young male larvae. Those scales which could not be dissected within 3 days were held at 10±1°C (16:8 L:D) for up to 2 days to prevent male larvae destroying features of the primary parasitoids necessary for identification. The features used to identify the two species were:

1. pigmentation of the abdomen dorso-anteriorally and the thorax dorsally
2. antennal morphology
3. sex (*E. inaron* laid male primary parasitoids).

These dissection data gave the ratio of *E. tricolor:E. inaron* offered in each replicate and the extent to which *E. tricolor* can discriminate between species during oviposition. There were 15 successful replicates.

Results

Results are presented in table 5.1. *E. inaron* pupae suffered over eight times more hyperparasitism than conspecific *E. tricolor*. The result was highly significant (Chi-squared P<0.005) and varied little among replicates: $\chi^2_1 = 30.8$.

Table 5.1: Species discrimination by *E. tricolor*

	Parasitoid species	
	E. tricolor	*E. inaron*
Total no. of pupae offered	137	213
Total no. of male larvae laid	10	82
Percentage of pupae attacked	7.2	38.5

A total of ten pupae were hyperparasitised but could not be positively identified. Both sexes of *E. inaron* suffered equally from male hyperparasitism.

There has been little previous work on host discrimination in facultative autoparasitoids. Dowell, Puckett and Johnson (1981) reported that *E. opulenta* showed a significant preference for citrus blackfly (*Aleurocanthus woglumi*) parasitised by the platygasterid *Amitus hesperidium* when offered mixtures of primary and secondary hosts. Data taken from their paper show that the overall sex ratio laid changed from 0.258 male in the presence of conspecifics to 0.431 when *A. hesperidium* was the only species available for male production. Gerling, Spivak and Vinson (1987) state that *Encarsia lutea* was incapable of interspecific host discrimination although they present data which show that *E. lutea* lays more males in *Bemisia tabaci* parasitised by *Eretmocerus mundus* than in nymphs previously parasitised by conspecifics; the percentage of hosts accepted for oviposition rose from 19.4% with conspecific hosts to 35.8% with *Eret. mundus*.

Table 5.2 shows the results of measuring the head capsules of male *E. tricolor* from conspecific and *E. inaron* hosts. Adult male *E. tricolor* which had been laid hyperparasitically in *E. inaron* were not significantly larger than those laid as hyperparasitoids of conspecifics. The preference for *E. inaron* as secondary hosts can therefore not be an adaptive response to a host which produces larger (fitter) male offspring (see host size effects in chapter 1).

Table 5.2: Effect of host species on size of male *E. tricolor*

Male host species	Mean head width (mm)	SE	no. observations
E. tricolor	0.207*	0.003	58
E. inaron	0.215**	0.007	39

$t_{(95)}$=1.40 P>0.05

* data from longevity experiment 4.2.1

** data from sex ratio experiment, chapter 6

Discussion

In addition to providing increased mating opportunities for sons, preferential exploitation of competitor species could also be seen as a mechanism by which females ensure that they are not hyperparasitising their own progeny, or those of closely related conspecifics. That is to say, if males are laid in another species they cannot, by definition, develop at the expense of conspecific females with whom they may share a high proportion of genes.

Avilla and Copland (1987) found that *E. tricolor* males laid in *E. formosa* pupae were significantly larger than when they developed at the expense of conspecific female pupae. Preferential exploitation of *E. formosa* could thus have benefits in terms of the fitness (mating ability and longevity) of sons as well as offering sons more mating opportunities and avoidance of hyperparasitism of kin, mentioned already.

In laboratory cultures it was completely impossible to distinguish melanised pupae of *E. tricolor* and *E. inaron* visually (without dissection). As mentioned in chapter 4 however, *E. tricolor* has been recorded from the same host material as *Euderomphale chelidonii* in England. This eulophid has quite different pupal case features to either of the *Encarsia* spp. and can also serve as host to *E. tricolor* males (pers. obs.). It is quite plausible therefore to believe that *Euderomphale chelidonii* will be similarly discriminated against for male production in natural populations.

The data presented above show for the first time a direct preference for hyperparasitism of a competing species. The strength of this preference led me to investigate the ability of female *E. tricolor* to recognize and discriminate in favour of their own progeny.

5.2 Kin Recognition

Normally reproducing female parasitoids have little reason to recognize their own progeny except during superparasitism of previously laid clutches. This self superparasitism has completely different consequences for female fitness compared to conspecific superparasitism.

Optimal foraging theory has been used to show that when the rate or chance of finding hosts falls, the optimum clutch size increases to that which maximises fitness per host rather than lifetime reproductive fitness (Charnov and Skinner 1984, Parker and Courtney 1984, Waage and Godfray 1984, Waage 1986). In this way self superparasitism

is adjustment of the size and sex ratio of a previously laid clutch, with associated fitness changes for the egg laid and competing siblings (van Dijken and Waage 1987; van Alphen, van Dijken and Waage 1987).

Only two studies testing recognition of progeny have been published. The first of these showed that the solitary endoparasitoid *Nemeritis canescens* could discriminate between hosts parasitised by her own progeny and that of conspecifics, and could avoid superparasitism of the former. This occurred by detection of an individual-specific marker chemical released from the Dufour's gland. The effects of this marker were detectable for approximately 48hrs (Hubbard, Marris, Reynolds and Rowe 1987).

The second study by van Dijken and Waage (1987) showed that the gregarious egg parasitoid *Trichogramma evanescens* did not alter sex and progeny allocation when self or conspecific superparasitism were compared, despite marked differences predicted under LMC theory.

They discuss the possibility that searching females may be using one or a number of cues to recognize their own clutches laid earlier. These have relevancy to the present study and are listed below:

1. an individual-specific marker pheromone
2. recognition of the configuration of the patch
3. time between ovipositions as a measure of distance from own progeny
4. contact with other searching females as an indicator of local conspecific clutches

An autoparasitoid remaining on or revisiting a patch in which she has already oviposited also changes the potential fitness of both sexes developing locally by subsequent oviposition, especially if females (sisters) suffer hyperparasitism from their male siblings. For this reason, the ability of *E. tricolor* females to discriminate between their own progeny and those of conspecifics pupating within the same patch was tested

Methodology

A mated female and a male were confined on each side of a leaf infested with third and fourth instar nymphs using a clip cage divided in half (as described in chapter 2). The parasitoids were allowed to oviposit for 24hrs at 25°C. Each female was then transferred to a glass vial containing honey and kept at 20±1°C (16:8 L:D). The females were given occasional access to whitefly nymphs for host feeding. The leaf half and parasitoid were labelled correspondingly so that later identification was possible.

The parasitised scales were allowed to develop (at 25°C) until pupation. All unparasitised scales were then removed and one of the females which had previously oviposited on the leaf was replaced and allowed to lay males over the whole leaf - both on the side of her own progeny and those of a conspecific female, for 24hrs. Parasitoid pupae from both sides were then placed individually into labelled gelatin capsules held at 25°C. Daily checks were made for male emergence. It was thus possible to know the degree to which hyperparasitism occurred on each side of the leaf i.e. in a female's own daughters or in those of an unrelated female.

Results

It was not possible to detect any preference of females to avoid laying males in kin (see table 5.3).

Table 5.3: Kin recognition in *E. tricolor*

	Own progeny	Conspecific progeny
Number of pupae offered	148	184
Number of males laid	26	33

(Chi-squared N.S.) No. of replicates=23

This type of investigation has not been previously reported for any species of autoparasitoid. Lack of maternal discrimination against related daughters has been mentioned by Gerling (1987). When *Encarsia lutea* was simultaneously offered unparasitised *Bemisia tabaci*, nymphs parasitised by another female and nymphs previously parasitised by herself, then parasitism of each host type occurred in much the same frequency as the hosts were offered.

Discussion

Copland (1976) describes the alkaline gland associated with the reproductive system of female Hymenoptera, thought to produce a pheromone important in marking attacked hosts. This gland is well developed in the Aphelinidae. Recently parasitised primary or secondary hosts can thus be distinguished from unparasitised neighbours.

Once the larva hatches however, it becomes liable to hyperparasitism. Thus the pheromone need only be detectable for a relatively short duration; much less than the 10 day break in this study.

The apparent inability of an *E. tricolor* female to recognize her own progeny can also tell us something about her natural search patterns. It seems quite probable that having encountered and laid eggs in a particular patch, the female has a very small probability of re-encountering that patch. Under such a scenario there would be little if any selective advantage to being able to recognize kin and avoid hyperparasitism therein.

5.3 Summary

The experiments in this chapter demonstrate clearly for the first time that *E. tricolor* shows no tendency to avoid hyperparasitism of its own progeny. The preference of females to exploit a competitor such as *E. inaron* for male production was notable for its magnitude and led to deeper investigation of the *E. tricolor*/*E. inaron* interaction, both in terms of sex ratio (chapter 6), interspecific competition (chapter 7) and field population dynamics (chapter 8).

CHAPTER 6

HOST AVAILABILITY AND SEX RATIO IN *E. TRICOLOR*

6.1 Introduction

There exists a wealth of papers on host selection by parasitoids (reviews by Vinson 1976; van Alphen and Vet 1986). Recently these have focused on optimality theory; the adaptation of parasitoids to gain maximal fitness by acceptance of those hosts which maximise the product of offspring fitness and number of progeny, per unit of maternal investment. This investment will be in the form of eggs and time.

Optimal host selection theory considers the relative gains in fitness from acceptance of different types of host. A searching parasitoid has to make a series of inter-related decisions in order to maximise her lifetime reproductive success:

1. which hosts to attack - what species, what size or stage, and whether to superparasitise
2. in which patches to search - high or low density patches, patch time allocation
3. the clutch size to produce
4. what sex ratio to lay in each host.

Charnov and Stephens (1988) developed fitness models to elucidate how ecological and biological constraints affected the range of hosts which the parasitoid acting optimally, can attack. Charnov and Stephens focussed on three life history parameters in particular:

a. fitness gain from offspring on a single average host
b. search and handling time for a single average host
c. instantaneous mortality rate.

They considered two types of environment: one with a low, and one with a high degree of host patchiness (contaigon). Egg laying constraints were also imposed on the parasitoid: in one, eggs matured at a fixed rate. In the other, the rate of egg production was negatively correlated with adult survival (increased egg laying increased the probability of adult mortality).

Their findings were not suprising. When host contagion was low, limiting egg production restricted the number of host types that can be attacked. When contagion was high and a female could rank all host types in a patch, the optimal range of host types was found using the method of Charnov's marginal-value theorum. With high host clumping a female is faced with two choices over egg laying:

1. to lay all her eggs in the patch, leave and search for more hosts

2. to lay all her eggs and remain in the patch laying eggs as they mature, then leave and search for more patches.

The optimal behaviour predicted by Charnov and Stephens depended on the interpatch travel time. Van Alphen and Janssen (1982) showed that a braconid endoparasitoid of *Drosophila* larvae was able to discriminate between host species and rank them according to the profitability of offspring survival in that species. This gives some qualitative support for these optimal diet type of models.

The situation is more complicated in autoparasitoids however, as acceptance of a particular host species for oviposition determines the sex of the progeny. The marked fitness penalties which a female may suffer from over-production of one sex simply due to host availability means that optimal host selection ***must*** be considered in the context of sex allocation.

Statements made by Flanders (1942,1956,1967) led to the belief that for autoparasitic species, female preferences for particular hosts changed radically upon mating from laying only male to laying only female eggs. Therefore the population sex ratio under this regime was dependent on the availability of males in the environment and the ratio of male hosts encountered before mating to female hosts encountered afterwards. However, successive demonstrations of the ability of fertilised females to lay males (Zinna 1962 for *Coccophagoides similis*, Gerling 1966 for *Encarsia pergandiella*, Bartlett [in Flanders 1969] for *Coccophagus lycimnia* and *C. scutellaris*, Williams 1972 for *Physcus seminotus* and *P. subflavus* and numerous others subsequently reviewed by Walter 1983; Viggiani 1984), led to general acceptance that post-mating male production was within the control of the female autoparasitoid.

A number of authors have suggested that heteronomous hyperparasitoids may have relaxed control over their sex ratio and lay each sex simply in proportion to the availability of parasitised and unparasitised hosts (Flanders 1939b, 1942, 1956, 1967; Zinna 1961,

1962; Williams 1977; Hassell *et. al.* 1983; Viggiani 1984; Donaldson 1985). The validity of this suggestion is obviously of crucial importance when investigating sex allocation behaviour in autoparasitoids.

When offering different host ratios to *Encarsia pergandiella* Hunter (in press) found that the sex ratio laid was always greater than the proportion of parasitised hosts (conspecifics) offered. This effect was statistically significant for the 25% and 50% parasitised host treatments in which mean sex ratios of 0.42 and 0.68 were laid respectively, but not significant for the 75% parasitised treatment which yielded a sex ratio of 0.83.

Donaldson (1985) obtained a completely different result with *Coccophagus atratus* when offered identical host ratios at intervals through the life-span of the parasitoid. Sex ratios close to the frequency of hosts available were recorded. The sequence of laying male and female eggs was random and sex ratio did not change significantly during the female's life.

To investigate the role of host availability has on the sex ratio laid by an autoparasitoid I examined the effect of offering different ratios of healthy and parasitised hosts to females which had four types of prior ovipositional experience.

6.2 Methodology

Young mated females were individually confined on a leaf area using a standard clip cage as described already. Each experimental area contained an abundance of parasitised and unparasitised hosts, in various ratios from 12-80% parasitised. All the parasitised hosts were young female *E. tricolor* pupae laid approximately 10 days previously. All early whitefly nymphs were removed leaving only a known ratio of parasitoid pupae and 3rd/4th instar whitefly. The total number of hosts per arena ranged from 25 to 375. Four types of prior ovipositional experience was offered to individual females with a young male for 24hrs at 25°*C*. These were:

1. none (access to honey only)
2. female *E. tricolor* pupae
3. healthy whitefly nymphs
4. *E. inaron* pupae

Between 2-4hrs after this experience both male and female were transferred to the experimental patch for 24hrs at 25°C. Following this period of experimental parasitism, parasitoid pupae were placed individually in gelatin capsules and held at 25°C. The experimental leaf area was also left at 25°C to allow female eggs laid in whitefly nymphs to develop until pupation, whereupon they too were transferred to gelatin capsules. Parasitoids were checked daily and sexed upon emergence. Mortality of both sexes occuring in the larval or pupal stage was detectable by dissection of scales which did not emerge.

For those females experienced with *E. inaron*, the same species was offered during the experimental period i.e. a choice of *E. inaron* pupae or unparasitised whitefly.

6.3 Statistical analysis

Data were analysed by fitting a series of generalised linear models (McCullagh and Nelder 1983) using a GLIM program (R. Stat. Soc. 1985). GLIM fits a model to pre-specified response and explanatory variables by maximum likelihood method which is the same as least squares when normal errors are assumed. Data are compared with model predictions, and differences minimised by adjustment of parameters. GLIM makes no distinction between continuous covariates (e.g. regressions) and discrete covariates (e.g. ANOVA). There is usually no need to transform non-normally distributed data as GLIM changes the model to fit the data rather than adjusting data to fit the values predicted by the model. Thus Poisson or binomially distributed data can be analysed unchanged.

The procedure for analysis is broadly as follows. Initially, a null model is fitted to give total deviance - a measure of the total variation in the data. The effect of different factors on the deviance between model and data values are assessed by fitting regression models of increasing complexity and specificity. The significance of any reduction in deviance can be calculated by construction of the appropriate F table using the values generated by the current GLIM model.

GLIM gives the results (r) of binomially distributed data analysis as a logit function $[\ln\left(\frac{p}{1-p}\right)]$ of a linear model, x. Transformation back to the natural state is achieved by the following equation:

$$r = \frac{e^x}{(1+e^x)}$$

The significance of GLIM answers from binomially distributed data are assessed using Chi-squared statistics to which they approximate in distribution.

For the analysis of the sex ratio data, binomial errors were assumed with the y-variate being the number of males laid in each replicate. For the analysis of egg number, Poisson errors were assumed with the y-variate being the total number of hosts parasitised in each replicate.

6.4 Results

The ratio of parasitised to unparasitised hosts did not explain a significant amount of the model deviance in any of the treatments. The reductions in deviance when fitting this parameter are given in table 6.1. The total number of hosts available, the number of parasitised or unparasitised hosts, and the number of eggs laid were not significant explanations of deviance either singly, together, or in any combination.

The data are presented graphically in figures 6.1-6.4 which show the difference between the observed sex ratio and the proportion of parasitised hosts available in each replicate, over the range of host ratios offered. Points should fall around the y-axis zero line if parasitoids were laying in direct proportion to host availability. The diagonal line describes the distribution of points expected from a parasitoid laying an unbiased sex ratio.

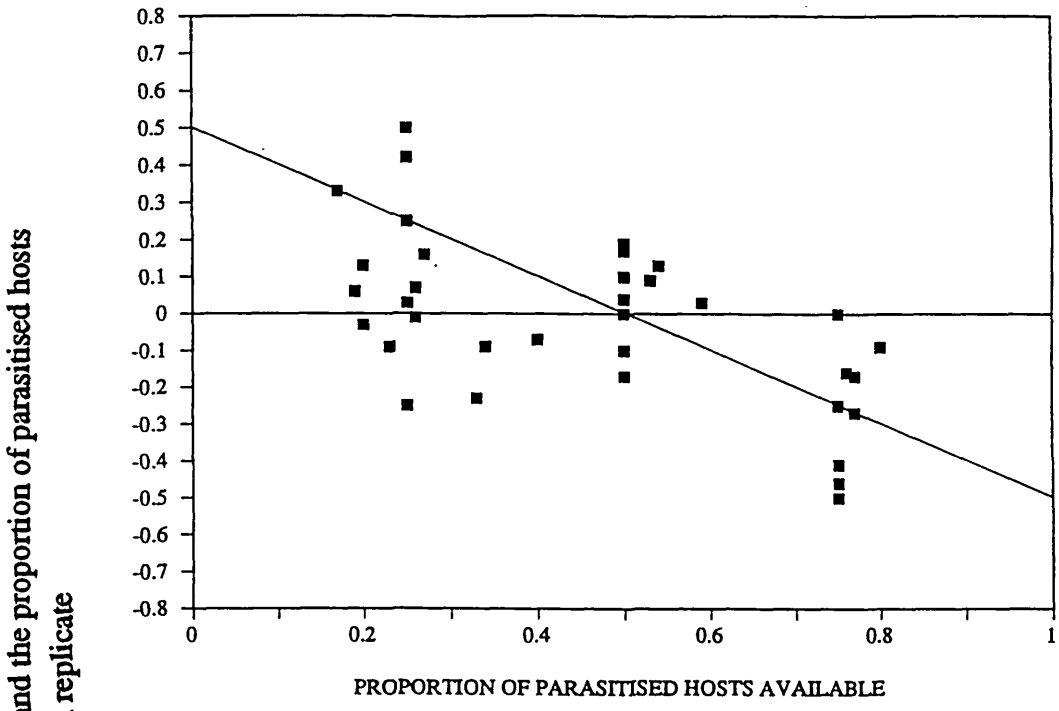
Table 6.1: GLIM Analysis of the effect of Host Availability on Sex Ratio.

TREATMENT	Total deviance	Deviance with $\frac{parasitised\ hosts}{total\ hosts\ available}$ fitted	Change in deviance
1) No Experience	34.78	31.14	3.64
2) Conspecific Pupae	8.27	7.91	0.36
3) Unparasitised Whitefly	48.64	48.03	0.61
4) *E. inaron* Pupae	13.14	13.13	0.01
Total	198.22	198.20	0.02

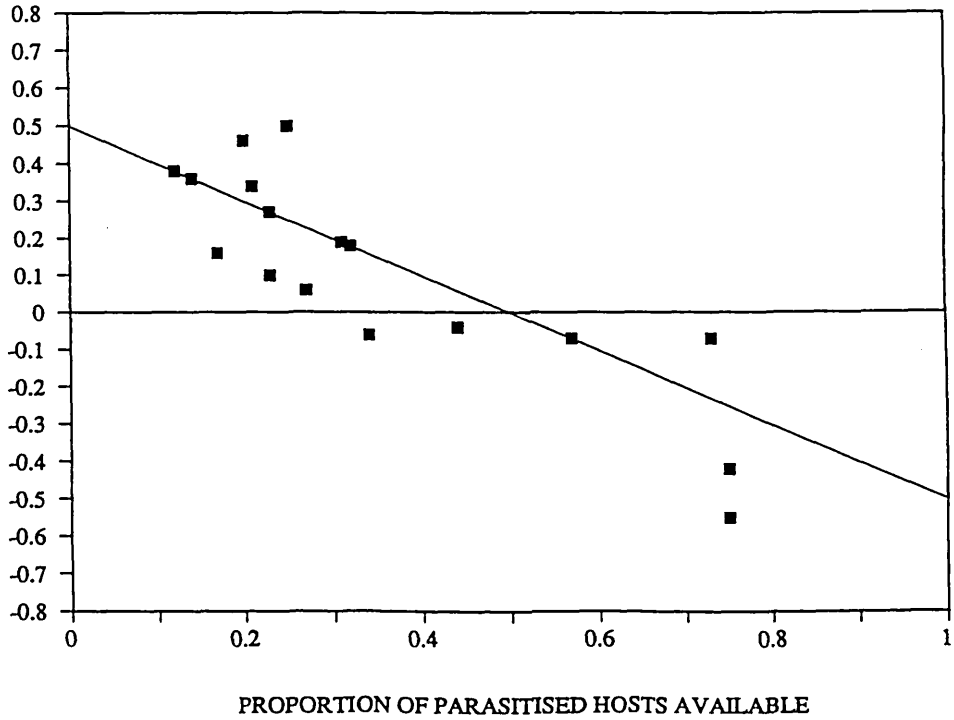
No changes in deviance significant, $\chi^2_1 P > 0.05$.

Figures **6.1-6.4:** Sex Ratios laid in each treatment. Data are plotted as the difference between the observed sex ratio in each replicate and that predicted by host availability. If hosts of both types were parasitised in direct proportion to their availability, all points should lie on the *x*-axis. If a sex ratio of 0.5 were laid independent of host availability, all points should lie on the diagonal line. (1 point=1 replicate)

6.1) NO EXPERIENCE

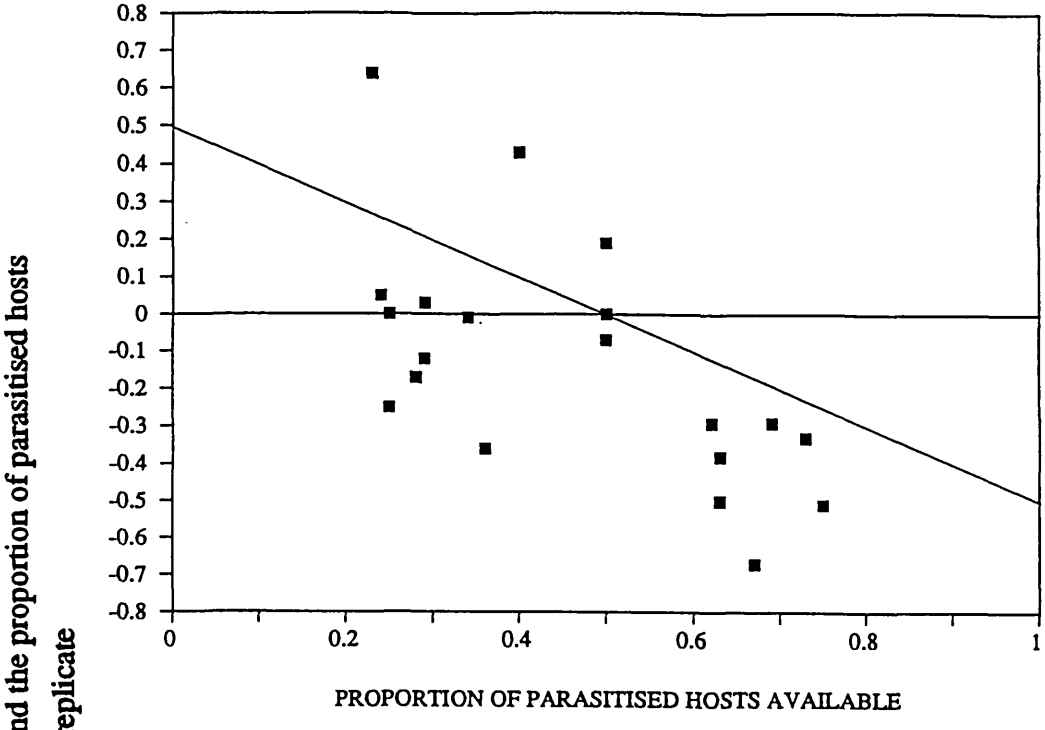


6.2) CONSPECIFIC PUPAE EXPERIENCED



Difference between the observed sex ratio and the proportion of parasitised hosts available in each replicate

6.3) UNPARASITISED HOSTS EXPERIENCED



Difference between the observed sex ratio and the proportion of parasitised hosts available in each replicate

6.4) E. INARON EXPERIENCED

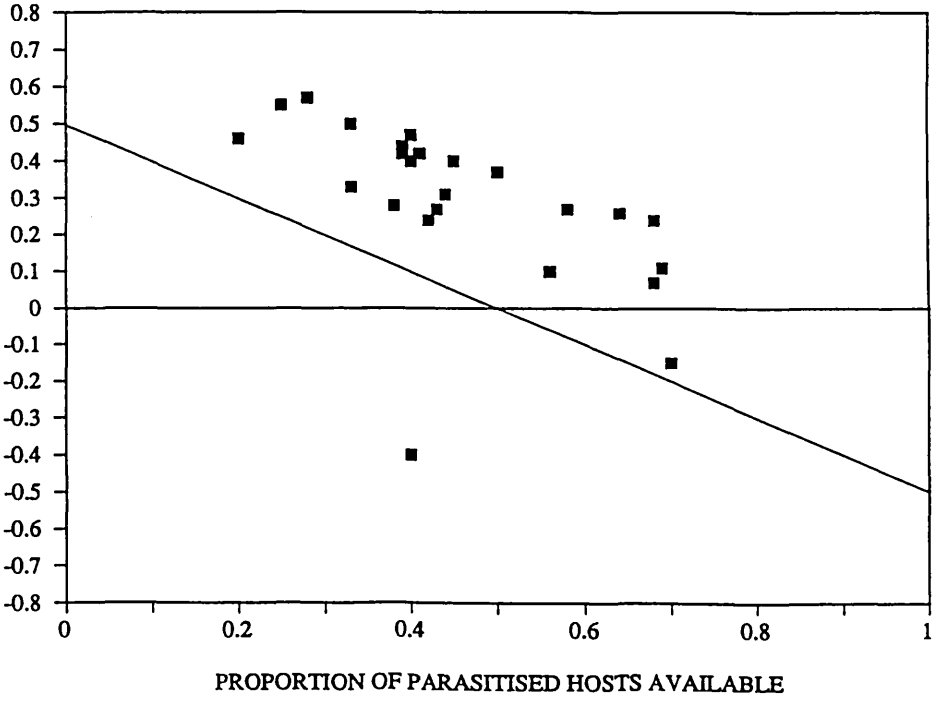


Figure 6.5: Mean sex ratio (with 95% confidence limits) laid following four types of prior ovipositional experience (summary of data from figures 6.1-6.4)

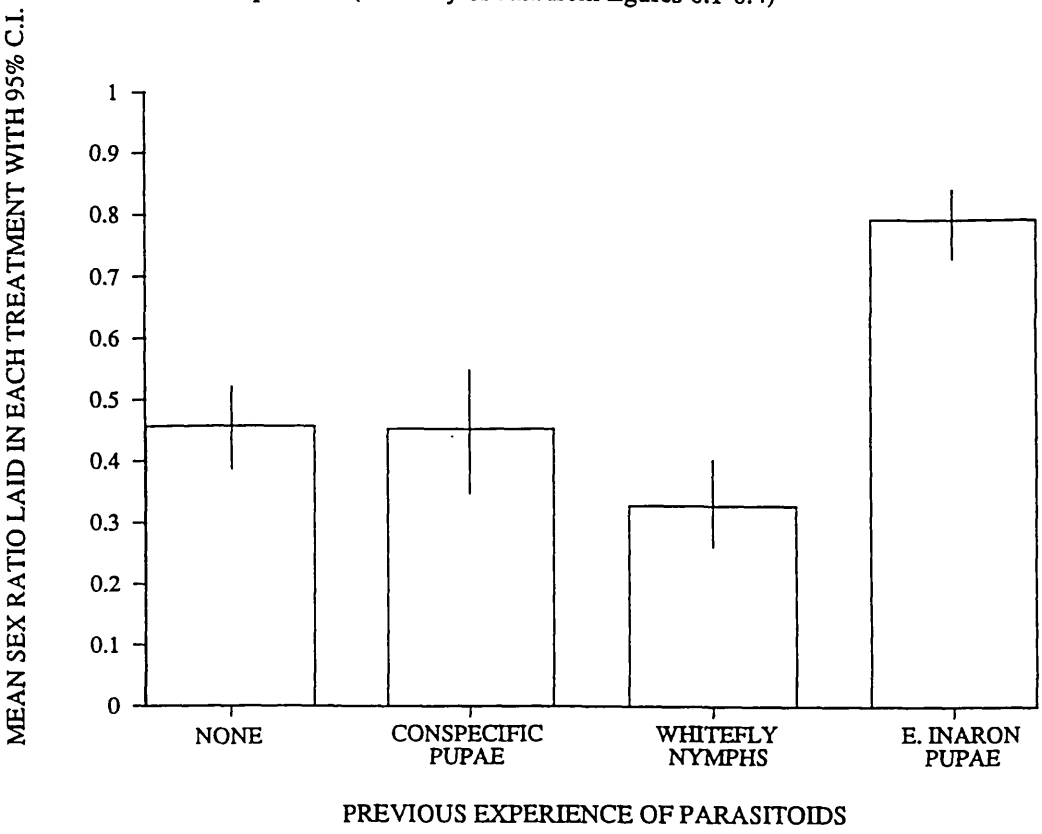
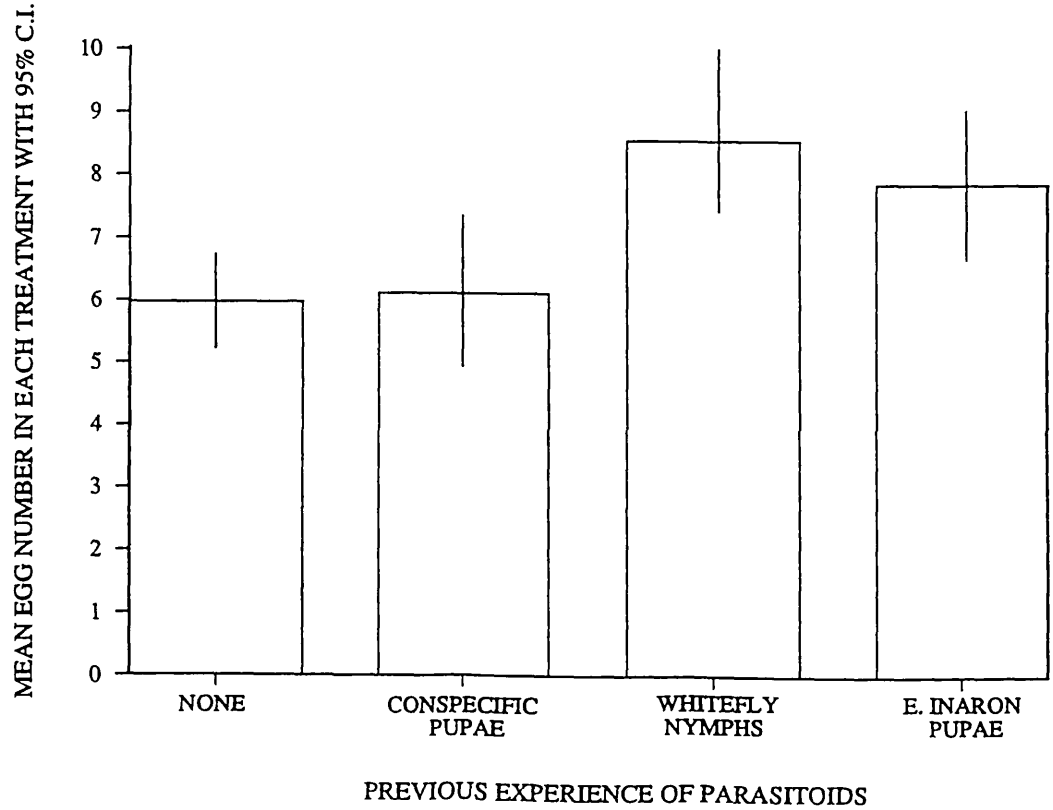


Figure 6.6: Mean number of hosts parasitised (with 95% confidence limits) following four types of prior ovipositional experience



However, experience had a marked effect on explaining the data. When the effect of different treatments was fitted, deviance was halved, as shown in table 6.2.

Table 6.2: GLIM Analysis of the effect of Experience on Sex Ratio

	Total deviance	Deviance when experience fitted	Change in deviance
Total	198.22	104.82	93.40*

*($\chi^2_3 = 93.4 P < 0.001$)

Table 6.3 gives the sex ratio and egg number laid in each treatment. Parasitoids with no experience and those with experience of laying males in conspecific pupae laid a mean sex ratio that was not significantly different from 1:1. Parasitoids experienced by laying females in healthy whitefly produced a sex ratio not significantly different from the above two treatments, but which was significantly female biased ($\chi^2_1 = 21.36 P < 0.001$). *E. inaron*-experienced females re-offered *E. inaron* laid a significantly male biased sex ratio ($\chi^2_1 = 65 P < 0.001$) which was also significantly different from any of the other treatments. The sex ratio data are presented graphically in figure 6.5.

The number of hosts available in each replicate had no effect on the number of eggs laid ($\chi^2_1 = 0.45 N.S.$) in any treatment. Examination of the 95% confidence limits of egg number in each treatment (figure 6.6) shows that wasps with experience of unparasitised whitefly laid significantly more eggs per replicate than inexperienced wasps or those with experience of conspecific pupae. This is most likely due to the host feeding opportunities inherent in treatment 2.

6.5 Discussion

In none of the above treatments were the parasitoids limited by the availability of hosts of either type (parasitised or unparasitised). The inexperienced wasps and those with experience of conspecific pupae laid virtually identical sex ratios insignificantly different from 1:1 as Godfray and Waage (in prep.) predicted given equal reproductive success of the sexes. The question to be answered therefore becomes: why did the other two treatments yield distinctly biased sex ratios?

Table 6.3: Effect of experience on sex ratio and mean number of eggs laid

Parasitoid experience	no. of reps.	Total no. males	Total no. females	Mean no. eggs per rep.	Mean sex ratio
1.None	37	101	120	5.97	0.46
2.Conspecific pupae	17	47	57	6.12	0.45
3.Fresh whitefly	21	59	121	8.57	0.33**
4.*E.inaron* pupae	24	150	39	7.88	0.79**

** Sex ratio significantly different from 0.5 ($P<0.001$ Chi-squared).

First, consider the effect of experience with unparasitised whitefly hosts. This had two effects, an increase in egg production due to host feeding opportunities, and the production of a female biased sex ratio.

Why should a female, having already encountered and laid eggs in a patch of unparasitised hosts, continue to lay a high proportion of female eggs in a patch offering opportunities to lay both sexes to a greater or lesser extent? This appears maladaptive from an immediate stand-point.

It may be, however, that in natural populations early female conditioning is important in governing future female oviposition. A female may use the absence of local parasitism as a cue to the presence of a low density of conspecific females in the population and an even greater scarcity of males. Under such a scenario, female offspring would almost certainly be mating locally with other members of the communal patch. It would be very unlikely that immigrant males would arrive to exploit the mating opportunities within the patch. A female parasitoid would therefore be quite safe in the assumption that her own sons would be major beneficiaries of the biased sex ratio. By favouring the production of females (and sufficient males to mate them), treatment 3 could then be seen as an adaptation by the female optimising her lifetime reproductive success.

Next, consider the male biased sex ratio laid when *E. inaron* pupae were available both prior to the experiment, and within the experimental arena.

No *E. tricolor* pupae were available in this experiment, so unlike the species discrimination experiment (chapter 5) the female was not making a relative decision of which hosts to exploit for the production of males, but an absolute decision of what sex ratio to lay in that patch. To understand the possible adaptive nature of producing a male biased sex ratio in such a situation, consider the following.

The sex ratio laid in treatment 4 (*E. inaron* experienced) was very different from that of treatment 2 (conspecific experienced). Thus the changes in behaviour must be due to the presence of the competing parasitoid species rather than purely the response of a female to a high local percentage parasitism. I can envisage two possible factors which may select for the male bias in this treatment:

1. A female emerging into a patch where she is surrounded by the progeny of a competing species may use this as a cue to probable intense local competition for primary hosts. When that female then encounters a patch already visited by a competitor she may decide to lay males in as many of the competitor's progeny as possible in order that her own females suffer less from future competition for primary hosts. Thus she may over invest in sons in order to indirectly increase her own reproductive success through future female function: her daughters. The main problem with this strategy is that it also benefits unrelated conspecific females that may visit the patch subsequently.

2. The marked preference for hyperparasitism of *E. inaron* by *E. tricolor* indicates that there are strong selective pressures ***not*** to attack conspecific larvae if non-conspecifics are available. In treatment 4, the advantage to an individual parasitoid comes only from certain avoidance of hyperparasitism of her own daughters.

Whether the second factor alone is truely strong enough to select for such a male bias, or whether both the advantages I have described act together is not possible to know at present. Intuitively, the second advantage appears of relatively minor importance; especially in the large patches used in these treatments.

This experiment rules out the possibility that autoparasitoids allocate sex in direct proportion to host availability when they are egg rather than host limited. To test the effects of host limitation it would be necessary to vary the relative proportions of host types ***and*** their absolute abundance, especially in the range of typical aphelinid fecundities (appendix 1) i.e. 0-20 hosts per patch.

The "no experience" treatment lies closest to the work of Hunter (in press) who found that *Encarsia pergendiella* consistently laid proportionately more males when offered conspecific pupae and unparasitised hosts. The methodology differed slightly in that Hunter used artificial experimental arenas. Parasitised and unparasitised hosts were offered on cut-out leaf discs on moist filter paper. It may be that parasitoids respond to damaged leaves adversely (as do *E. tricolor*, pers. obs.) or that unparasitised whitefly (female hosts) suffer from, or react to leaf damage. The whitefly may then appear of a lower quality to the parasitoid which produces more males in response.

None of the treatments were similar to Donaldson (1984) in which fixed ratios of unparasitised and parasitised (conspecific) pupae were offered at intervals through the life of the parasitoid *Coccophagus atratus*. Therefore there is little point in drawing comparisons between his work and this study.

Treatments 1 and 2 lend support to the Godfray and Waage argument that, given an abundance of hosts and equal reproductive success of the sexes, no bias should be observed in the overall sex ratios. The marked deviation from equal sex ratios seen in the other two treatments indicates that the factors determining sex allocation decisions in these wasps are more involved than present theories have explained.

The realism of these ideas are reviewed in the light of data on interspecific competition (chapter 7) and field studies (chapter 8).

CHAPTER 7

INTERSPECIFIC COMPETITION

7.1 Introduction

It is common to find several parasitoid species attacking a particular host. The size of such complexes can be highly variable ranging from just one or two, to some twenty species and interspecific competition between species in large complexes, has repeatedly been cited as an intense force in structuring parasitoid communities (Askew and Shaw 1986).

Heteronomous hyperparasitoids are regularly recorded from complexes involving several species of primary parasitoid (Clausen and Berry 1932; Cowland 1934; Priesner and Hosny 1934; Russell 1934; Poinar 1964; Dysart 1966; Oatman 1970; Flanders 1971; Watve and Clower 1976; Williams 1977; Dowell et. al. 1981; Fidalgo 1983; Viggiani 1984; Thompson et. al. 1987; Williams in press). Consider the simplest situation of a facultative autoparasitoid and a single species of primary parasitoid in competition for the same host. This cannot be viewed as classical 2-species, one resource competition, for as the autoparasitoid population grows, a competing primary parasitoid suffers from loss of resource ***and*** from direct hyperparasitism. Presumably it was this reasoning that led Williams (1977) to suggest that facultative autoparasitism constituted "...an inherent advantage to a species when competing with primary parasitoids that reproduce in the orthodox manner...".

Competition between species in a complex containing one or more heteronomous hyperparasitoids should be particularly intriguing. For a facultative autoparasitoid, differential use of competing primary parasitoids over conspecifics for hyperparasitic male production will have implications at the individual and population level. Hyperparasitism of competitors rather than conspecifics within a patch will ensure greater mating opportunities for the male progeny of an individual female. It may also permit an autoparasitoid to co-exist with a competitively superior primary parasitoid at elevated population densities, in habitats where normal reproduction would result in competitive exclusion.

Obviously, such competitive interactions will also have implications for the use of heteronomous hyperparasitoids in biological control programs. It will become clear that, if introductions of parasitoid species are being made to control a particular pest, the

question of whether or not to introduce a heteronomous hyperparasitoid could be critically important to the success of the program. The role of these wasps in homopteran control is reviewed in the discussion.

To evaluate the success of a heteronomous hyperparasitoid in competition with a normally reproducing parasitoid, competition experiments were run using *E. tricolor* and the primary parasitoid with which it occurs in the UK, *E. inaron*. Specifically, two questions were addressed:

1. what is the initial trajectory of the two parasitoid populations when in competition?

2. does the marked preference for hyperparasitism of *E. inaron* play an important role in interspecific competition?

7.2 Methodology

Four Brussels sprout plants lightly infested with all stages of whitefly were placed in a muslin-walled cage as described in chapter 3. Within 48hrs of emergence, 50 mated female and 5 male *E. inaron* were collected and added to the cage. After 2 weeks, 3 leaves were removed at random. The following were recorded for each leaf sampled:

a. Number of apparently healthy scales and the number of whitefly pupal cases
b. Number of parasitoid pupae and the number of parasitoid emergence cases
c. The number of parasitoids that pupated in the 10 days following the sample.

Parasitoids were returned to the culture cage within 24hrs of emergence from the sampled leaves.

Identical samples were taken at weekly intervals thereafter. At 3 weeks, however, a standard 3-leaf sample was taken and 35 mated female *E. tricolor* (all less than 48h old) were added to the culture cage. Parasitoid pupae from weekly samples following the addition of *E. tricolor* were placed individually in gelatin capsules and allowed to emerge. In this way the change in parasitoid populations in each cage was determined. Each cage was sampled for a minimum of 8 weeks. A final sample was taken 3 weeks after the weekly sampling ceased (11 or 12 weeks after the start of the experiment). Fresh lightly infested plants were added to the cage as necessary to compensate for death and defoliation of experimental plants. The experiment was repeated three times.

The reciprocal experiment was also carried out. Initially, 50 mated female *E. tricolor* were allowed to reproduce within the cage and after 3 weeks, 35 mated *E. inaron* were added. This was replicated only once.

All experiments ran continuously at $25\pm1°C$, ambient humidity.

7.3 Results

In all cases the facultative autoparasitoid was able to displace *E. inaron* even when populations of the latter species were very large. Results are shown graphically in figures 7.1-7.4, and are plotted as the mean(±SE) number of parasitoids emerging from each 3-leaf sample (figures 7.1-7.4b,c) and the mean percentage parasitism of each sample over the period of the experiment (figures 7.1-7.4a). The three cages in which *E. tricolor* was added to *E. inaron* populations showed differences in the population dynamics of both parasitoid species. For this reason I will consider each cage separately.

a. In cage 1: (Figure 7.1) The *E. tricolor* population grew steadily with a continually female biased secondary sex ratio. This was concurrent with a steady decline in the *E. inaron* population. By week 8 just eight *E. inaron* individuals emerged from the 3-leaf sample, and by week 11 there were no *E. inaron* at all.

b. In cage 2: (Figure 7.2) The *E. inaron* population quickly attained a far larger size than in cage 1. When *E. tricolor* was added however the *E. inaron* population began to decline, reaching very low levels by week 9. The sex ratio of *E. tricolor* was also markedly different from cage 1, in that for 4 weeks after *E. tricolor* was added to the cage, male production far exceeded female production. Fluctuations in male numbers also tended to follow changes in the percentage parasitism of the sample. Female *E. tricolor* did not appear in any numbers until week 8. By week 12 however, the sex ratio was female biased and only five *E. inaron* individuals emerged from the sample.

c. In cage 3: (Figure 7.3) Production of the sexes by *E. tricolor* in cage 3 followed a similar pattern to cage 2, in that male production surpassed female production, which only featured at the end of the experiment. The *E. inaron* population reached its greatest levels in this cage and remained high until week 8 when it decreased by an order of magnitude. Three weeks after the weekly sampling ceased, a final sample showed no *E. inaron*.

Figure 7.1: Mean percentage parasitism (a) and changes in the number of *E. inaron* (b) and *E. tricolor* (c) in each sample of the competition experiment - cage 1

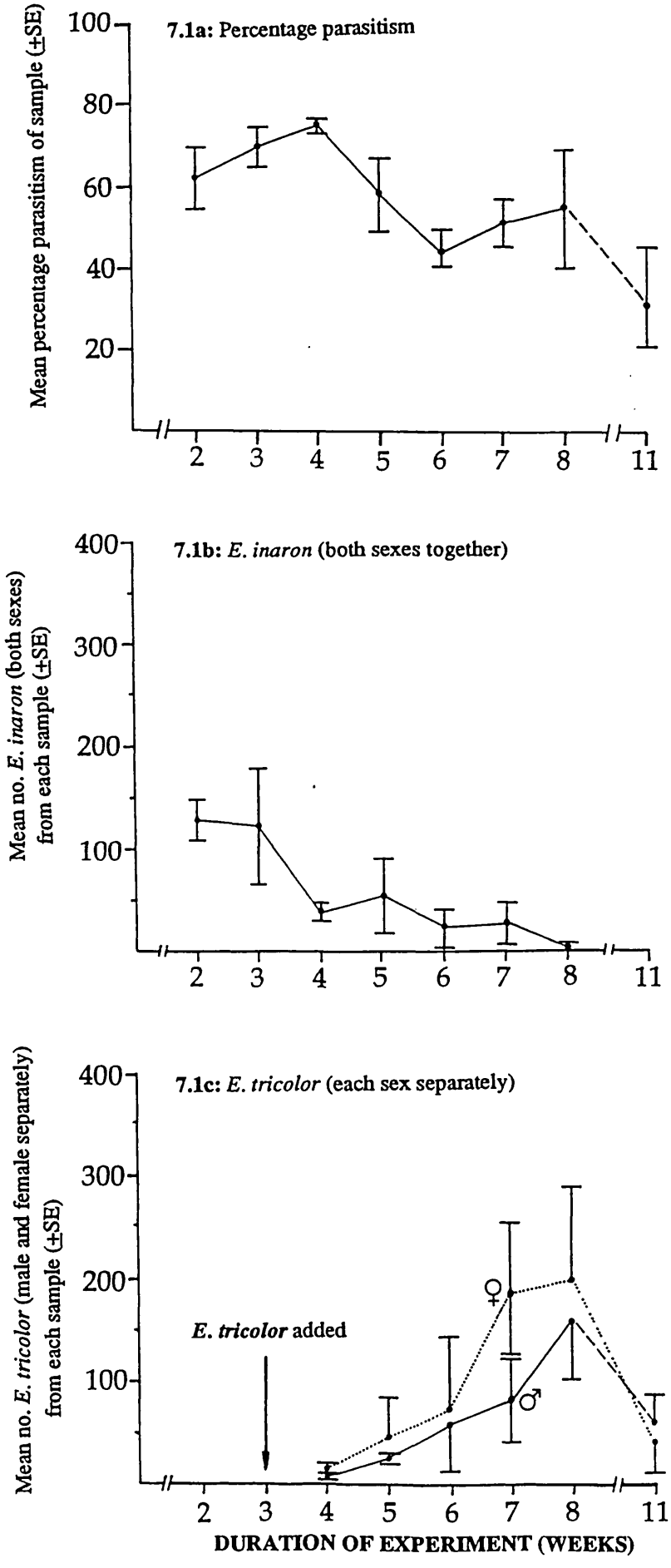


Figure 7.2: Mean percentage parasitism (a) and changes in the number of *E. inaron* (b) and *E. tricolor* (c) in each sample of the competition experiment - cage 2

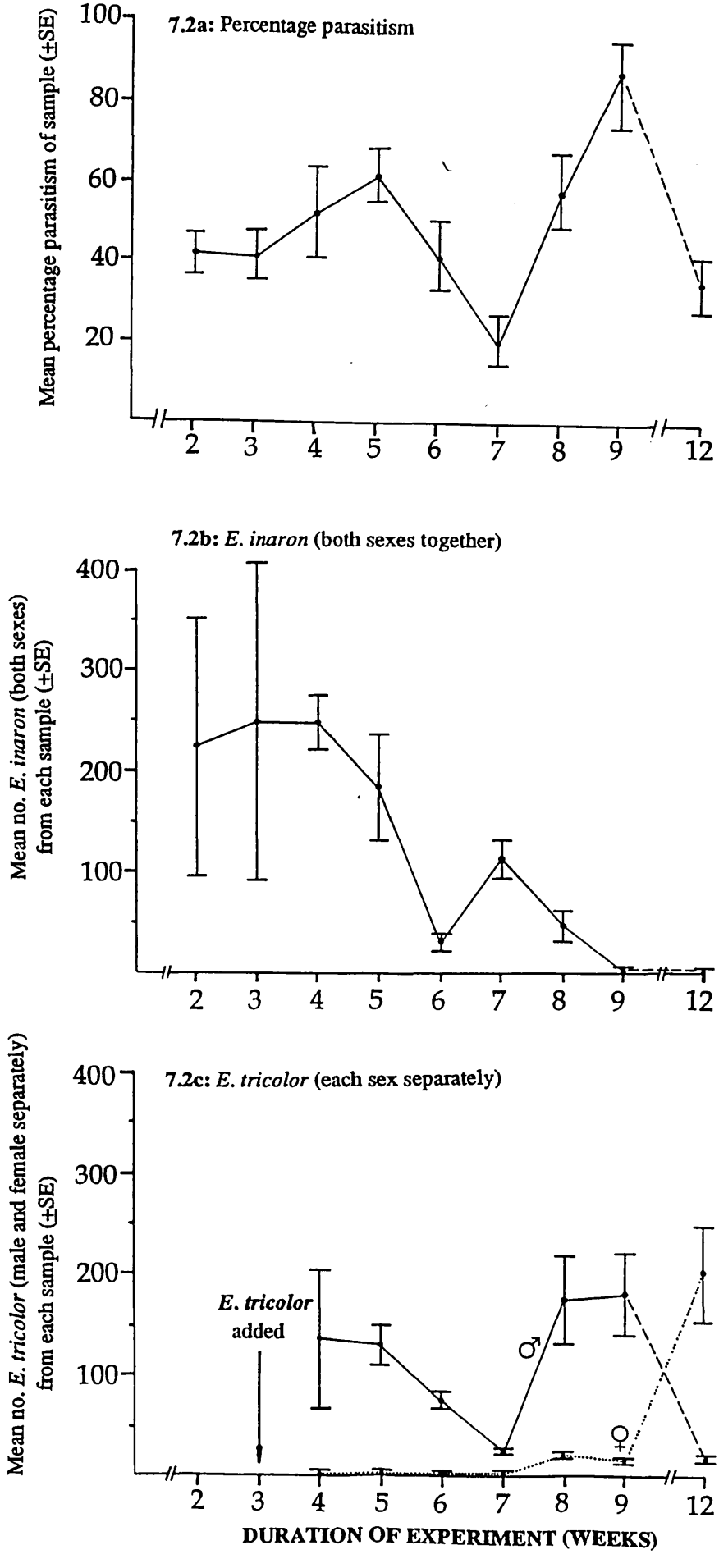


Figure 7.3: Mean percentage parasitism (a) and changes in the number of *E. inaron* (b) and *E. tricolor* (c) in each sample of the competition experiment - cage 3

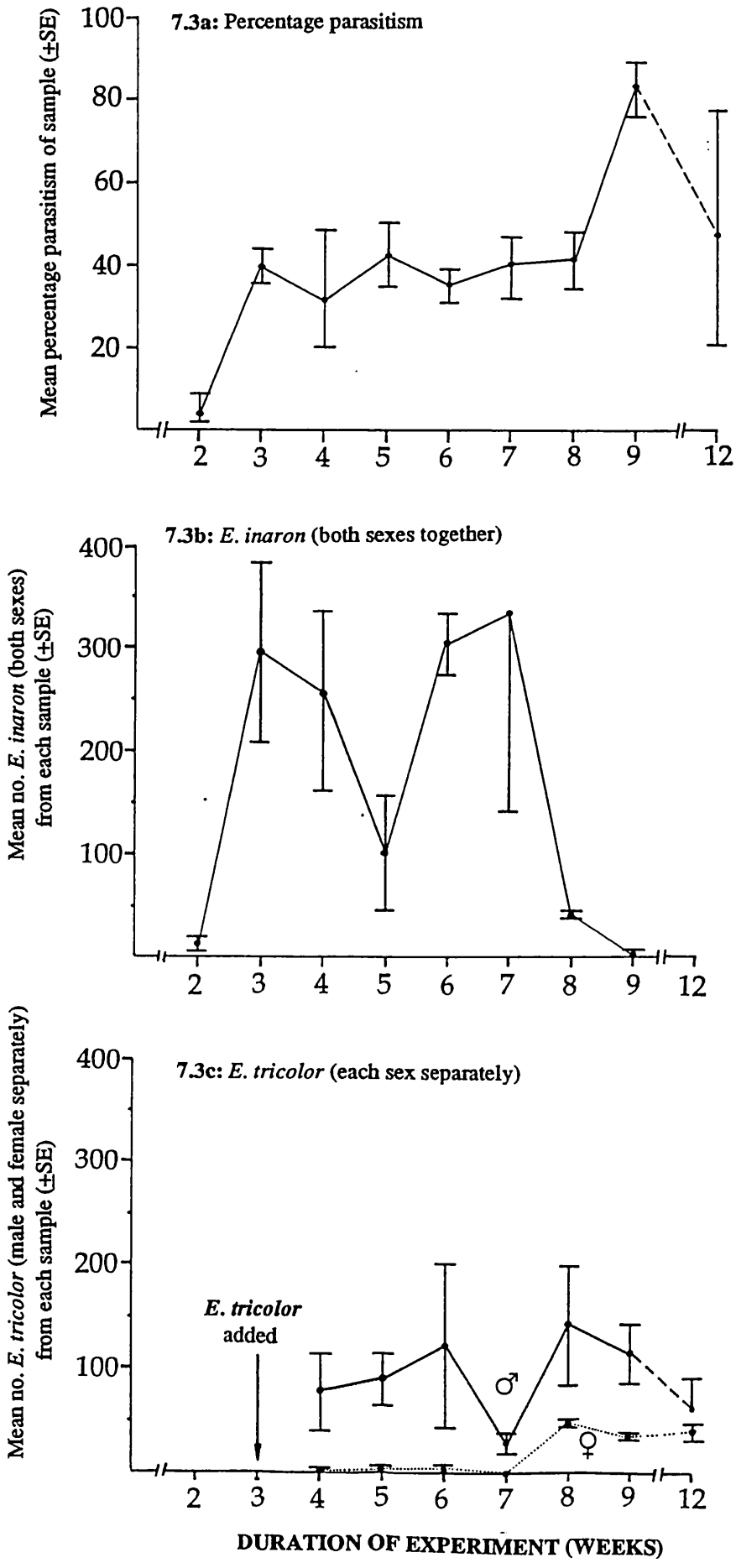
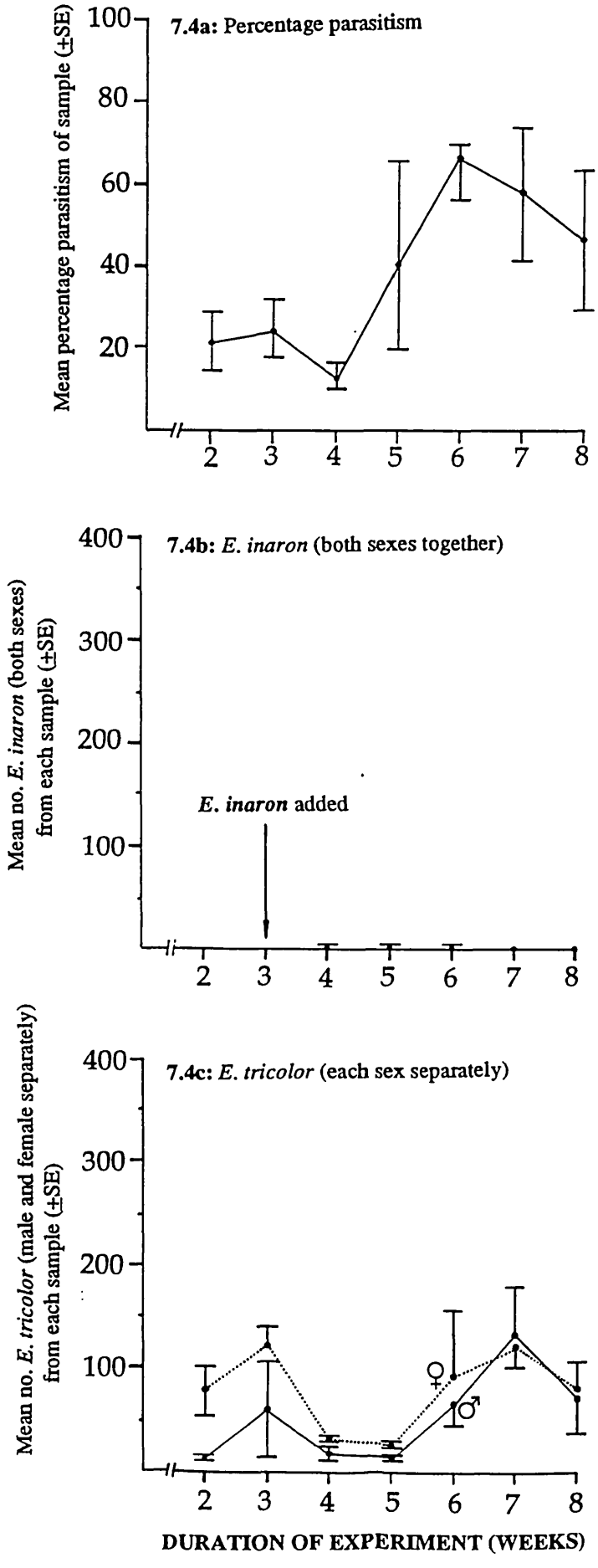


Figure 7.4: Mean percentage parasitism (a) and changes in the number of *E. inaron* (b) and *E. tricolor* (c) in each sample of the competition experiment - cage 4



d. In cage 4: The results of the reciprocal experiment in which *E. inaron* was allowed to challenge an established population of *E. tricolor* are shown graphically in figure 7.4. The population if *E. tricolor* in cage 4 did not reach the high levels of *E. inaron* in cages 2 and 3. *E. tricolor* also maintained a female biased sex ratio for almost all of the experimental period. The number of *E. inaron* never rose above 2 in any sample, and by 7 weeks had returned to zero.

The pattern of population growth and sex allocation was rather different in each of the replicate cages. It did not appear to be a consistent function of the availability of primary or secondary hosts. A slightly different sampling or management regime may have helped elucidate the important factors affecting the dynamics of each cage. The overall result was nevertheless, highly consistent: the *E. inaron* population fell from extreme domination to virtual complete elimination in the space of a few generations. Once the autoparasitoid had established itself, no further resurgence of *E. inaron* was seen. In cages 2 and 3, the strong male bias in the *E. tricolor* populations was almost certainly due to hyperparasitism of *E. inaron* hosts which were many times more abundant than female *E. tricolor*. In this respect, cage 1 gave an unexpected result. The only possible causitive factor which may account for the altered trajectories in this cage is the early population levels of *E. inaron*. At week 3, when *E. tricolor* was innoculated into each of the cages, the density of scales parasitised by *E. inaron* was approximately twice as great in cages 2 and 3 compared to cage 1. This difference was even visually obvious when the experiments were being tended on a daily basis. Therefore, the high densities of non-conspecific secondary hosts in cages 2 and 3, appear to have induced conspicuous male production by the autoparasitoid.

In cage 4, *E. inaron* was unable to invade the population of *E. tricolor* despite the fact that primary hosts were continually available in substantial numbers (as shown by the low percentage parasitism). This indicates either that interference by *E. tricolor* prevented *E. inaron* parasitising hosts or that hosts parasitised by *E. inaron* were almost certain to be hyperparasitised by *E. tricolor*. Given the results of the other three replicates, the latter point is more probable.

7.4 Discussion

One of the most valuable roles which heteronomous hyperparasitoids play is that of control of homopteran pests. The competitive interactions investigated in this chapter are not only interesting clues to the competitive advantage of a facultative autoparasitoid in complexes containing conventional parasitoids, but also have a number of implications for the use of these wasps in biological control programs. Not suprisingly, the literature

on biocontrol frequently describes competitive displacement of one species of parasitoid by another species introduced at a later date. The reasons for these displacements are usually attributable to:

1. better survival of the larvae of one species in multiply parasitised hosts (intrinsic superiority)
2. greater searching ability of the second parasitoid (extrinsic superiority)
3. differences in fecundity
4. the ability to exploit younger host stages or attack the host earlier in its life cycle (Doutt and DeBach 1964; Huffaker, Simmonds and Laing 1976).

These effects tend only to affect population dynamics of the respective species when the pest populations are reduced and competition for hosts increases. Some particularly well studied cases are those of *Opius*/Oriental Fruit Fly in Hawaii and parasitoids of the Red Scale in California.

7.4.1 Some classic examples of competition in biological control

Initially, *Aphytis chrysomphali* was accidentally introduced to Californian citrus groves, along with the California Red Scale (*Aonidiella aurantii*) prior to 1900. Later, *Aphytis lingnanensis* was introduced from China as part of a classical biological control program. This species replaced *A. chrysomphali*, but only gave economic control in selective areas. Subsequently, *Aphytis melinus* was released and spread rapidly, displacing *A. lingnanensis* in all but the southern coastal habitats. Recent studies have suggested that one of the reasons *A. melinus* outcompeted the other species lies in its ability to exploit small hosts for progeny production. It appears that two *A. melinus* individuals can develop in a smaller host than can *A. lingnanensis*. The sex ratio of progeny from these small hosts is also different for the two species; *A. lingnanensis* produces a male biased sex ratio, whereas *A. melinus* produces an unbiased or slightly female biased sex ratio(Luck, Podoler and Kfir 1982; Luck and Podoler 1985; Luck 1986; Reeve 1987).

Force (1974) reviewed competition among parasitoids in terms of *r* and *K* strategies (MacArthur and Wilson 1967). He described an inverse relationship between the reproductive capacity and competitive ability of three species of Red Scale parasitoids. The encyrtid *Comperiella bifasciata* had a very low rate of reproduction, but outcompeted both of the other species in laboratory experiments. Seemingly, this allowed the species to maintain viable populations in the field. The thelytokous endoparasitoid *Encarsia perniciosi* was an *r*-strategist with a reproductive capacity some 140 times

greater than *Comperiella*, but was displaced by both the other species in the laboratory systems. The ectoparasitic *Aphytis* was intermediate in both respects, but was the most successful species in the field. Force appreciated Flanders' (1971) observation that potentially, *E. perniciosi* could be a far more effective control agent in a situation where it was not suffering competitive pressures from *Aphytis*. Unfortunately, *E. perniciosi* had almost always been introduced into areas already containing such ectoparasitoids.

Similarly to the Red Scale program, three parasitoids were used in succession against the Oriental Fruit Fly, *Dacus dorsalis*. All the parasitoids belonged to the genus *Opius* and originated, as did the pest, from the Philippines and Malaysia. *Opius longicaudatus* being easily propagated on late instar fly larvae, was released in large numbers and rapidly became established. *O. vandenboschi* was released at the same time but in much smaller numbers. This species remained relatively scarce for about a year before displacing *O. longicaudatus* from its dominant position. This success of *O. vandenboschi* was attributed to its ability to attack first instar larvae (easily accessible) and intrinsic superiority in multiply parasitised hosts. Last, *O. oophilus* was released and became dominant, proving to be the most intrinsically superior species and able to attack the youngest and most accessible host stage: *D. dorsalis* eggs (van den Bosch and Haramoto 1953; Clausen, Clancy and Chock 1965).

7.4.2 Competition involving heteronomous hyperparasitoids

Heteronomous hyperparasitoids have played an extremly important and frequently successful role in biological control of Homopteran pests. It is in the introductions of alien species as part of a biocontrol program that the dynamics of systems containing heteronomous hyperparasitoids have been most (if not best) studied.

In California, the Olive Scale *Parlatoria oleae* was only partially controlled by *Aphytis paramaculicornis* because of the hot, arid summer climate, which destroyed *Aphytis* populations. In a series of papers, Doutt (1966), Broodryk and Doutt (1966), Kennett, Huffaker and Finney (1966), Huffaker and Kennett (1966) and Finney (1966) made a detailed study of biocontrol of the olive scale and the role of a recently successful control agent *Coccophagoides utilis*, originating from Pakistan. *Aphytis paramaculicornis* being ectoparasitic, was not a suitable host for male *C. utilis* which could only produce males from conspecific females in California.

The total level of parasitism by these two species appeared to be in a state of flux during the 1963/64 period when the group carried out their sampling program. Parasitism by *A. maculicornis* fell from 75% to 49% with a concurrent increase in parasitism by

C. utilis from 9% to 39%, despite the fact that *A. maculicornis* had a higher fecundity, a greater larval competitive ability in superparasitised hosts, and several more generations per year. Kennett et. al. (1966) attribute the success of *C. utilis* to its ability to exploit younger hosts than *Aphytis* and its greater synchronisation with the host life cycle. Thus, exploitation of secondary hosts by *C. utilis* was not a feature of the competitive interaction between the two species.

In a further paper more than 20 years after the original work, Huffaker, Kennett and Tassan (1986) described the status of the two parasitoids from samples taken in 1978 and 1982. Remarkably, by 1982 biocontrol of the olive scale was two orders of magnitude better than in the 60's. The degree of parasitism by *Aphytis* had fallen from 75% in 1963 to 30% in 1982. Huffaker et. al. did not detect any significant differences in the level of parasitism by *C. utilis* over time or between geographical locations (3 counties in California) for the 20 years of the study. This suggests the host-parasitoid population dynamics of this species to be remarkably stable; as predicted by Hassell, Waage and May (1983) in their host-autoparasitoid model described in chapter 2.

Thompson, Cornell and Sailer (1987) recently published the results of a series of release programs for biological control of the Citrus Blackfly, *Aleurocanthus woglumi* in the citrus groves of Florida. Parasitoid releases were made in 1976. The platygasterid, *Amitus hesperidium* was released in the greatest numbers and bought about a rapid decline in blackfly populations. The direct facultative autoparasitoid, *Encarsia opulenta* subsequently became dominant and displaced *Amitus* throughout the original release area. An ***indirect*** facultative autoparasitoid, *Encarsia smithi* was also found to be established in Florida and was additionally released at several locations. Males of indirect autoparasitoids are laid in primary hosts in anticipation of attack by a primary parasitoid permitting the development of the hyperparasitic male larvae.

When in 1979 blackfly was found in several other parts of the state, all three species were available for release (Nguyen et. al. 1983). When *A. hesperidium* was released alone, or together with *E. opulenta*, control of blackfly outbreaks was achieved in about 6 months. At one particular site however, *E. smithi* was also released and control was delayed by about 12 months. The reason for this appears to be the host relations of *E. smithi* which is normally an indirect autoparasitoid. Thompson et. al. state however, that ***both*** sexes can develop hyperparasitically at the expense of *E. opulenta*. This facultative hyperparasitic development of female *E. smithi* would be remarkable if verified. It could also account for the apparently adverse interaction between *E. smithi* and *E. opulenta* in the biocontrol program which is summarised below.

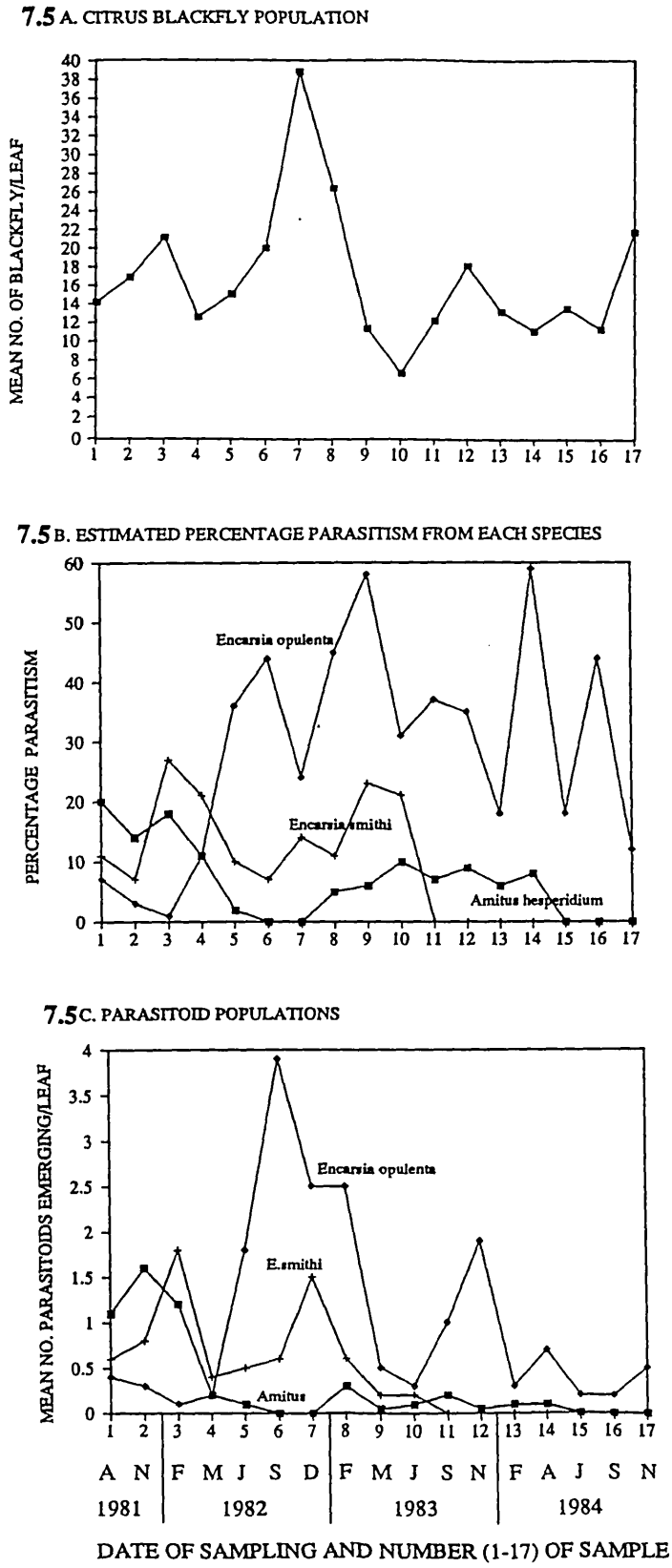
Following releases made in 1979, sampling began in August 1981. *A. hesperidium* populations peaked initially but declined rapidly thereafter. *E. smithi* then became dominant briefly but subsequently declined except for a small rise again in December 1982 following an upsurge in its secondary host, *E. opulenta*. Populations of *E. opulenta* were initially very low. In mid 1982 however, *E. opulenta* increased dramatically and it remained the dominant species completely eliminating both of its competitors by the middle of 1984. Figures 7.5a,b,c were drawn from data given by Thompson et. al. and show graphically the fluctuations in host and parasitoid populations and the percentage parasitism attributable to each species.

It is regretable that Thompson et. al. do not differentiate the sexes of *Encarsia* they monitored nor the presence or degree of hyperparasitism. Nevertheless, the interaction between these three species give the best published support of the competition experiments between an orthodox parasitoid and an autoparasitoid which I have described above.

Furthermore, in another paper on biocontrol of citrus blackfly, Summy et. al. (1983) describe *E. opulenta* as dominant in the Lower Rio Grande Valley of Texas, having competitively displaced an *Encarsia* species, *E. clypealis* and reduced *A. hesperidium* populations to the point of several local extinctions. It was fortunate that the elimination of competitors by *E. opulenta* did not appear to diminish the degree of pest control.

This brings us to an important point: the high interspecific competitive ability of heteronomous hyperparasitoids has consequences not yet fully defined. By adding a heteronomous hyperparasitoid to a system containing one or a number of reasonably successful primary parasitoids, the populations of the original parasitoids may decline severely as the heteronomous species asserts its hyperparasitic nature. A consequence of this may be that the pest population, relieved of its burden of parasitoids to a greater or lesser extent, may be capable of reaching economically damaging levels. This depends on whether the heteronomous hyperparasitoid can make up for the primary parasitism which would have occured from the parasitoids which it itself exploited when producing males. The example of *E. opulenta* above, suggests that this may not be as unlikely as it first appears. The distinction that needs to be made is that a high interspecific competitive ability is not a prerequisite for good biological control, and in cases such as species showing a hyperparasitic habit, may be positively detrimental.

Figure 7.5(abc) Competitive exclusion of *Amitus hesperidium* and *Encarsia smithi* by *Encarsia opulenta* during a Citrus Blackfly program in Florida (Data from Thompson et. al. 1987). Figures represent the primary host population (a), estimated percentage parasitism by each parasitoid species (b) and the number of each species of parasitoid emerging from leaf samples over the duration of the study (c).



7.4.3 Parasitoid complexes containing a heteronomous hyperparasitoid

In natural communities of parasitoids attacking a host(s), a heteronomous hyperparasitoid appears to have a competitive advantage over her competitors which she can exploit hyperparasitically. To assess the importance of this ability in field populations, a literature search was carried out for papers which described a species of facultative autoparasitoid in a complex containing one or more conventional primary parasitoids, and which gave information on which species was most abundant/important.

Two sources were used for the literature search:

1. the Review of Applied Entomology (series A) from 1930 to present day,
2. the BIOCAT database of species introduced in biocontrol programs. This database is still being developed and updated by CAB International, Institute of Biological Control.

For both the above sources, searches were made for references to the genera showing heteronomous host relations. When the abstract suggested that the original paper offered information on relative abundance of species within a host-parasitoid complex, the reference was consulted fully. Descriptions of complexes of parasitoids which did not simultaneously contain a heteronomous hyperparasitoid and one or more primary parasitoids (or gave information on relative abundance of each parasitoid species) were not included in the review.

The information is presented in two tables. Table 7.1a,b gives a list of references to natural populations in which a facultative autoparasitoid was dominant (table 7.1a), or in which a normal primary parasitoid was dominant (table 7.1b). Table 7.2a,b gives the same arrangement for species introduced in biological control programs. In 40 cases where a dominance hierarchy was described from natural communities, 27 were headed by a facultative autoparasitoid. When introduced species from biocontrol programs were examined, 8 out of 10 such programs were dominated by an autoparasitoid.

More care is necessary if introduced species are considered as the selective nature of the introductions means that less effective species in their native habitats tend to be disregarded for control programs abroad whereas the more apparent species are favoured for foreign introductions. In other words, both the pest and the parasitoid communities in these programs are an artifact of man's doing and should not be seen as in too great a way as a "natural" interaction ecologically. Nevertheless, the domination of these

programs by autoparasitoids is again striking and has obvious implications for the release of heteronomous hyperparasitoids into biocontrol systems already containing one or more standard parasitoids. This is discussed further in chapter 9.

It is evident from the results of this experiment that a facultative autoparasitoid able to exploit a normally reproducing primary parasitoid for the production of its own males has a singular advantage over the latter species. Once established in sufficient numbers, an autoparasitoid population may also be highly resistant to invasion by orthodox parasitoids. These results give a vivid illustration of the true benefits of heteronomous hyperparasitism; a method of sex allocation allowing continual reproduction, even in highly competitive environments.

List of parasitoid complexes containing a heteronomous hyperparasitoid

Dominant Facultative Autoparasitoid	Primary Host Species	Percentage Domination by Autoparasitoid	Other Species In Complex	Reference

Table 7.1a: ***Natural Communities - Facultative Autoparasitoid Dominant***

Coccophagoides similis	*Diaspidiotus viticola* and *Targiona vitis*	"majority"	*Azotus celsus*	Zinna (1962)
Coccophagus atratus	*Filippia gemina*	"always the most dominant"	*Metaphycus* sp.	Donaldson, Clark and Walter (1986)
Coccophagus basalis and *Coccophagus fallax*	*Saissetia oleae*	dominant in approx. equal numbers	*Scutellista cyanea* *Lecaniobus utilis* *Coccophagus cardei* *Euaphagus* sp. 3 *Aneristus* spp.	Flanders, Bartlett and Fisher (1961)
Coccophagus gurneyi	*Pseudococcus gahani*	Most	*Tetracnnemus pretiosus* *T. peregrinus* *Pseudaphycus angelicus* *Chrysoplatycerus splendus* *Lepomastidea abnormis*	Bartlett and Lloyd (1958)
Coccophagus insidiator	*Gossyparia spuria*	97%	*Trichomathus cyanifrons*	Flanders (1952)

Coccophagus rusti	*Sassetia oleae*	93%	*Metaphycus hemilecanii* (m)	Flanders (1965)
Coccophagus rusti	*Sassetia oleae*	88%	*Scutellista cyanea* (m)	Flanders (1965)
Encarsia aleurochitonis(a)	*Aleurochiton aceris*	72%	*Encarsia margaritiventris* *Euderomphale secreta*	Hulden (1986)
Encarsia asterobemisae	*Asterobemisia carpini*	commonest	*Encarsia longicornis* *E. coryli*	Viggiani (1981) Iaccarino and Viggiani (1983)
Encarsia clypealis(b)	*Aleurocanthus woglumi*	dominant	*Encarsia opulenta* *Amitus hesperidium* *Eretmocerus serius*	Flanders (1969)
Encarsia clypealis(c)	*Aleurocanthus woglumi*	dominant	*Encarsia merceti* *E. divergens*	Flanders (1969)
Encarsia elongata	*Lepidosaphes gloverii*	84%	*Encarsia citrinus (n)*	Flanders (1971)
Encarsia lahorensis	*Dialeurodes citri*	dominant(d)	"many others"	Ortu and Prota (1983)
Encarsia lahorensis	*D. citri* and *Aleurocanthus spiniferus*	"main species"	"others"	Chen (1985)
Encarsia lutea	*Bemisia tabaci*	66%	*Eretmocerus mundus*	Gameel (1969)
Encarsia margaritiventris	*Aleurotuba jelineki*	dominant(e)	*Amitus aleurotubae* *Cales noaki* *Encarsia aleurotubae* *Encarsia tricolor* *Eretmocerus longicornis*	Laudonia and Viggiani (1984)
Encarsia meritoria(f)	*Bemisia tabaci*(l)	59% in 1962 51% in 1963 1964-1965(g)	*Encarsia formosa* *Encarsia* sp. *Eretmocerus haldemani*	Gerling (1967)

Encarsia moffsi(a)	*Pealius quercus*	61%	*Encarsia borealis* *Eretmocerus zippanguiphagus* *Amitus longicornis*	Hulden (1986)
Encarsia pergandiella	*Aleyrodes spiraeoides*	By far the most abundant	*Encarsia coquilletti* *Encarsia meritoria* *Signiphora aleyrodis* *Eretmocerus haldemani* *Euderomphale flavimedia*	Oatman (1970)
Encarsia pergandiella	*Trialeurodes packardi*	87% (h) at peak popln.	*Encarsia quaintancei* *Eretmocerus corni*	Kuenzel (1975)
Encarsia quaintancei	*Trialeurodes abultilonea*	63%	*Encarsia pergandiella* *Amitus aleurodinus* *Eretmocerus haldemani*	Dysart (1966)
Encarsia tricolor	*Aleyrodes proletella*	dominant	*Encarsia lutea* *Trichaporus partenopeus*	Dansig (1964)
Encarsia sp. "G" (q)	*Aonidiella citrina*	46%	*Encarsia lounsburyi* *Aphytis* spp. *Comperiella bifasciata*	Flanders (1971)
Physcus debachi	*Aonidiella aurantii*	dominant	others in Burma	Fisher (1961)
Physcus flavus	*Lepidiosaphes beckii*	67%	*Encarsia citrinus* *Aphytis lepidosaphes*	Flanders (1971)
Physcus testaceous	*Lepidosaphes ficus*	51%	*Archenomous* sp. *Aphytis mytilaspidis* 2 *Encarsia* spp.	Flanders (1971)
Physcus testaceous (o)	*Lepidosaphes ulmi*	74%	2 *Encarsia* spp. *Aphytis mytilaspidis*	Flanders (1971)
Physcus testaceous (p)	*Lepidosaphes ulmi*	56%	*Archenomus* sp. *Aphytis mytilaspidis* 2 *Encarsia* spp.	Flanders (1971)

Table 7.1b: ***Natural Communities - Primary Parasitoid Dominant***

Dominant Primary Parasitoid	Primamry Host Species	Percentage Domination by Primary Parasitoid	Other Species In Complex (autoparasitoid marked *)	Reference
Amitus hesperidium	*Aleurocanthus spiniferus*	"generally more abundant"	*Encarsia smithi* *	Lin, Wei and Tao (1975)
Anarhopus sydenyensis	*Pseudococcus adonium*	Most	6 other species including *Coccophagus gurneyi* *	Bartlett and Lloyd (1958)
Aphytis fusipennis	*Lepidosaphes gloverii*	53%	*Encarsia elongata* *	Flanders (1971)
Aphytis lepidosaphes	*Lepidosaphes beckii*	67%	*Encarsia citrinus* *Physcus fulvus* * *Adelencyrtus* sp.	Flanders (1971)
Aphytis spp.	*Aonidiella messengeri*	31%	*Encarsia lounsburyi* *Encarsia* sp. "G" * *Pteroptrix albocinctus*	Flanders (1971)
Blastothrix longipennis	*Lecanium tiliae*	80%	*Coccophagus lycimnia* * *Metaphycus kincaidi*	Rubin and Beirne (1975)
Cardiogaster hyalina	*Aleurocybotus* sp.	approx. 90%	*Encarsia luteola* *	Poinar (1964)
Chrysoplatycerus splendus	*Pseudococcus maritimus*	Most	5 other species including *Coccophagus gurneyi* *	Bartlett and Lloyd (1958)
Comperiella bifasciata	*Aonidiella citrina*	46%	*Aphytis* spp. *Encarsia* sp. "G" * *Pteroptrix wanhsiensis* *Pteroptrix albocinctus*	Flanders (1971)
Encarsia inaron	*Aleyrodes proletella*	59%	*Encarsia tricolor* * *Euderomphale chelidonii*	Pers. obs.
Eretmocerus haldemani	*Trialeurodes abutilonea*	70%	*Encarsia* sp. *E. quaintancei* * *E. pergandiella* *	Watve and Clower (1976)
Euderomphale chelidonii	*Aleyrodes lonicerae*	76%	*Encarsia tricolor* *	Pers. obs.

Euderomphale secreta	*Aleurochiton aceris*	45%	*Encarsia margaritiventris* * *Encarsia aleurochitonis* * (a) *Amitus minervae*	Hulden (1986)

Table 7.2a: ***Introduced/Biocontrol Parasitoid Complexes - Facultative Autoparasitoid Dominant***

Encarsia clypealis	*Aleurocanthus woglumi*	"largely replaced other spp."	*Encarsia smithi* *Amitus hesperidium*	Flanders (1969)
Encarsia lingnanensis	*Unaspis citri*	"by far the most abundant"	*Encarsia* nr.*funicularis* *E. ?citrinus* *Encarsia* sp.	DeBach and Rosen (1976)
Encarsia lutea	*Bemisia tabaci*	variable(i)	*Eretmocerus mundus*	Gerling, Motro and Horowitz (1980)
Encarsia opulenta(j)	*Aleurocanthus woglumi*	v. dominant	*Eretmocerus serius*	van Whervin (1968)
Encarsia opulenta	*Aleurocanthus woglumi*	dominant	*Amitus hesperidium* *Encarsia clypealis* *Eretmocerus serius*	Summy et. al. (1983)
Encarsia smithi	*Aleurocanthus spiniferus*	65%	*Amitus hesperidium*	Peterson (1955)
Encarsia sublutea(k)	*Bemisia tabaci*	dominant at 7 out of 9 sites counted	*Eretmocerus mundus*	Gerling (1985)
Physcus seminotus	*Anlacaspis tegalensis*	80%	*Adelencrytus miyarai* *Tetrasticlus* sp.	Williams (1977)

Table 7.2b: ***Introduced/Biocontrol Complexes - Primary Parasitoid Dominant***

Dominant Primary Parasitoid	Primary Host Species	Percentage Domination by Primary Parasitoid	Other Species In Complex (Autoparasitoid marked *)	Reference
Eretmocerus haldemani	*Bemisia tabaci*	77%	*Encarsia meritoria* * (f) *Encarsia formosa* *Encarsia* sp.	Gerling (1967)
Eretmocerus serius	*Aleurocanthus woglumi*	large majority	*Encarsia divergens* * *Encarsia smithi* *	Clausen and Berry (1932)

Notes

(a) Hulden states that these species are probably autoparasitic

(b) In Poona and Madras, India

(c) On lemon trees near Assam, India

(d) Accounted for 22.5% parasitism of dominant host

(e) Accounted for 34% parasitism of primary host

(f) Gerling believes this species to be a facultative autoparasitoid

(g) *Encarsia meritoria* dominant early summer, *Eretmocerus haldemani* dominant late summer (1964 and 1965).

(h) Degree of dominance estimated from graph

(i) Approximately equally abundant in 1977, but *E. lutea* overtly dominant in 1978

(j) In Jamaica

(k) Previously described as *Prospaltella transvena* (Gerling 1983)

(l) *Aleyrodes spiraeoides* and *Trialeurodes abutilonia* also present

(m) Described by Flanders (1965) as the least abundant species

(n) Thelytokous primary endoparasitoid (Flanders 1953)

(o) In 1940

(p) In 1948-1950

(q) Autoparasitoid in Flanders (1953)

CHAPTER 8

FIELD WORK

There exist only two field studies of heteronomous hyperparasitoids in natural communities: a very brief account of host and parasitoid population growth over one season (Kuenzel 1975) and an unpublished, but more detailed account by Donaldson (1985) focusing on sex ratio and host availability. The main findings of both these studies have been described in chapter 2.

Direct field observations of these tiny wasps is almost impossible. They do however have a peculiar trait in that the pupal case from which autoparasitoids emerge can display clues about its previous occupant(s); hyperparasitoids leave the emergence case in a different condition to a primary endoparasitic species. By following the history of parasitised patches and examining the living and empty pupal cases in the patch, it is possible to accumulate data on the parasitoid spp. which have exploited the patch. In certain cases, when all the members of a complex can be recognised by their pupal and/or emergence cases features, the patterns of parasitism by the heteronomous hyperparasitoid can also be recorded.

In this chapter I describe natural populations of three parasitoid species on two species of whitefly in the grounds of Silwood Park, Ascot, Berkshire. Initially, I looked at natural patterns of parasitism by *E. tricolor*. Later I addressed questions about hyperparasitic preferences in *E. tricolor* and ovipositional decisions within discrete patches containing conspecific and non-conspecific pupae.

8.1 Methodology

Field work was undertaken at two sites in Silwood Park. The first was a birch-dominated woodland known as Nash's copse, some 2 hectares in area. Numerous stands of honeysuckle *Lonicera periclymenum* of various sizes grow in this woodland. The honeysuckle is host to the whitefly *Aleyrodes lonicerae* Walker. The second site was a 400m^2 plot of Brussels sprout plants (var. Winter Harvest) which were planted annually as part of a cereal/legume/brassica/fallow rotation in a plot from which rabbits were excluded. The Brussels plants were host to the Cabbage Whitefly, *Aleyrodes proletella* (Linnaeus). The only other major host to this whitefly is Greater Celandine, *Chelidonium majus* which occured as a very small number of discrete patches at the edges of the Silwood grounds. No parasitoids were reared from whitefly occuring on this host plant in 1987 or 1988. The following descriptions of my field work are

divided according to the host plant species which was used. Work monitoring the Brussels Sprout crop over the summer and autumn of 1986 failed to produce useful results due to serious bird damage of the crop and very low populations of whitefly.

8.1.1 Honeysuckle: 1986-1987

In the woodland site I selected 3 patches of honeysuckle, all of which were discrete and separated from each other by 50-100m. The patches differed in area, plant size and density. Monthly samples of 20 stems were taken covering the range of stem sizes available. All whitefly stages on each leaf were counted under a binocular microscope and any parasitised scales allowed to emerge at a constant 25°C. If it was not possible to count all the stems immediately, they were kept with their cut ends in a flask of water at 10°C for up to 3 days. This sampling program ran from 1/8/86 to 19/8/87.

8.1.2 Brussels Sprout: 1987

The 1987 season was used as an opportunity to investigate the natural patterns of parasitism in the Brussels crop and to show the pattern of exploitation of other parasitoid species by *E. tricolor* repeatedly seen in the laboratory experiments. To test this, I adopted the null hypothesis that *E. tricolor* lays a male in whatever species of parasitised host it encounters.

Between 24/7/87 and 14/12/87, fortnightly examinations were made of Brussels plants naturally infested with whitefly. Initially, 50 of the most infested leaves were selected and marked. No more than three leaves were selected from any one plant. When it became apparent however that some plants had far greater levels of parasitism than others, up to 31 leaves on a single plant were marked and monitored. All the whitefly stages on each leaf were counted and recorded. Likewise any parasitised scales. These were recorded as being in a discrete group or solitary (4cm or more from any other scale) on the leaf. It was possible to distinguish the three species of parasitoids present by examination of the pupae or empty pupal case following emergence of the wasp. The diagnostic features used are described in table 8.1. In this way it was possible to record the history of individual leaves and the patches of parasitised and unparasitised hosts on these leaves.

In some cases it was not always possible to discriminate between the two *Encarsia* species which were host to hyperparasitic *E. tricolor* males. This was usually due to the pupal case failing to show diagnostic features clearly enough to be certain of the identity of its recently departed occupant. Such ambiguous male host records were not used in the analysis of results.

Table 8.1: Diagnostic features of pupae/pupal cases used in field studies.

Species	*Pupal Stage*	*Empty Emergence Case*
E. inaron	Usually flattened dorso-ventrally. Completely black.	Ring of pupal fragments often extending around inner circumfrence. Many pupal fragments remaining attached to inside of whitefly case.
E. tricolor Females	Grey with transparent circumference to whitefly puparium.	Semicircular pupal fragments - thicker posteriorally. "Clean" appearance of whitefly case.
E. tricolor Males	Same as *E. tricolor* or *E. inaron* females. No identification possible at this stage.	Contains melanised remains of primary parasitoid. Exit hole small and often off-centre of whitefly case.
Euderomphale chelidonii	Bright yellow with black pupa.	Bright yellow. No pupal remains.

The species of primary parasitoid present were easily assessed at the pupal stage. Empty pupal cases however required examination under a x40 microscope. When emergence of wasps from the patches was particularly extended, the leaf was not disturbed until emergence had ceased i.e. the same count of empty pupal cases on two consecutive occasions.

Between 5/11/87 and 14/12/87 all the pupae remaining unemerged were transferred individually to gelatin capsules and allowed to overwinter under cover of an open building subject to natural temperature and photoperiod changes. The capsules were checked weekly for emergence. These individuals were indentified and sexed. Pupae remaining unemerged on 2/5/87 following 9 weeks of zero emergence were disected.

8.1.3 Brussels Sprout: 1988

The initial plan for 1988 was again to show preferential attack of *E. inaron* by *E. tricolor* under an experimental regime, wherein the availability of each species was manipulated following a whitefly and *E. tricolor* release program. In this way, I attempted to answer these two questions:

1. Was *E. inaron* hyperparasitised to a greater extent than *E. tricolor* even though both were available at high densities?
2. Did the pattern of hyperparasitism differ in patches with a high density of parasitised hosts when compared to other patches available in the environment?

The population of Cabbage Whitefly was artificially elevated early in the season by putting out potted Brussels plants heavily infested with whitefly. A total of 14 such plants were put out over the month of June. By September, substantial populations of whitefly were to be found on many plants in the plot.

At the beginning of September, plants which had been exposed to primary parasitism by either *E. tricolor* or *E. inaron* were also placed randomly in the plot. Approximately 100 young female *E. tricolor* were released at this time. Unfortunately, these lab-infested plants were persistantly eaten by rabbits entering the site through holes in the fencing. The plan was to destructively sample from these plants over the course of the season. Consequently, this plan was abandoned and instead a total of 9 lab-infested plants were placed within a $16m^2$ area securely fenced with chicken wire 1m high in the centre of the plot. Plants had previously been exposed to primary parasitism by each parasitoid species separately for 48h at 25°*C*: 5 plants were placed in the *E. inaron* culture and 4 plants in the *E. tricolor* culture.

The following samples were taken from plants of the Brussels crop as an indicator of the background patterns of parasitism within the plot.

Date	Number of leaves examined
6/9/88	20 mature leaves
20/9/88	25 mature leaves
14/11/88	87 mature leaves

The lab-infested plants were put out on 15th/16th September and left until 14th November when they were taken in. Parasitised scales were allowed to develop until pupation, whereupon they were placed individually in gelatin capsules and allowed to emerge in the 25°*C* C.T. room. Unemerged scales were disected 2 weeks after parasitoid emergence had ceased. The usual reason for non-emergence was mechanical damage to the pupae during transfer into the gelatin capsules. A few pupae suffered fungal attack; their contents were usually unidentifiable and were not included in the results.

8.2 Results

8.2.1 Honeysuckle: 1986-1987

Two parasitoid species were found attacking *A. lonicerae* on honeysuckle: *E. tricolor* and the eulophid *Euderomphale chelidonii* Erdös (identified by Z. Boucek of C.I.E.). The percentage parasitism of 3rd and 4th instar *A. lonicerae* by each parasitoid species is given in table 8.2.

Table 8.2: Percentage parasitism of 3rd and 4th instar *A. lonicerae* in samples from 3 sites in Nash's Copse, Silwood Park.

	E. tricolor	*Euderomphale chelidonii*	Both Species
Site 1	1.60	0.85	2.42
Site 2	0.75	1.94	2.66
Site 3	0.00	2.89	2.89
Total	0.85	1.82	2.64

Changes in the whitefly numbers in each 20-stem sample are plotted in figures 8.1-8.3. The total number of parasitoids reared from the sample are also given. The overwintering whitefly pupae emerged and lay the first generation which appear as the small April surge of eggs. This produces the summer generation which starts egg-laying in July, producing the pupae that will overwinter. Peak parasitism appeared at, or shortly after the number of 4th instars peaked in September/October. The field sex ratio of *E. tricolor* was strongly female biased (mean of sites 1 and 2: 0.15 proportion male).

Such very low levels of parasitism by *E. tricolor* made continual study of this particular system too work-intensive to be worthwhile; especially when the substantially higher levels of parasitism in the Brussels crop were discovered during the 1987 field season.

8.2.2 Brussels Sprout: 1987

A total of 137 patches were monitored, the details of which are given in tables 8.3 and 8.4. The overall percentage parasitism of 3rd and 4th instar *A. proletella* by both species combined was 12.3%. Only four patches of *Euderomphale chelidonii* were found and these never exceeded 6 parasitised scales per patch. This species was of very minor importance in the Brussels crop and is not considered further.

Figure 8.1: Fluctuations in the number of *Aleyrodes lonicerae* nymphal stages and number of parasitised scales over the 1986/7 honeysuckle sampling program - site 1

SITE 1

TOTAL NUMBER OF PARASITOIDS FROM SAMPLE														
Encarsia tricolor	3F	0	0	10F:3M	0	0	0	0	0	0	0	0	3F	0
Euderomphale chelidonii	6	0	1	3	0	0	0	0	0	0	0	0	0	0

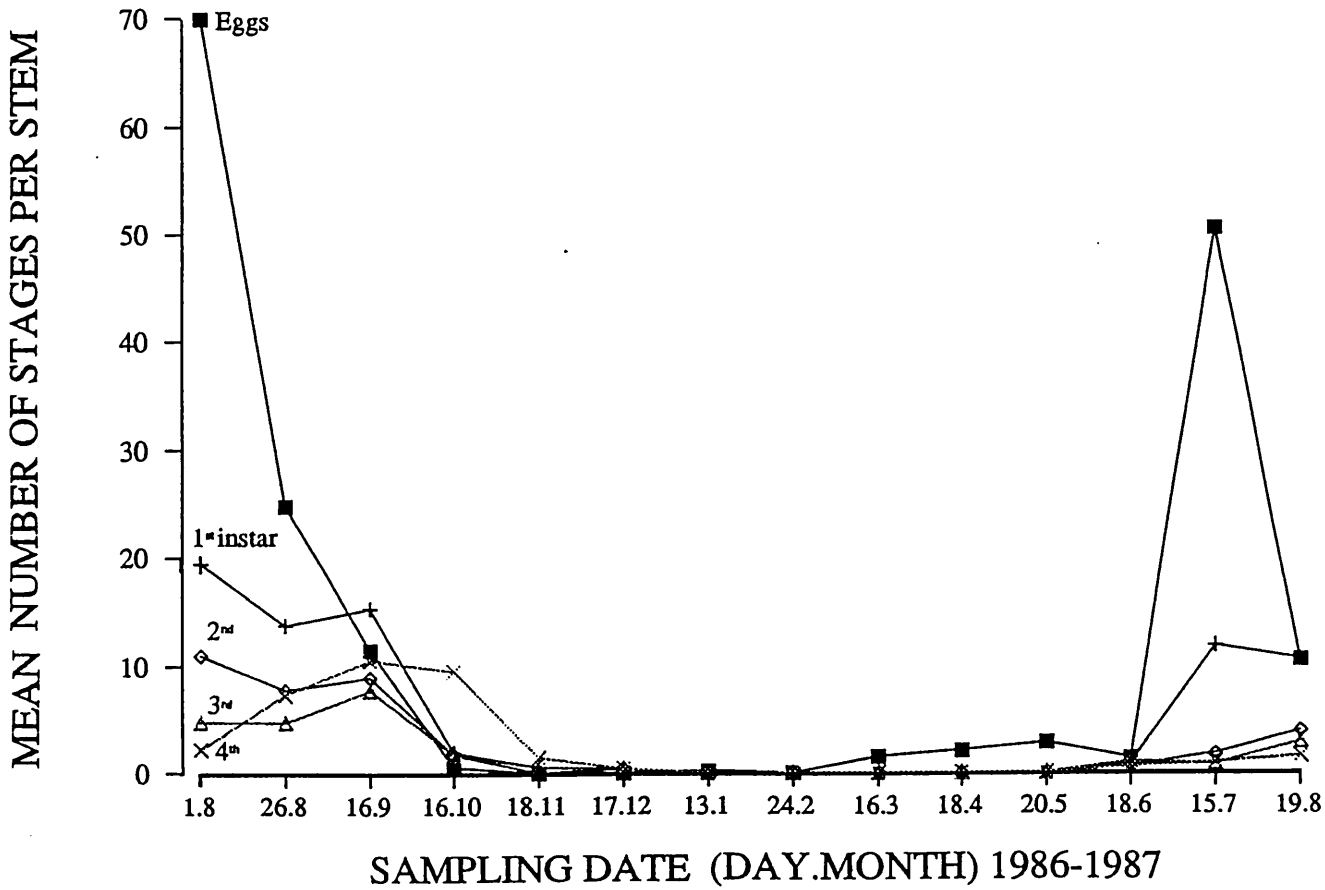


Figure 8.2: Fluctuations in the number of *Aleyrodes lonicerae* nymphal stages and number of parasitised scales over the 1986/7 honeysuckle sampling program - site 2

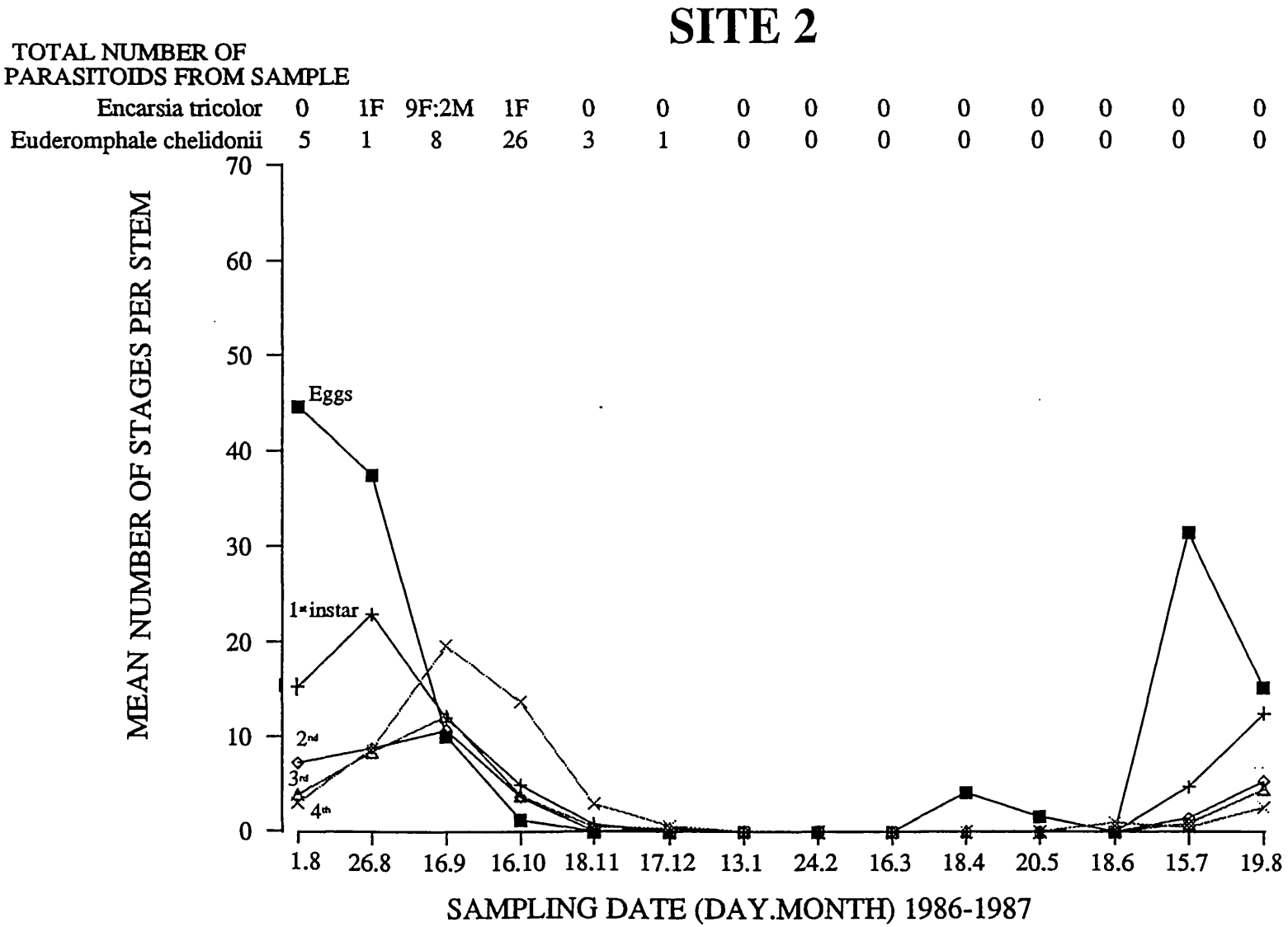


Figure 8.3: Fluctuations in the number of *Aleyrodes lonicerae* nymphal stages and number of parasitised scales over the 1986/7 honeysuckle sampling program - site 3

SITE 3

TOTAL NUMBER OF PARASITOIDS FROM SAMPLE														
Encarsia tricolor	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euderomphale chelidonii	0	1	4	14	2	1	0	0	0	0	0	0	3	0

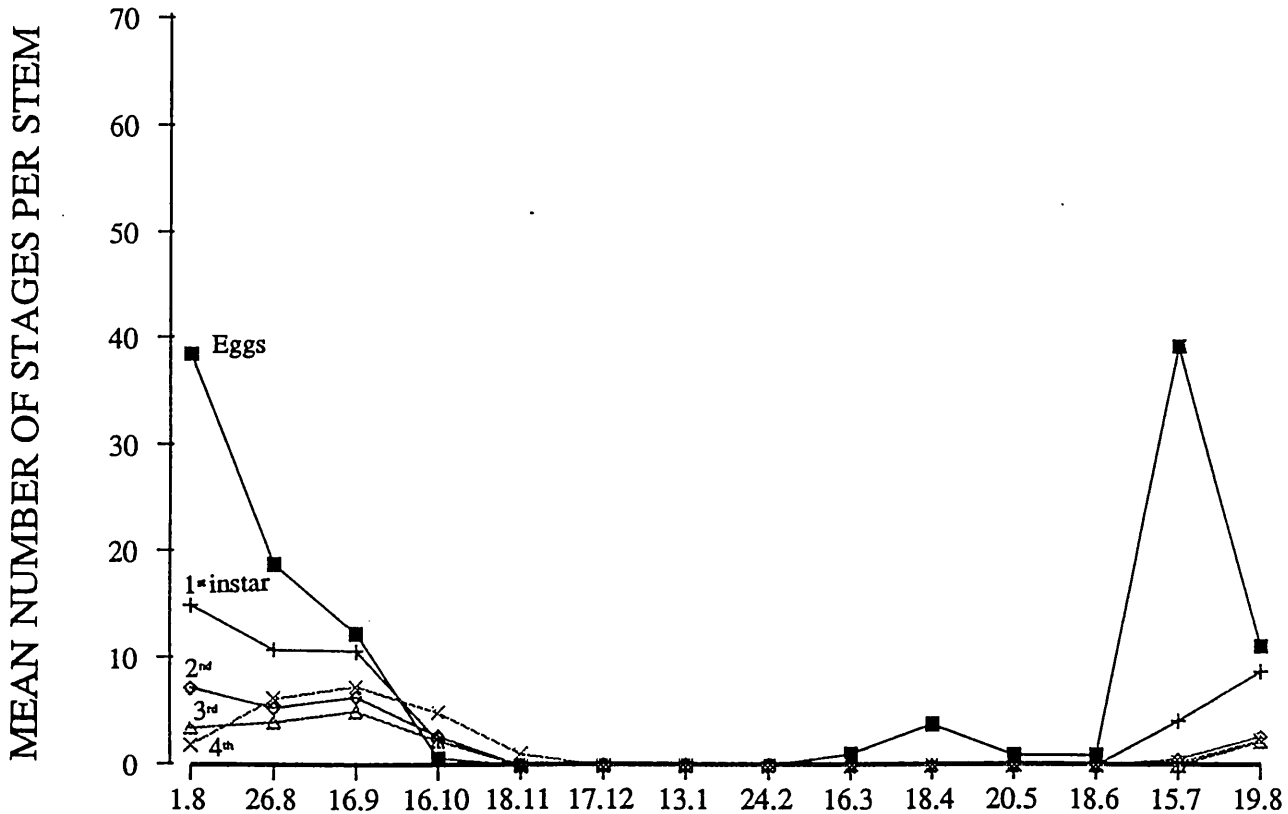


Table 8.3 showns the number of each type of patch monitored in the Brussels crop in 1987. Half of the patches examined initially developed parasitised scales although the method of sampling was biased in favour of patches showing parasitism, as described in the methods section. *E. inaron* was more common than *E. tricolor*, occurring in a total of 40 patches. A Chi-squared test of association between *E. tricolor* and *E. inaron* showed that the frequency of patches containing both species together was significantly higher than would be expected, given random occurrence of the two species ($\chi^2_1 = 4.04 \quad P < 0.05$).

Table 8.3: Number and Types of Patch recorded in Brussels Crop, 1987.

	Number of Patches
Total recorded	137
Total showing parasitism	67
Total sampled on at least 2 occasions	53
Patches parasitised by *E. inaron* only	26
Patches parasitised by *E. tricolor* only	16
Patches parasitised by both species together	14
Patches lost before any information could be gathered (contents unknown)	11

A fraction of the parasitised pupae disappeared over the course of the season. As the leaves themselves aged, they dried-out, becoming brittle and distorted. Extreme care was necessary when handling such leaves. Often they were placed for a few hours in a plastic bag with moist tissue to rehydrate the leaf allowing it to be unfolded and examined. Loss of parasitised material was inevitable given such circumstances. This meant that for a large proportion of the patches data on the history and exact make-up of the patch were incomplete. For example, if 3 parasitised pupae disappeared from a patch of *E. inaron* pupae, it was not possible to know if the lost pupae were themselves *E. inaron* or had been hyperparasitised by *E. tricolor*. This generated a substantial number of missing values which made statistical analysis using GLIM (for fitted regressions) valueless. GLIM was used however, for calculation of means and standard errors or 95% confidence limits (given below as asymmetrical ranges) and for simple comparison of means with Poisson or binomial errors.

Table 8.4 gives details of the average (and 95% confidence limits) size of patches and their degree of exploitation by both parasitoids singly and together. The sex ratio of *E. tricolor* from both types of patch is also given. For patches of each species singly,

Table 8.4: Patch Size, Percentage Parasitism and Sex Ratio for Mono-Specific and Mixed Patches in Brussels Crop, 1987.

	Patches of E. inaron only		*Patches of E. tricolor only*		*Patches of both species together*	
Variable	Mean	Range of 95% CL	Mean	Range of 95% CL	Mean	Range of 95% CL
Size of patch attacked (number of whitefly nymphs/patch)	12.2[a]	10.7-13.9	18.1[b]	15.7-20.8	20.5[b]	18.1-23.2
Number parasitised hosts/patch	6.9[a]	5.9-8.0	5.2[a]	4.0-6.7	12.3[b]	10.4-14.4
% parasitism in patch	42.9[ab]	30.5-56.3	28.6[a]	17.4-43.3	59.8[b]	46.6-71.6
Sex Ratio of *E. tricolor* in patch (percent male)	N/A	N/A	9.6[a]	5.1-17.3	41.0[b]	20.1-65.8

Means followed by the same letters (a,b) are not significantly different (binomial t-test).

there were no significant differences in the number of hosts parasitised or the percentage parasitism within each patch. Mixed species patches had, on average, significantly more parasitised hosts per patch and a higher percentage parasitism than the mono-specific patches. The sex ratio of *E. tricolor* in patches where *E. inaron* was also present, was significantly more male biased than in patches containing only conspecifics; i.e. significantly more hyperparasitism occurred in patches with *E. inaron*.

Two interesting and important points arrise from the data of table 8.4. The first is that the patches of *E. tricolor* alone can be divided into those patches in which only females developed and patches containing both sexes. The details are given in table 8.5 which shows that *E. tricolor* males tended to be laid in larger than average patches of conspecifics.

Table 8.5: The relationship between *E. tricolor* sex ratio and size of patch

	No. parasitised by *E. tricolor*		
Sex of *E. tricolor* in patch	No. patches	Mean	Range of SE
Where only Females developed	12	4.8	4.2-5.5
Where both sexes developed	4	15.5	13.7-17.6
All Patches	16	5.2	4.5-5.9

The second point is similar in that the mixed patches can also be divided into:

1. those patches in which males/both sexes of *E. tricolor* developed
2. those patches in which only females developed.

Table 8.6 shows that the sex ratio of *E. tricolor* changes according to the number of *E. inaron* present in the patch. That is to say, larger patches of *E. inaron* tended to attract more hyperparasitism than their smaller (less conspicuous) counterparts.

There was no significant change in the sex ratio of *E. tricolor* sampled early in the season compared to later on. An arbitary split of the data was made at 8/10/87 (the second sample that yielded information on sex ratio) and compared to the sex ratio of all *E. tricolor* discovered subsequently (see table 8.7).

Table 8.6: The relationship between sex ratio in *E. tricolor* and the number of *E. inaron* present in each mixed-species patch

	No. *E. inaron* in each patch		
Sex of *E. tricolor* in patch	No. patches	Mean	Range of SE
Where only Females developed	5	4.8	3.9-5.9
Where males/both sexes developed	9	12.9	11.7-14.1

Table 8.7: *E. tricolor* Sex Ratio in Early and Late part of 1987 Field Season

	Prior to 8/10/87	After 8/10/87
Number of *E. tricolor* discovered	52F:14M	73F:21M
Sex Ratio (percent male)	21.2%	22.3%

($N.S.$ $\chi_1^2 = 0.03$ $P > 0.05$)

8.2.3 Brussels Sprout: 1988

The results of this work can be divided into two catagories:

1. parasitism on the lab-infested plants
2. the natural parasitism within the Brussels crop

8.2.3a Parasitism on the lab-treated plants

The series of failures in defending the infested plants from rabbits, caused the experiment to be delayed until quite late in the season. Probably because of this, the lab-treated plants did not show significant levels of field hyperparasitism. Only three patches of *E. tricolor* were found on the *E. inaron* parasitised plants (see table 8.8).

8.2.3b Parasitism within the Brussels crop

The early whitefly releases achieved a high primary host population relative to previous years. This resulted in the numbers of primary parasitoids appearing greater than in previous field seasons, although the proportion of whitefly parasitised remained very low. Thus, no useful data on secondary parasitism were collected. Analysis of the pattern of primary parasitism was possible however. The higher populations of whitefly meant that patches of nymphs on each leaf were overtly less discrete than in 1987. Consequently, data were collected as parasitism occuring per leaf.

Table 8.8: Summary of results from lab-parasitised Brussels plants.

	Plants previously exposed to *E. tricolor*	Plants previously exposed to *E. inaron*
Number of plants (leaves) used	5 (18)	4 (16)
Mean (±SE) number of unparasitised scales available per leaf	96.0±18.6	113.7±27.7
Mean (±SE) number of parasitised scales available per leaf	14.6±3.5	40.4±9.7
Overall percentage primary parasitism	13.1	26.2
Number of leaves parasitised in the field	1*	3**

* A single *E. inaron* developed on one leaf of one of the *E. tricolor* infested plants.
** The composition of these patches was:

Number Unparasitised	Number of *E. inaron*	Number of *E. tricolor*
111	76	7F:1M
329	74	6F:9M
170	136	1F

Of the 132 leaves sampled between 6/9/88 and 14/11/88, 15 showed parasitism by *E. tricolor* and 18 by *E. inaron*. The overall percentage parasitism by these species was 1.56 [SE 1.25-1.96] and 2.47 [SE 1.95-3.14] respectively. Only four mixed patches were found: too few for analysis. The composition of these patches was:

Number Unparasitised	Number of *E. inaron*	Number of *E. tricolor*
46	1	3F:1M
54	6	4M
103	1	10F
600	18	2F

The mean size of patches (number of scales/leaf) showing no parasitism was 109.4 [n=90, 95% confidence limits: 108.3-110.0]. The mean size of patches showing parasitism by *E. tricolor* was 136.2 [n=15, 95% CL: 133.0-139.5], i.e. larger patches of whitefly were more likely to attract primary parasitism by the autoparasitoid.

When the regression of number of parasitised scales per leaf was fitted against density of whitefly (total number of scales per leaf) assuming binomial errors, this parameter explained a significant amount of model deviance, and the slope of the regression was significantly different from zero. The changes in model deviance are shown in table 8.9. There was a negative relationship between the percantage primary parasitism of whitefly by *E. tricolor* and the number of whitefly on leaves in the Brussels crop. The regression and fitted line are shown in figure 8.4.

Figure 8.4: Regression of proportion primary parasitism by *E. tricolor* on number of whitefly per leaf, for the Brussels crop in 1988.

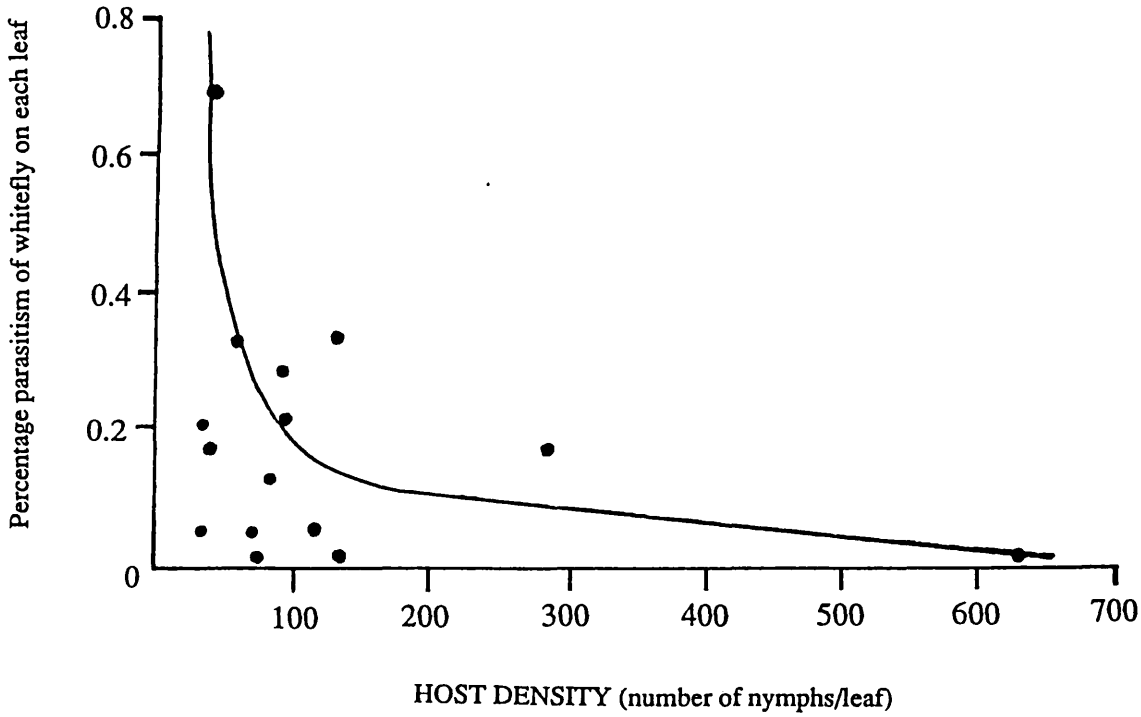


Table 8.9: GLIM model: Regression of percentage parasitism by *E. tricolor* on whitefly density

Y-variate	Total Deviance	Deviance with Total hosts/leaf Fitted	Change in Deviance
No. parasitised hosts	357.9 (df=14)	217.0 (df=13)	140.9*

* 39.4% reduction in model deviance (χ^2_1 $P < 0.01$)

8.3 Discussion

Natural patterns of parasitism over the two complete field seasons of these studies have given more information on ovipositional decision making by *E. tricolor* in the natural environment, than the manipulative approach. Data from such studies was highly work-intensive to collect and this often accounts for the relatively small sample sizes used in analyses.

For all the host plants studied, the field sex ratio of *E. tricolor* was highly female biased. To find a patch where both sexes were developing together and in which males outnumbered females was exceptional save in patches previously exploited by *E. inaron*. In the honeysuckle, hyperparasitism of *Euderomphale chelidonii* was never recorded, despite the fact that *E. tricolor* females readily and successfully hyperparasitised this species in the laboratory. The apparent absence of exploitation of the eulophid by *E. tricolor* is most likely to be due to the very lowoccurrenceof both species and the low density of whitefly on this plant. Sampling in such a situation would need to be very extensive to record hyperparasitism accurately.

Manipulative experiments were clearly hampered by factors unrelated to wasp behaviour (rabbits, length of season). Despite the failure of these experiments, natural patterns of parasitism gave considerable validation of the lab findings regarding the ovipositional preferences of *E. tricolor* for male production. The results of the 1987 field season clearly allow us to reject the null hypothesis that *E. tricolor* lays males without preference as to host species. *E. tricolor* was attracted to *E. inaron* patches even though

patches parasitised by conspecifics offered a similar number and density of secondary hosts. The sex ratio laid in the presence of *E. inaron* was also significantly more male biased than in patches containing only conspecifics.

It is unfortunate that little data could be collected which gave insights into the factors which attract secondary parasitism from *E. tricolor* (apart from the presence of *E. inaron*). Certainly patch size seems to play an important role. Males were only laid on *E. tricolor* females in larger than average conspecific patches. Similarly, larger patches of *E. inaron* tended to be exploited for male production more frequently than small patches of *E. inaron*. This may simply reflect their relative degree of apparency to the searching female *E. tricolor*: the area which the patch covers on the leaf surface, the density of secondary hosts, the emission of an attractant kairomone, etc.

Intuitively, we may expect a long-lived patch to have a higher probability of being being visited by more than one *E. tricolor* female. Thus, the probability of hyperparasitism (of conspecifics or of *E. inaron*) would be higher in these patches compared to their more ephemeral counterparts. GLIM analysis of sex ratio with the duration of individual patches fitted as an explanatory variable could have potentially been valuable in indicating whether longer-lived patches tended to suffer more hyperparasitism more than short-lived patches. As mentioned earlier however, missing values meant that the data were not suitable for GLIM analysis.

The lack of a change in *E. tricolor* sex ratio over the season contrasts with the findings of Kuenzel (1975) and Donaldson (1985), who both found that changes in autoparasitoid sex ratio were concurrent with changes in the availability of primary and secondary hosts. The density of hosts in both of these studies and the percentage parasitism by the autoparasitoid were both many times greater than the values seen in the present study. Thus, the magnitude of the changes in these two parameters was also many times greater and may have accounted for the temporal differences in autoparasitoid sex ratios.

Finding that the percentage primary parasitism was inversely related to the density of whitefly in the Brussels crop in 1988 was intriguing. Such a pattern of parasitism would be seen if a female encountering a patch of hosts laid all her available (mature) eggs and then departed in search of more patches whilst she matured a fresh batch of eggs. This was one of the predictions made by Charnov and Stephens (1988) in their paper on host selection described at the start of chapter 6. They considered how egg maturation and an egg-production/mortality rate tradeoff affected host selection by a

parasitoid in environments with high/low host contaigon. A female wasp in a patchy environment could lay all her eggs and leave the patch, or remain in the patch, mature and lay more eggs before leaving. The interpatch travel time was an important factor in deciding which strategy to adopt: long travel times favoured greater local patch exploitation and less migration.

An alternative explanation of this "parasitise - move on, parasitise - move on" type of behaviour is that the wasp is reducing the risk of her progeny being discovered by a conspecific *E. tricolor*. In this way, she could avoid extensive hyperparasitism of her offspring which a large patch of parasitised scales may attract. This idea assumes that ***secondary*** parasitism is density dependent. A similar reason has been suggested for why Pierid butterflies disperse their eggs over plants in an inversely density dependent manner. Courtney (1986) cites density dependent canabalism of eggs as the reason why *Pieris rapae* leave a patch after laying just a few eggs; far less than could theoretically develop from the patch (but see Root and Kareiva 1984, 1986 for alternative explanation).

Further support for the avoidance-of-hyperparasitism hypothesis would come from showing that secondary parasitism is positively density dependent in the field.

Despite the low population density of all parasitoid species attacking the two native whiteflies, sufficient observations were made to corroborate the laboratory findings described in chapters 5-7. The fact that male production can occur on the two conventional parasitoids, *E. inaron* and *Euderomphale chelidonii* must have implications for the dynamics of natural *E. tricolor* populations in the UK. There are 6 additional species of whitefly primary parasitoids native to the UK which occur at comparable levels to the species recorded in this study (A. Polaszek, pers. comm.). Assuming that *E. tricolor* could successfully hyperparasitise most or all these species, then in their absence, conspecific females would have to be utilised for ***all*** male production. This would reduce the population, possibly to the point where *E. tricolor* was no longer viable in this country; although for an individual wasp there are no benefits to preferential hyperparasitism of other species over conspecifics other than those described previously (increased local mating opportunities and certain avoidance of attacking close relatives).

CHAPTER 9

DISCUSSION

Heteronomous hyperparasitism is a remarkable method of development found only in eight genera of a family of minute hymenopteran parasitoids, the Aphelinidae. The biology and ecology of many heteronomous species remains poorly studied. The small size of these wasps makes classification problematic, manipulative experiments difficult, and direct field observations of searching females virtually impossible. Contained in chapters 4-8 of this thesis are the results of work which set out to explore the possible adaptive consequences of heteronomous hyperparasitism. Initially I examined the biology of *Encarsia tricolor*, a native facultative autoparasitoid in which both sexes can develop in several of their respective host species. *Encarsia tricolor* has a typical heteronomous parasitoid biology. Both sexes are long lived for such small wasps. They can develop in any instar of their respective hosts although larger hosts are preferred, especially for primary parasitism. Daily fecundity is low and requires host feeding activity throughout the life of the female if maximal egg production is to be achieved. All primary host instars are utilised for host feeding. Oviposition of male eggs takes significantly longer than female eggs, probably because the ovipositor must penetrate the integument of the developing primary parasitoid in addition to that of the homopteran host. In the remaining chapters I went on to investigate the relationship between *E. tricolor* and *E. inaron*, a parasitoid of the Cabbage Whitefly in which both sexes develop as primary endoparasitoids.

9.1 Decision Making during Oviposition

E. tricolor preferentially exploits *E. inaron* for male production. This discrimination has a marked impact on sex ratio and competition between the two species. The fact that experience affects the decisions made during oviposition, strongly suggests that experience plays an important part in giving the female clues as to parasitism in the field. The preference for hyperparasitism of non-conspecific primary parasitoid species may have two benefits. It increases the local mating opportunities for males and is a mechanism to avoid exploitation of related female larvae for male production. To what extent each of these factors is advantageous to females searching for patches under field conditions, is difficult to assess without detailed knowledge of the search patterns of individual females. Following the reasoning in the kin recognition section of chapter 5, it seems unlikely that a female will revisit a patch in which eggs have been laid previously. Only if a female commonly remains in a patch long enough to encounter her own

daughters at a stage suitable for hyperparasitism, will preferential attack of non-con-specifics be a very effective way of avoiding intra-familiar hyperparasitism. This assumes that males reared as hyperparasitoids on other species have a similar fitness to those males reared from conspecific hosts, or that the average reduction in fitness of a male with an alternative species food source is not as great as the potential gain in maternal fitness from avoiding hyperparasitism of conspecifics. Both points seem plausible. There is an obvious parent-offspring conflict in this latter situation, but the male offspring are powerless to influence maternal decision making!

9.2 Host Selection and Sex Ratio

The most appealing of theories explaining heteronomous hyperparasitoid sex ratios is also the most recent. Godfray and Waage (in prep.) have approached the problem of adaptive sex ratios in these wasps by considering the role of egg and host limitation on parasitoid searching and sex allocation. For a wasp encountering an abundance of hosts of both types, in a situation where both LMC and the probability of hyperparasitism of relatives are negligible, Fisher's rule holds and equal investment in the sexes is predicted; leading to an unbiased sex ratio. Given that there is an abundance of parasitised and unparasitised hosts available in the environment these assumptions are quite likely to be correct. Aphelinids have a low fecundity and so a large number of parasitised hosts is indicative of the patch having been visited by a large number of foundress females which decreases LMC. In addition, there may well be a substantial number of immigrant males arriving from other, equally well exploited patches. The probability of attacking kin is clearly low if one's own progeny are diluted in a large patch of unrelated conspecific larvae.

For a wasp searching at low host densities, the search time required to find each host would be high and reproduction would probably be limited by the rate of finding hosts. Given such a situation, there will be no benefits for a wasp to reject laying an egg in each and every host she discovers, as long as such oviposition does not hinder future egg-laying opportunities. Such a strategy would lead to the deposition of male and female eggs in the same proportions as the respective hosts occur in the environment. Support for this prediction of Godfray and Waage, comes from work done by Donaldson (1984) and Keunzel (1975).

Kuenzel (1975) monitored a small complex of parasitoids (*Encarsia pergandiella* and *Eretmocerus corni*) on the Strawberry Whitefly, *Trialeurodes packardi*. The sex ratio of *E. pergandiella* initially fluctuated around 50% but increased rapidly in late August

with an increase in the number of secondary hosts of both species. This was after an exponential rise in the whitefly population in late July. *E. pergandiella* was by far the most dominant parasitoid.

Donaldson found the sex ratio of *Coccophagus atraus* fluctuated according to host availability, being female biased early in the season and male biased later when male hosts (*Coccophagus* and *Metaphycus* spp.) were more abundant than unparasitised scales. Percentage parasitism of the scale insect *Filippia gemina* fluctuated between 30-92% or 0-77% (estimated from graphs) depending on the host plant sampled. The density of second instar scales (preferred stage) was around 25-50 scales per leaf for both host plants for most of the sampling period. The test of the Godfray and Waage hypothesis comes from knowing whether the rate of host finding at these kinds of host densities is limiting to the reproduction of the wasp. Donaldson does not provide sufficient data to decide one way or another. The fact that percentage parasitism of the scale was high and the population of scale insects fell dramatically between the second and third instars suggests that the scale was being highly exploited. Thus, we may expect the searching wasp to be limited in its reproduction by the rate of finding hosts rather than its rate of egg maturation. Given such a situation, Godfray and Waage predict that an egg should be laid in each and every host found, leading to a sex ratio reflecting the relative abundance of each host type, as was apparently seen.

As already stated, Godfray and Waage have argued that for a heteronomous hyperparasitoid limited in its exploitation of hosts by search time, equal investment in the sexes should result in parasitism matching the relative abundance of the two host types. Consider the following situation, however. If for example, unparasitised hosts were abundant while parasitised hosts were rare, reproduction of the parasitoid would not be limited by the rate of finding (female) hosts whereas reproduction through male function would be completely host-limited. For this situation, the argument that to reject any host would be maladaptive, fails. Instead, equal investment in the sexes should lead to equal time spent searching for each host type. The proportion of male hosts parasitised would thus be greater than the relative frequency with which they occurred in the environment. This in turn would lead to a more even sex ratio than predicted by Godfray and Waage when wasp reproduction was limited by both host types.

Godfray and Waage predict an identical strategy of equal search time allocation for host types occurring in different environments, such as that for heterotrophic parasitoids searching for homopteran (female) and lepidopteran (male) hosts.

The 1987 field season data showed that the overall mean sex ratio of *E. tricolor* (mean 21.6% male, range of 95% CL: 11.4-37.2%) in patches of *E. tricolor* alone and together with *E. inaron* was less than, but not significantly different from the mean percentage parasitism in these patches (mean 45.8%, range of 95% CL: 34.8-57.3%). That is to say, these data were in line with the Godfray and Waage prediction of sex ratio under host limitation reflecting host availability. The confidence limits on the means are very large however, and this could not be considered as a particularly stringent source of support for their theory.

Undoubtedly the pivotal question which determines the validity of the Godfray and Waage hypothesis is that the rate of host finding in the field determines the sex ratio. As the rate of finding both host types increases and the parasitoid becomes egg-limited, equal (search time) investment in the sexes should result in parasitism of the two host types to an equal extent and an unbiased sex ratio is predicted. A low rate of host discovery should result in parasitism of each and every host found and the sex ratio should reflect the relative frequencies of each host type in the environment.

An assumption implicit in this model is that the costs of producing a male are identical to that of a female. Differences in the time or energy needed to lay a haploid as opposed to a diploid egg and any mortality risks associated with oviposition in the different host types will result in an imbalance of costs of producing each sex. Under Fisher's rule of equal investment, the sex ratio will become biased in favour of the sex that is cheaper to produce. Whether the difference in ovipositional times for male and female eggs is a significant factor in differential investment in the sexes is doubtful. Even drilling times of around 11 minutes necessary for a male egg against some 4 minutes for a female egg are small compared to the time required to locate and assess hosts. At her maximal egg-laying rate a typical female heteronomous hyperparasitoid is unlikely to lay more than 10 eggs per day. Thus, she may successfully oviposit in hosts for between 40 minutes (primary hosts only) and 110 minutes (secondary hosts only) in a 24h period. Other factors such as fertilisation cost for diploid eggs should, intuitively, be negligible.

The field work endorsed the lab findings of preferential hyperparasitism of *E. inaron* over conspecific females and gave clues as to the factors (mainly patch size) which attracted primary and secondary parasitism from *E. tricolor*. Patches of parasitism were highly discrete. When individual patches of whitefly were discovered by *E. tricolor*, parasitism appeared to be inversely density dependent. This finding lent qualitative support to the Chamov and Stephens (1988) model in which an egg-limited parasitoid in a patchy environment was predicted to deposit all her eggs in a patch and either leave

(to search for more patches), or remain in the patch, mature and lay more eggs before leaving. The difficulty of finding new patches was important in determining the optimal strategy. Such behaviour by an aphelinid like *E. tricolor* with only a handful of eggs to lay, would produce the negative relationship between patch size and percentage parasitism seen in this study.

9.3 Autoparasitoid Classification

The act of laying males hyperparasitically on conspecific females is bizarre. As mentioned several times already, there are advantages to preferential exploitation of competitors over conspecifics for male production. There are no advantages to obligate autoparasitism, however. There are no adaptive reasons why this, highly limiting type of sex allocation, should ever have evolved; except in situations when a heteronomous hyperparasitoid had become geographically, temporally or otherwise isolated from non-conspecific competitors (alternative male hosts).

During the course of this research, it emerged that Walter's (1983) classification of heteronomous hyperparasitoids may require revision. In particular, it becomes evident when examining in detail the references cited by Walter, that there exist no documented cases of true obligate autoparasitism and ***very*** few, of alloparasitism. I will examine the evidence for each group in turn.

First, consider the obligate autoparasitoids, in which a male larvae can only complete its development in/on a ***conspecific*** female hymenopteran. In his 1983 paper, Walter lists 11 species of "obligate autoparasitoids". Of these 11, three possess footnotes to the effect that the references cited are contradictory to other authors, or the exact nature of the host relations are less than certain. Of the remaining 8, two have been subsequently shown to be facultative autoparasitoids (*Encarsia lahorensis* - Hudson and Williams 1986; *Encarsia formosa* - Gerling 1983). *Encarsia formosa* is amphitokous, males being produced in low numbers only under crowded culture conditions. For five of the remaining six species, the nature of the male dependency has been stated solely as fact (references listed in table 9.1 below) with no references made to experimental evidence or field data in the presence of alternative secondary hosts i.e. the ***ability*** of males to develop on conspecifics is shown but not the ***necessity*** to do so. The only paper to do this is that of Broodryk, Kennett and Tassan (1986) who clearly state that *Coccophagoides utilis* does not exploit its competitor *Aphytis paramaculicornis* for male production. Unfortunately, both of these species are exotic introductions of the U.S. Olive Scale biocontrol program and so would not be a natural pair of species found

interacting in their natural habitats (*A. paramaculicornis* originates from Iran whereas *C. utilis* is native to Pakistan). Thus this single verified "obligate" host record is in effect, spurious.

Table 9.1: Species of suspected obligate autoparasitoids for which the host relations are uncorroborated or ambiguous

Species	Reference
Coccophagoides abnormicornis	Doutt according to Flanders in Zinna (1962)
Coccophagoides utilis	Huffaker, Kennett and Tassan (1986)
Coccophagus gossypariae	Sumaroka (1965)
Encarsia perniciosi (bisexual form)	Rice (1937); Chumakova and Goryunova (1963)
Physcus flavus	Clausen (1956)
Physcus intermediatus	Taylor (1935); Flanders (1936c)

Secondly, consider the alloparasitoids, in which males only develop as hyperparasitoids of ***non-conspecific*** hymenopterans. Walter (1983) lists 5 species, which it must be said, benefit from a slightly better quality of past literature. Indeed, there seem to be genuine cases in which the male only develops as a hyperparasitoid of species other than his own. It can be shown however, that as before, this is usually a result of differences in female ovipositional behaviour rather than any inate inability to utilise conspecifics for male production. Of the five species listed by Walter, two appear at best to be alloparasitoids simply by ovipositional preference of the maternal female: *Aneristus ceroplastae* and *Coccophagus pulvinariae* do not appear to have statements of obligate host relations in any of the references cited by Walter (1983). The interesting species are *Coccophagus malthusi*, *Coccophagus basalis* and *Lounsburyia trifasciata* in which the males are indirect hyperparasitoids of non-conspecifics. In *C. malthusi*, the female deposits fertilised diploid eggs into waxy scale insects of the genus *Ceroplastes*, whereas haploid male eggs are laid in various lecaniine coccids other than those used as

female hosts (Annecke 1964; Annecke and Insley 1974). This clearly results in a mechanism by which males can only develop as secondary parasitoids of non-conspecific species. This behaviour also has the same advantage as preferential hyperparasitism of competitors in that by laying male eggs in a completely different ***primary*** host species a maternal female avoids the problem of hyperparasitism of her own daughters.

Another more physiological problem of development arises in *Coccophagus basalis* and *Lounsburyia trifasciata*. The reason that males of these species cannot successfully develop on conspecific female larvae according to Flanders (1936c) and Flanders et. al. (1961) is that conspecific female juveniles fail to consume sufficient of the primary host body fluids to stimulate hatching of the quiescent male larva laid in the primary host earlier. Species such as those in the genus *Metaphycus* however, totally consume the primary host and thereby stimulate hatching of the fluid-sensitive male.

It seems much more appropriate to view both obligate (habitual) autoparasitism and alloparasitism as a range of facultative responses to hyperparasitise conspecifics over competitors or *vice versa*. Such responses may be based on behavioural preference (such as *E. tricolor* exploiting *E. inaron*) or ecological necessity (such as an autoparasitoid isolated from alternative male hosts and forced to exploit conspecific females for male production). The response of individual females to their environment can thus be viewed as a spectrum of ovipositional decisions based on the available choices. Although less aesthetically attractive; this is a more ecologically realistic way of considering these groups of heteronomous hyperparasitoids, rather than a strict obligate/facultative/allo classification. Indeed, the term "heteronomous hyperparasitoid" itself becomes of much more use if the autoparasitoid divisions break down to a continuum of female ovipositional preferences rather than a kind of strict phylogenetic classification.

9.4 Biological Control

Of the 860 successful establishments of parasitoids in biological control programs reviewed by Greathead (1986) the majority (185 or 22%) are aphelinids, followed by braconids (18%) and encyrtids (15%). The aphelinids have also given effective control much more frequently than any other parasitoid family.

The marked competitive ability of heteronomous hyperparasitoids in complexes of normally-reproducing primary parasitoids could be vitally important when considering their use in biological control. In some systems involving hyperparasitism, such as that of *Aphytis paramaculicornis* and *Coccophagoides utilis* on Olive Scale, one can be certain that no interactions are occurring at the secondary level (Huffaker, Kennett

and Tassan 1986). The introduction of *C. utilis* only increased competition for primary hosts. In other systems such as *Encarsia opulenta/E. clypealis/Eretmocerus serius/Amitus hesperidium* on the Citrus Blackfly, a series of introductions and competitive displacements initially bought about very effective control of the pest population. The final displacement of other species by the heteronomous hyperparasitoid, *E. opulenta* did not reduce the level of pest control (Summy et. al. 1983) indicating that this species had a higher degree of efficiency in the field as well as a high interspecific competitive ability.

The dynamics of such novel systems are almost completely unpredictable, although the end result, the attainment of a dominant position by a heteronomous hyperparasitoid, may actually be quite predictable. This could potentially be dangerous if a heteronomous hyperparasitoid displaces a more effective control agent through preferential hyperparasitism of the latter species. Nevertheless, the remarkable success of many heteronomous hyperparasitoids in biological control programs (shown in the discussion of chapter 7) suggests that generally, it may be better to introduce the autoparasitoid species into systems in which orthodox parasitoids are already effectively suppressing homopteran pests, despite the possible negative effects which male hyperparasitism may have on populations of the established primary parasitoid species.

Just how effective this group of parasitoids has been in biocontrol introductions is indicated by tables 7.1b and 7.2b in chapter 7. One should however, view the literature showing the frequency of heteronomous hyperparasitoid dominance in complexes containing orthodox parasitoids with a degree of caution. As well as the artificial nature of biocontrol programs in creating particularly efficient natural enemy systems, because heteronomous hyperparasitoids have been important in biocontrol and because their life histories are particularly interesting, then complexes in which they figure prominently may have been better described and so are more likely to offer the information necessary for inclusion in these tables. Also, as mentioned in chapter 7, the dominant position of a parasitoid in a complex resulting from a high interspecific competitive ability has no bearing on the capacity of the parasitoid to effectively control the pest population. Sub-dominant species with lower competitive abilities may potentially reduce host populations to a greater extent in the absence of a competitively superior rival. The *r*-selected *Encarsia perniciosi* in competition with *Aphytis* and *Comperiella* spp. described by Force (1974) and cited in chapter 7 is a good example. Theoretically, *E. perniciosi* was the most potent control agent but multiple introductions of highly competitive ectoparasitic species seem to have prevented *E. perniciosi* from realising its full potential.

The subject of multiple introductions in biological control has been modelled several times (Hassell and Varley 1969; May and Hassell 1981; Münster-Swendsen 1982; Kakehashi, Suzuki and Isawa 1984; Hassell 1986; May and Hassell 1988). Kakehashi et. al. (1984) addressed the problem of niche overlap between two parasitoid species and how this affects the predicted host populations. The assumption that the distribution of attacks by each parasitoid species was of critical importance in deciding whether to make a multiple or single species introduction. They showed that for two species with independent (segregated) niches, multiple introduction was always favoured, whereas if the niches overlapped completely, single introduction of the parasitoid with the greater area of discovery was always most effective. Single introduction was also favoured when the degree of niche overlap between the two species was greater than 30% (values below this were statistically independent). Hassell and Waage (1984) point out however, that in the real world, two species are unlikely to respond in exactly the same way to host cues and that more or less independent distributions of parasitism are probably more usual.

The high degree of success which heteronomous hyperparasitoids have achieved in biological control programs and the sub-economic levels at which they frequently maintain their homopteran hosts suggests that the area of discovery of these parasitoids is usually large. Therefore, a heteronomous hyperparasitoid in the context of the Kakehashi model, may be seen as "the best" parasitoid to introduce for many situations. A major disadvantage occurs however, when heteronomous hyperparasitoids are introduced alone: they are forced to derive all male production from conspecific females. This would inevitably result in a slowing of the initial population growth and an extended period before control of the pest population could be accomplished. Given this problem, it is intuitive that multiple introduction of a heteronomous hyperparasitoid together with a conventional primary endoparasitoid which could act as a male host during the important establishment period, could enhance the rate and possible effectiveness of control by the heteronomous hyperparasitoid. Whether or not the conventional parasitoid remained at viable population levels in the control program is of secondary importance, for unlike the Kakehashi model, this species does not exclude hosts from utilisation by the heteronomous hyperparasitoid. Parasitism by the conventional species simply changes the frequency distribution of male and female hosts for the heteronomous hyperparasitoid.

9.5 The Evolution of Heteronomous Hyperparasitism

The probable evolutionary sequence of the different types of heteronomous parasitoids according to Walter (1984) is as follows. Diphagous parasitoids initially evolved due to strong competition for primary hosts. For aphelinids, an ectoparasitic larva will almost always outcompete an endoparasitoid developing internally even if the latter species is in a much more advanced state of development (Flanders 1971). Thus, the development of males ectoparasitically can be seen as a method of reproduction when the probability of superparasitism of hosts is high.

In such a highly competitive environment with a shortage of primary hosts, it is not a large step for the males to become facultatively hyperparasitic: developing ectoparasitically on Hymenoptera within the same primary host. Indeed, the majority of heteronomous hyperparasitoids are ectoparasitic in their development (Walter 1984). Presumably the male suffers less in fitness terms from being hyperparasitic than would a conspecific female. Primary hosts with their greater store of resources continued to be utilised for female production (host size effects: see chapter 1).

Once male hyperparasitism had evolved, it would be difficult to return to the bisexual orthodox endoparasitoid, diphagous, or even perhaps to the facultatively hyperparasitic male state due to the highly competitive ability of heteronomous hyperparasitoids and the degree of success they appear to achieve in complexes of orthodox parasitoids. All other things being equal, a heteronomous hyperparasitoid in such a complex cannot do better than ***remain*** as such.

Walter (1983a) also suggested an alternative selection pressure leading to obligate male hyperparasitism. He postulated an isolation event in which a diphagous parasitoid found itself devoid of its usual hosts. Thus it became necessary to exploit a new primary host species. Walter imagined that this new host species provided cues which closely resembled those of the original host evoking a female egg-laying response. When parasitised however, the cues changed to those which induced a male egg-laying response from the parasitoid.

The selection pressures leading to *endo*parasitic male development and indirect heteronomous hyperparasitism are not tackled by Walter, who considered them "easy subsequent speciations" once ectoparasitic male hyperparasitoids had evolved.

Actually, major disadvantages of endoparasitism come from dealing with the host immune response and competition with secondary *ecto*parasitoids. So why be an *endo*parasitic hyperparasitoid? The only advantage to endoparasitism seems to exist in highly competitive environments when an endoparasitic species can oviposit in a particularly young hymenopteran larva. The egg then hatches but allows the host to develop further before the hyperparasitoid larva completes its development. A secondary *ecto*parasitoid larva could not synchronise its development with that of its hymenopteran host. Thus, only well developed secondary hosts would be worth attacking by an ectoparasitic hyperparasitoid. The secondary *endo*parasitoid can thereby attack a substantial proportion of the available parasitised hosts before they reach a stage suitable to the hyperparasitic *ecto*parasitoid. In this way the endoparasitoid devalues the host to a competing secondary ectoparasitoid causing it, in effect, to become a tertiary parasitoid with the decrement in fitness (loss of food resource) that comes with consumption at a higher trophic level.

Indirect heteronomous hyperparasitoids have two advantages over their direct counterparts:

1. Male larvae always develop ectoparasitically. They therefore ***all*** have a high intrinsic competitive ability.

2. They can lay males directly in/on parasitoid hosts when such hosts are available or, if secondary hosts are rare, they can deposit male eggs in primary hosts in anticipation of subsequent attack by a primary endoparasitoid.

It is not clear in what kind of situation either of these advantages may have been important in selecting for indirect hyperparasitism. The first advantage is shared by over half of the heteronomous hyperparasitoids listed by Walter (1983a). Therefore, the second point, or some factor relating to it, must be more important.

Imagine that secondary host are rare. Time spent searching for them will be very unprofitable compared to time spent exploiting primary hosts which could harbour eggs of both sexes. The fact that secondary hosts are rare however, is indicative of a low density of searching females and so re-discovery of the patch containing quiescent male eggs by another searching parasitoid would carry a low probability.

Alternatively, imagine that secondary hosts are available but competition for them is intense. Under such a scenario, indirect males stand a much improved chance of discovery by a primary endoparasitoid. Laying males in primary hosts which develop ectoparasitically sets a trap for subsequent visitors to the patch which relieves the indirect heteronomous hyperparasitoid from the competition occurring for secondary hosts. By remaining quiescent until the primary parasitoid has almost developed, the indirect hyperparasitoid will find itself automatically in the most advantageous situation to exploit its hymenopteran host.

A clue to the evolution of heterotrophic parasitism, in which males develop as primary endoparasitoids of lepidopterous eggs, comes from the classical observations of Beingolea (1959) who studied 2 species of *Encarsia* which he imaginatively called "A" and "B". The host relations for both species appeared to be facultative. Males of species "A" were regularly recorded emerging from eggs of the noctuid *Anomis texana* and occasionally as hyperparasitoids. Males of species "B" on the other hand, were frequently recorded from hymenopteran hosts and infrequently as primary endoparasitoids of lepidopterous eggs.

A similar situation exists with *Encarsia lutea*; recorded both as a heteronomous hyperparasitoid (Viggiani 1984; Gerling and Foltyn 1987) and as a heterotrophic parasitoid utilising *Trialeurodes* spp. for female production and eggs of the bollworm *Heliothis zea* and the Cabbage Looper, *Trichoplusia ni* for male production (Stoner and Butler 1965).

Walter (1983a) suggested that heterotrophic parasitism was an extreme development of heteronomous host relations in which parasitoids search for completely different types of host. Placing male and female eggs in different host species is not unknown in heteronomous hyperparasitoids. The example of *Coccophagus malthusi* was mentioned earlier. Female eggs of this species are laid in scales of the genus *Ceroplastes* whereas male eggs are laid in *Coccus* or *Saissetia* spp. Walter did not dwell on the selective pressures leading to heterotrophic parasitism, although both of the above cases clearly point to the origin of the habit as being hyperparasitic male development. Further support for this would come from finding that when heterotrophic parasitoids develop on Hymenoptera, they do so endo rather than ecto-parasitically. The step from being a secondary endoparasitoid to being a primary endoparasitoid of a different host would be simpler than a simultaneous change in host ***and*** method of development. Males of *Encarsia lutea* are endoparasitic (Gerling and Foltyn 1987).

Furthermore, the two types of host are likely to be found in the same, or similar habitats. For *Encarsia lutea* and the two species studied by Beingolea, both hosts were found in cotton fields. For *Encarsia porteri* many plants were sampled for several species of Lepidoptera and the highly polyphagous *Trialeurodes vaporariorum* (Rojas 1968). For the species of *Encarsia* mentioned by Flanders (1925) codling moth (*Carpocapsa pominella*) eggs were mainly found on the fruits of the Walnut whereas the diaspid scale *Aspidiotus juglansregiae* was found on the leaf petioles and young growth of the tree.

So why be heterotrophic? The reasons for the evolution of such host relations are not obvious. Judging from the often facultative nature of this type of development in several of the species studied to date, the move from heteronomous hyperparasitism to exploitation of lepidopterous eggs is clearly not as difficult or unlikely a task as it initially appears. Indeed, to a developing endoparasitoid, the two host types may be physiologically quite similar. The selective advantage to heterotrophic parasitism comes from two sources:

1. as with preferential hyperparasitism of non-conspecifics, it is a mechanism of avoiding hyperparasitism of one's own relatives

2. the exploitation of a resource enabling male production, which may at times, be less intensively parasitised (more freely available) than secondary hymenopteran hosts.

9.6 Conclusions

To summarise, a number of important adaptive consequences arise from heteronomous hyperparasitism which is a method of sex allocation seen only in a group of aphelinid parasitoids. Initially, I set out to answer the questions:

1. In what situations does being a heteronomous hyperparasitoid constitute a selective advantage over parasitoids that reproduce in the orthodox manner?

2. What factors are most important in determining heteronomous hyperparasitoid sex ratios?

3. What are the implications of 1 and 2 for population dynamics of systems containing a heteronomous hyperparasitoid?

The data in this thesis covered and gave insights into the answers to all three questions. To consider each question in turn...

1. The selective advantage to being heteronomous hyperparasitoid occurs when in competition with conventional parasitoids that develop in the normal manner. When competition for primary hosts is high, a normal endoparasitoid becomes restricted in its reproduction and may have to resort to superparasitism as it's only chance of producing offspring. Eggs laid in superparasitised hosts will clearly have a poorer chance of survival than when laid singly in previously undiscovered hosts. Heteronomous hyperparasitoids suffer no such reproductive restrictions. They can leave offspring in all types of patches whether they have been completely parasitised or whether they contain only parasitised hosts. The only limitation comes from the sex of offspring laid in each patch. Aphelinids are good fliers however, and dispersal from single-sex patches, to find mates is unlikely to be problematic.

There are also selective advantages for a heteronomous hyperparasitoid to attack a competitor rather than a conspecific female when laying a male egg. By preferentially attacking these secondary hosts for male production, an individual female ensures a greater number of local matings for her sons and avoids hyperparasitism of her own offspring. There are no advantages to obligate autoparasitism. Such behaviour should only be seen when a heteronomous hyperparasitoid has become temporally or geographically isolated from non-conspecific male hosts, or is physiologically incapable of developing at the expense of a competing endoparasitoid. Most heteronomous hyperparasitoids actually appear to be facultative autoparasitoids rather than strict obligate autoparasitoids or alloparasitoids.

2. The sex ratio of the facultative autoparasitoid *E. tricolor* given an abundance of both host types was determined by previous ovipositional experience. In two treatments an unbiased sex ratio was produced as predicted recently be Godfray and Waage (in press). When experienced with unparasitised hosts, the mean sex ratio became significantly female biased. When experienced with *E. inaron* pupae and offered *E. inaron* as male hosts, the mean sex ratio became significantly male biased. Reasons for these biases were proposed as the adaptive responses by the ovipositing female to the environment in which she initially found herself (her previous experience).

3. The implications for preferential hyperparasitism of competitors, when adopted by all the members of a species of heteronomous hyperparasitoid were clearly demonstrated by caged competition experiments. In all cases, virtually complete elimination of the conventional parasitoid was observed. A culture of *E. tricolor* proved uninvadable by the normal endoparasitoid, *E. inaron*. This high competitive ability was echoed in the dominant status of heteronomous hyperparasitoids recorded from complexes containing orthodox parasitoids that reproduce in the normal way.

Clearly, heteronomous hyperparasitism carries with it some disadvantages. If it did not, presumably this pattern of development would be a far more common phenomenon than, in fact, it is. The exploitation of previously undiscovered patches appears, to begin with, to be a major drawback. In these patches a female autoparasitoid can only lay female offspring. For a species which mates locally, the mother must either linger in the patch until her daughters have developed sufficiently to permit hyperparasitism, or she must rely on one of her daughters emerging and hyperparasitising sibling females to some extent. For those species with highly variable female development times, females emerging last of all can be mated by the males which were laid on their sisters. These males will, in effect, be their own "nephews". Further primary parasitism within the patch could then ensue from these newly mated females. *E. tricolor* however, does not show highly variable female development times and probably has little difficulty in dispersing to find mates. The whole process actually seems somewhat too tenuous to have been positively selected for. If it does occur, it is more likely to be as an incidental result of the unusual host relations of heteronomous hyperparasitoids.

In the absence of alternative male hosts, a heteronomous hyperparasitoid must practice autoparasitism of conspecifics and with high levels of autoparasitism, comes the increasing risk of hyperparasitism of one's own progeny. This will clearly reduce the overall production of offspring by individual females. For the species as a whole, the rate of population growth would also reduced as the level of autoparasitism increases. If alternative male hosts become available and autoparasitism falls, the rate of population growth should rise accordingly. The dynamics of these 3-species host-parasitoid-heteronomous hyperparasitoid systems should prove to be a particularly interesting future field of investigation both from the theoretical and empirical perspective. Hopefully, such studies could elucidate the true importance of the various factors revealed, to some extent, by this study.

CHAPTER 10

SUMMARY

1. The family Aphelinidae contains eight genera which show heteronomous host relationships. Females ***always*** develop as primary endoparasitoids of Homoptera. Male host relations differ from those of the female and can be divided into three categories: diphagous parasitoids, heteronomous hyperparasitoids and heterotrophic parasitoids. Males of heteronomous hyperparasitoids develop as hyperparasitoids of Homoptera parasitised by conspecifics or closely related primary parasitoids. *Encarsia tricolor* Förster is a heteronomous hyperparasitoid and occurs at low levels on a number of whitefly species in Britain.

2. The study set out to explore the adaptive consequences of heteronomous hyperparasitism; to discover in which situations heteronomous hyperparasitoids would have a selective advantage over conventional parasitoids, and the implications of this for the sex ratio and population dynamics of such species.

3. Initially, the biology of *E. tricolor* was investigated. Aphelinids such as *E. tricolor,* typically have a low fecundity and lay some 4-8 eggs per day in warm conditions. *E. tricolor* was able to exploit all host instars of both host types (parasitised and unparasitised) for reproduction, although larger whitefly instars were preferred for female eggs. Neither sex showed overtly variable development times. Oviposition of male eggs in conspecific female pupae took approximately three times longer than for female eggs in late instar whitefly.

4. *E. tricolor* showed a high species discrimination ability and a high preference to lay male eggs in *E. inaron* pupae over conspecifics. *E. inaron* was a conventional parasitoid in which both sexes develop as primary endoparasitoids. This behaviour may have two benefits:

a. it may increase the local mating opportunities for sons
b. it is a certain method of avoiding hyperparasitism of one's own progeny.

This discriminatory ability did not extend to kin recognition. A female *E. tricolor* did not avoid hyperparasitism of her own progeny when offered a choice of daughters or non-related conspecifics for male production.

5. When offered an abundance of parasitised and unparasitised hosts in different ratios, the mean sex ratio laid by *E. tricolor* was found not to be dependent on the availability of each type of host, but was changed by the previous ovipositional experience of the female wasp. For wasps with no experience, and for those with experience of laying male eggs in conspecifics, an unbiased sex ratio was produced. When offered unparasitised whitefly and *E. tricolor* pupae, a female biased sex ratio was laid after wasps had been experienced laying female eggs in whitefly hosts. When *E. inaron* pupae and unparasitised whitefly nymphs were offered together to *E. tricolor* with prior experience of laying male eggs in *E. inaron* pupae, a significantly male biased sex ratio was produced. Results from the first two treatments were in line with those predicted recently by Godfray and Waage (in press.) for a heteronomous hyperparasitoid limited in its reproduction by its egg supply. Adaptive reasons for the biased sex ratios of the other two treatments were proposed. These involved responses to local mate competition and changing reproductive success of the sexes.

6. Caged competition experiments between *E. tricolor* and *E. inaron* were run, and sampled at weekly intervals, to investigate the dynamics of competitive interactions between a heteronomous hyperparasitoid and an orthodox parasitoid wasp, like *E. inaron*. *E. tricolor* showed a remarkable competitive ability. Very small numbers of the heteronomous hyperparasitoid successfully invaded established populations of *E. inaron*. The reciprocal experiment was carried out: a caged population of *E. tricolor* proved completely resistant to invasion by small numbers of *E. inaron*.

7. Three field studies were done. The percentage parasitism by any species of parasitoid was always very low. The most significant findings were as follows.

a. *E. tricolor* occurs in patches with *E. inaron* significantly more often than expected given random occurrence of the two species i.e. *E. tricolor* appears attracted to patches of *E. inaron*, or vice versa.
b. Larger patches of *E. inaron* or conspecific *E. tricolor* were more likely to attract hyperparasitism than their smaller counterparts.
c. Male production in patches containing *E. inaron* was significantly higher than in patches containing only conspecifics.
d. Primary parasitism by *E. tricolor* was found to be inversely density dependent. Such a pattern of primary parasitism would be expected if a female laid her full complement of eggs in a patch and then left in search of new patches.

8. In the discussion, the classification of heteronomous hyperparasitoids described by Walter (1983a) was modified. It was proposed that his divisions into obligate/facultative autoparasitoid/alloparasitoid were in fact a result of ovipositional preferences and in the vast majority of cases of a ***facultative*** nature. Such flexibility broadens the ovipositional options and allows a more plastic response on the part of the parasitoid. Breaking down these divisions in turn overcomes some of the problems which arise when trying to explain the adaptive significance of obligate autoparasitism and obligate alloparasitism. The sequence of evolution of heteronomous hyperparasitoids was extended from that described by Walter (1984), although the lack of detailed studies on heteronomous hyperparasitoid biology and host relations made the proposed pathways speculative rather than analytical.

9. A literature search of references to parasitoid complexes containing a heteronomous hyperparasitoid and one or more conventional parasitoids revealed that in the majority of such systems, the heteronomous hyperparasitoid was dominant. This was true for both natural complexes and those created in biocontrol programs, suggesting that heteronomous hyperparasitoids are also highly competitive in the natural state. The benefits and risks associated with introducing a heteronomous hyperparasitoid in biological control programs were also addressed.

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APPENDIX

APHELINID FECUNDITIES FROM THE LITERATURE

Species	Temp (°C)	Mean lifetime fecundity(±SE)	Mean rate eggs/day (±SE)	Sex laid	Reference
Aphelinus flavus	?	48	?	M+F	Hamilton (1973)
Aphytis aonidiae	24.2	85.1	4.5	F	Gulmahmad and DeBach (1978)
A. chrysomphali	27	13	6-7	M+F	Rosen and DeBach (1978)
A. hispanicus	22.3	23.8	?	F	Gerson (1968)
A. holoxanthus	27	33.8	?	M+F	Rosen and DeBach (1978)
A. maculicornis	27	30	1.3	M+F	Rosen and DeBach (1978)
A. melinus	27	24	?	M+F	Rosen and DeBach (1978)
Coccophagoides utilis	20	26.4±3.67	?	F	Broodryk and Doutt (1966)
Coccophagus atratus	26	46(a)	?	M+F	Donaldson (1985)
C. bartletti	24	26.93(b)	?	M+F	Walter (1984)
Encarsia sp.	17	85.4±7.84(c)	4.27	F	Vet and van Lenteren (1981)
E. adrianae	25	70.88	4.66	F	Lopez Avila(1988)
E. deserti	25	57.8±4.74(d)	4.82	F	Gerling (1987)
	22-30	63.0±4.16(d)	5.25	F	Gerling (1988)

Species	Temp (°C)	Mean lifetime fecundity(±SE)	Mean rate eggs/day (±SE)	Sex laid	Reference
E. formosa(e)	15	15.58±1.05	0.85±0.04	F	Burnett (1949)
	15	75.8(i)	?	F	Kajita (1979)
	17	165.6±10.95(c)	8.3±0.35	F	Vet and van Lenteren (1981)
	18	28.2±2.43	1.68±0.14	F	Burnett (1949)
	18	69±13.28	3.3	F	Madueke (1979)
	18	223.0(i)	9.29	F	Christochowitz et. al. (1981)
	20	290	5.6	F	van Lenteren et. al. (1987)
	21	28.77±1.90	1.81±0.09	F	Burnett (1949)
	21-24	50-120	?	F	Agekyan (1981)
	22.5	160.2±17.09	11.2	F	Madueke (1979)
	25	59.5(i)	?	F	Kajita (1979)
	25	141.28	7.55	F	Lopez Avila(1988)
	25	442.2±21.9	12.01	F	Arakawa (1982)
	27	91.1(i)	7.99	F	Madueke (1979)
	30	23.0(i)	?	F	Kajita (1979)
	32	23.1(i)	?	F	Di-Pietro (1977) In Artigues *et al* (1987).
E.(Prospaltella) inquirenda	22.3	20.6	?	F	Gerson (1968)
E. lutea	27-32	28-50	?	?	Gameel (1969)
E. neomaskelliae	18.5	80-110	?	?	Prasad (1955)
E. partenopea	23-25	12	4.0	M+F	Mazzone (1983)

Species	Temp (°C)	Mean lifetime fecundity(±SE)	Mean rate eggs/day (±SE)	Sex laid	Reference
E. pergandiella	17	124.9±7.59(c)	6.24	F	Vet and van Lenteren (1981)
	24	49.4±8.35	4.99	F	Gerling (1966)
	24	45.5	?	M+F	Gerling (1966)
E. (Prospaltella) perniciosi	27	46	?	M+F	Rosen and DeBach (1978)
E. tricolor	7/18	10.92(f)	0.78±1.22	F	Christochowitz et. al. (1981)
	14	40.8(g)	2.04	F	Artigues et. al. (1987)
	18	90.6(g)	4.53	F	Artigues et. al. (1987)
	22	193	6.4	F	Castresana et. al. (1979)
	24	103.1	4.06	F	Artigues et. al. (1987)
	24	113.7(g)	5.68	F	Artigues et. al. (1987)
	25	85.36±13.85	7.31±0.27	F	This study
Eretmocerus sp.	17	149.9±21.87(c)	7.49	M+F	Vet and van Lenteren (1981)
Eret. mundus	14	18.4	1.30	M	Sharaf and Batta (1985)
	14	20.0	1.66	M+F	Sharaf and Batta (1985)
	18	14.5	1.91	M+F	Twafik et. al. (1979)
	25	26.3	3.17	M	Sharaf and Batta (1985)

Species	Temp (°C)	Mean lifetime fecundity(±SE)	Mean rate eggs/day (±SE)	Sex laid	Reference
	25	27.4	3.43	M+F	Sharaf and Batta (1985)
	27-32	42-63	?	?	Gameel (1969)
	30	48.0	4.57	M+F	Tawfik et. al. (1979)
	30	42.2	4.17	M	Tawfik et. al. (1979)
Eret. serius	?	200	20-28.6	M+F	Clausen (1978)
Physcus debachi	26	66(h)	4.71	?	Fisher (1961)
P. seminotus	24-26	79.8(j)	2.22	F	Williams (1972)
	24-26	98.7(j)	5.19	M+F	Williams (1972)
P. subflavus	24-26	62.3(j)	1.56	F	Williams (1972)

Notes:
(a) estimated from graph
(b) alternate access to hosts and honey
(c) length of experiment 20 days
(d) length of experiment 12 days
(e) thelytokous species
(f) alternating temperature regime, experiment lasted 14 days
(g) access to hosts 8h/day for 20 days
(h) wording ambiguous in reference
(i) in Artigues et. al. (1987)
(j) length of experiment 19 days