

How to behave?
Evolution of host-handling behaviour in
the whitefly parasitoid *Encarsia formosa*

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How to behave?
Evolution of host-handling behaviour in
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Stellingen Propositions

- 1 Elk model, van Nicholson-Bailey tot Claudia Schiffer, is een versimpeling van de werkelijkheid.
Every model, from Nicholson-Bailey to Claudia Schiffer, is a simplification of reality.
- 2 De kunst is kort, het leven lang (contra *Ars longa, vita brevis*).
Art is short, life is long (versus Ars longa, vita brevis).
Schopf, J.W. (ed.), 2002. *Life's origin: the beginnings of biological evolution*. University of California Press.
Klein, R.G. & B. Edgar, 2002. *The dawn of human culture*. John Wiley & Sons.
- 3 Wat 'Moneymaker' is voor tomatentelers, is nieuwsgierigheid voor de wetenschap.
What 'Moneymaker' is to tomato growers, is curiosity to science.
- 4 Veldgegevens maken het verschil tussen bezigheids therapie en betekenisvolle theorie.
Field data make the difference between occupational therapy and sensible theory.
- 5 Het hiernamaals is een placebo.
The hereafter is a placebo.
- 6 Pas als de lusten zijn bevredigd is het tijd voor beschaving.
Only after lust has been satisfied it is time for civilisation.
Martin Bril, de Volkskrant 30 juli 2002

- 7 Tevredenheid is de abortus van het ongeboren idee.
Content is the abortion of the unborn idea.
- 8 De keerzijde van een ideaal is altijd eerezucht.
The seamy side of an ideal is always ambition.
Maarten 't Hart, *Een vlucht regenwulpen* (1978)
- 9 De eerste cadens in het derde pianoconcert van Rachmaninov is een voorbeeld van zinvol geweld.
The first cadence in the third piano concerto by Rachmaninov is an example of meaningful violence.
- 10 Oranje onder!
Down with the Crown!
- 11 Het geven van een kadotip is erger dan het krijgen van goedbedoelde rotzooi.
To give a gift tip is worse than to get well-meant trash.

Stellingen behorend bij het proefschrift van Joep Burger: "How to behave? Evolution of host-handling behaviour in the whitefly parasitoid *Encarsia formosa*."
Wageningen, 10 december 2002

Voor “de wilde spinnen”

Contents

| | | |
|---------------------------------------|--|-----|
| Abstract | | 9 |
| Chapter 1 | General introduction | 11 |
| Chapter 2 | Host feeding in insect parasitoids: why destructively feed upon a host that excretes an alternative? <i>J.M.S. Burger, T.M. Reijnen, J.C. van Lenteren & L.E.M. Vet</i> | 23 |
| Chapter 3 | An evolutionary advantage of destructive host feeding over non-destructive feeding on honeydew <i>J.M.S. Burger, A. Kormany, J.C. van Lenteren & L.E.M. Vet</i> | 41 |
| Chapter 4 | Natural history of whitefly in Costa Rica: an evolutionary starting point <i>J.M.S. Burger, G. Gort, J.C. van Lenteren & L.E.M. Vet</i> | 53 |
| Chapter 5 | Reproduction now or in the future: optimal host-handling strategies in the whitefly parasitoid <i>Encarsia formosa</i> <i>J.M.S. Burger, L. Hemerik, J.C. van Lenteren & L.E.M. Vet</i> | 81 |
| Chapter 6 | Effect of host distribution and parasitoid experience on foraging decisions of <i>Encarsia formosa</i> <i>J.M.S. Burger, Y. Huang, L. Hemerik, J.C. van Lenteren & L.E.M. Vet</i> | 119 |
| Chapter 7 | General discussion | 137 |
| Appendix | Natural life expectancy and the value of future reproduction | 145 |
| Bibliography | | 157 |
| Nederlandse inleiding en samenvatting | | 177 |
| Dankwoord | | 191 |
| Curriculum vitae | | 193 |
| List of publications | | 195 |

Abstract

The main aim of evolutionary ecology is to explain the adaptation of form, function and behaviour of organisms to their environment. In this thesis, I studied host-handling behaviour of the whitefly parasitoid *Encarsia formosa* from such an evolutionary point of view. This parasitoid is applied as biological control agent against whitefly pests in greenhouses. Previous studies revealed mechanistic explanations for the parasitoid's behaviour. Here I focussed on functional explanations for the parasitoid's decision to reject, feed upon or parasitise a host. Since host feeding yields nutrients for egg maturation but destroys an opportunity to oviposit, the decision between host feeding and oviposition reflects a life-history trade-off between current and future reproduction.

First, manipulation experiments were conducted to seek for evolutionary benefits of destructive host feeding over non-destructive feeding on host-derived honeydew. The first experiments suggested that honeydew could be an advantageous alternative to host feeding. Honeydew did have a positive effect on egg load and estimated number of eggs matured within 20 and 48 hours, whereas a host feeding did not. Host feeding did not positively affect survival when honeydew was supplied. In the second experiments the observation period was extended. Even in the presence of honeydew, host feeding did have a positive effect on the number of ovipositions per hour of foraging per host-feeding attempt, without affecting parasitoid survival or egg volume.

To understand the conditions under which *E. formosa* has evolved, fieldwork was carried out in Costa Rica to quantify natural whitefly densities and distributions. The number of hosts on the lower side of a leaflet of an average plant within an average spot along an average transect could be described by a Poisson distribution with mean and variance equal to 0.241. This mean was most variable at plant level. Spatial dependence between numbers of whiteflies on leaves was detected within individual plants and within a 100-m transect. Thus, host density in the field was low compared with pest densities, but aggregation occurred at several spatial scales.

A dynamic state-variable model was developed to predict optimal host-handling decisions, which maximise lifetime reproductive success, in relation to host density and parasitoid's life expectancy. Random decisions resulted in only 35 to 60 % of the lifetime reproductive success from optimal decisions. Host feeding was predicted to be maladaptive at presumable field conditions of low average host density and short parasitoid's life expectancy. Nutrients from the immature stage should be sufficient to prevent egg limitation. Both host density and parasitoid's life expectancy had a positive effect on the optimal host-feeding ratio. Explaining evolution of host-feeding behaviour

under natural conditions may require incorporation of variation in host density, incorporation of parasitised host types or field data showing that parasitoid's life expectancy in the field is longer than assumed.

In a semi-field set-up, parasitoids were allowed to forage at field host density in either a uniform or an aggregated host distribution. Contrary to predictions by the optimal-foraging model, parasitoids did host feed, i.e. upon about 11 % of the accepted hosts, even when host aggregation was ignored. In the model, host encounter rate was underestimated from host density. Nevertheless, there was also a host distribution by time interaction on host-feeding tendency. Host encounter decreased the leaving tendency on an average leaflet when time since latest host encounter was short, but increased the leaving tendency when time since latest host encounter was long, independent of host distribution. This suggested that parasitoids can forage efficiently at different host distributions.

The value of future reproduction and the decision whether to host feed or parasitise strongly depends on the parasitoid's life expectancy. I hypothesised that a parasitoid's life expectancy in the laboratory is an overestimate of a parasitoid's life expectancy in the field. Several age determination methods were tested, using pteridine, wing fray and relative residual longevity. Unfortunately, none proved a useful technique to estimate the age of a field-caught specimen.

The main conclusion is that host-handling behaviour in the whitefly parasitoid *Encarsia formosa* may have evolved as an adaptation to a spatially and possibly temporally heterogeneous environment. To truly understand the evolution of host-handling behaviour, future studies should focus on physiological, biochemical and genetic mechanisms; the effect of spatial and temporal heterogeneity in host availability on the risk of egg limitation; and the parasitoid's life expectancy in the field.

1

General introduction

Prologue: the Last Judgement and evolution by natural selection

"Shh! Silence please!" The hubbub dies down. Not for long, however, because the visitors cannot keep their excitement for themselves. Soon the whispering crescents back to unorthodox levels until the attendant urges people again to behave respectfully. Respectfully because they are standing in the Sistine Chapel in Rome, looking at one of the masterpieces of the Renaissance that Michelangelo frescoed on the altar wall between 1537 and 1541. It depicts the Last Judgement, where a beardless and muscled Christ decides at the end of time whether people's behaviour has been good or bad. Good people go to heaven, bad people to hell. At the lower centre of the painting, angelic trumpeters show books of good and evil deeds on how to behave if not to be doomed forever.

This thesis is about how to behave. Not from a Christian point of view, however, but from an evolutionary one. The main aim of evolutionary biology is to explain the adaptation of form, function and behaviour of organisms to their environment. The central concept in evolutionary biology was published by Darwin (1859) in *The origin of species*. The first point in the argument is that living organisms reproduce and will infinitely increase in number if not limited by resources (e.g. energy, time, nutrients). Second, reproducing organisms produce offspring that resemble their parents because at least some characteristics are heritable. Mechanisms of heredity are for example genes or nurture. Third, organisms vary in characteristics some of which affect survival and reproduction, i.e. they vary in fitness. Darwin argued that if organisms have to compete for limited resources, organisms with heritable characteristics most favourable for survival and reproduction will have most offspring and the offspring will also have these favourable characteristics by heredity. As a result, the frequency of characteristics changes (evolves) between generations within a population of a species by natural selection. Darwin's theory predicts that organisms will be replaced by organisms with

characteristics that make them better able to survive and reproduce in their environment. It can therefore explain adaptation of organisms to their environment (within phylogenetic, physical, genetic, physiological and ecological limits).

The Last Judgement illustrates some principles of evolutionary biology of behaviour. The mortal beings may be the organisms that vary in behaviour. Christ can be seen as natural selection that favours one type of behaviour over another. The books of good and evil deeds symbolise the environmental conditions that determine the relative fitness value of the different types of behaviour. Heaven depicts the environment where the fittest organisms survive and reproduce. An important difference between the Christian and evolutionary point of view on how to behave is that the Last Judgement takes place at the end of times, whereas evolution is a continuous process because selection pressures change with changing physical or biological environments.

Life in time and space

We have developed from the geocentric cosmologies of Ptolemy and his forebears, through the heliocentric cosmology of Copernicus and Galileo, to the modern picture in which the earth is a medium-sized planet orbiting around an average star in the outer suburbs of an ordinary spiral galaxy, which is itself only one of about a million million galaxies in the observable universe.

Hawking (1996)

The aim of science is to understand the universe in time and space. Biology is the science of life. Exobiology studies the origin, evolution and distribution of life in the universe (e.g. Brack *et al.* 2001; McKay 1997). Most biologists focus on the earth, where life is apparent, can be studied most easily and provides inside in our own (human) origin. Life as we know it is the product of a long evolutionary history. The age of the universe is about ten or twenty thousand million years (the time it takes to travel from Wageningen to Rome at a velocity of 0.1 mm per year!); our sun and the planets orbiting it formed some five thousand million years ago (Hawking 1996). The earliest direct evidence of life on earth comes from 3465 ± 5 million-year-old microbial fossils found in the Pilbara region of north-western Australia (Schopf 1993). They resemble cyanobacteria, photosynthetic prokaryotes that can still be found nowadays in habitats ranging from hot springs to hot and cold deserts (Whitton & Potts 2000). The fossils are already cellular and suggest that life has originated substantially earlier (Schopf 1994; Lazcano & Miller 1994).

The first detailed and testable hypothesis on the origin of life was published by Oparin (1953; first English edition in 1938). He proposed that life emerged from a prebiotic soup of organic compounds that were synthesised in a reducing atmosphere of methane (CH_4), ammonia (NH_3), water (H_2O) and hydrogen (H_2). Miller (1953) circulated these substances past an electrical discharge and identified several amino acids after a week. Abiotic synthesis of carbon-based molecular building blocks of organisms (amino acids, sugars, fatty acids and nucleotides) has received considerable

attention ever since (reviewed by Miller 1992). Alternative sources of organic compounds to energy sources like electrical discharges are delivery by asteroid and comet impacts or interplanetary dust particles, and synthesis from impact shocks (reviewed by Chyba & Sagan 1992; Cooper *et al.* 2001). The dominant source depends strongly upon the composition of the early atmosphere (Chyba & Sagan 1992), which is still under dispute.

Given for instance high concentrations of inorganic polyphosphates, the organic monomers can join to form polymers like polynucleotides and polypeptides (Cavalier-Smith 2001), which are able to act as a template for their complement. Such capacity of self-reproduction is one of the main properties of life (see e.g. Luisi 1998 for a discussion about definitions of life). Once replicating molecules originated, evolution by natural selection was an inevitable consequence resulting in an increase of molecular complexity and organisation such as in cellular organisms.

Life-history theory

Organisms that we observe today are thus the result of a long evolutionary history. Natural selection is believed to be the main mechanism that has shaped an organism's form, function and behaviour, although gene frequencies or traits can also change through chance (non-selective pressures like predators, catastrophes or random genetic drift in founder populations; see e.g. Dobzhansky & Pavlovsky 1957; Mayr 1963), or sexual selection (Andersson 1994). Life-history theory (Stearns 1992; Roff 1992) aims to explain the interspecific and intraspecific variation in life-history traits, such as growth rate; age and size at maturity; number, size and sex ratio of offspring; and longevity. The optimal value of a life-history trait is the value that maximises lifetime fitness and will be favoured by natural selection, where fitness is defined as the contribution of an individual to the gene pool in the next generation (Daan & Tinbergen 1997). Resources (e.g. energy, time, nutrients) invested into a trait that increases fitness, however, cannot be invested into another trait that also increases fitness. When resources are limiting, this results in a physiological trade-off. For instance, in field grasshoppers there is a negative correlation between clutch size and egg weight (Stearns 1992). In addition, changing the value of a life-history trait that increases fitness may change the value of another life-history trait that decreases fitness. This results in a microevolutionary trade-off. For instance, sexual activity reduces life span in male fruitflies (Partridge & Farquhar 1981). This thesis deals with the microevolutionary trade-off between current and future reproduction.

Modelling

Mathematical modelling is a technique that can help to explain and predict natural phenomena. Optimisation models enable us to calculate what the optimal form, function and behaviour of an organism is under certain constraints and how the organism should deal with trade-offs, given that natural selection leads to the survival of the fittest. The

four essential components of an optimisation model are an optimisation currency ("What is optimised?"), a strategy set ("What is the range of decisions?"), a state space ("What internal factors affect the decision?"), and a set of constraints ("What factors prevent perfect adaptation?") (Oster & Wilson 1978). For example, imagine your bank account. What should you do to increase your balance: save your money at the bank to yield a certain interest or invest it in stocks and shares to make an uncertain but possibly higher profit? Your balance would be the optimisation currency. The strategy set consists of saving and investing. The state space could consist of the initial balance and your dependence on the balance for subsistence. Constraints that prevent you from becoming infinitely rich are the interest rate and your mortality.

Although lifetime fitness is the ultimate optimisation currency, most biologists resort to indirect measures or proxies such as body size, fecundity or longevity (Roitberg *et al.* 2001). Classical foraging theory usually optimises the average rate of net energy gain (Stephens & Krebs 1986). Dynamic state variable modelling (Mangel & Clark 1988; Houston & McNamara 1999; Clark & Mangel 2000) includes state dynamics as a fifth component of an optimisation model. Dynamic state variable models are more flexible than rate-maximising models, and use expected lifetime reproductive success as an optimisation currency, which is closely related to phenotypic fitness. Compared with static optimisation models more quantitative and testable predictions can be made. I used this technique to model the microevolutionary trade-off between current and future reproduction.

Insect parasitoids

Insect parasitoids have proven an ideal model system to study behavioural and evolutionary ecology and their biology provides valuable insight into many aspects of natural selection and adaptation (Godfray 1994; Price 1997). The adult female possesses an ovipositor that is used to deposit one or more eggs on or in certain stages of another arthropod, its host. The parasitoid larva develops by destructively feeding on the body of its host. Behavioural decisions by an adult female parasitoid to find and parasitise a host therefore have a direct impact on her reproductive output and are under strong selection. Godfray (1994) thoroughly reviews theoretical and empirical work on behavioural decisions that insect parasitoids are confronted with during the foraging process. (It should be noted that throughout this thesis "foraging" includes searching for both food and hosts.) The foraging process has been divided into habitat location, host location and host acceptance. Behavioural decisions that are made during foraging include the decision to search for food or hosts, where to forage, how long to stay, to reject or accept an encountered host, to feed upon or (super-) parasitise an accepted host, how many eggs to lay, and what sex ratio to produce. The microevolutionary trade-off between current and future reproduction is reflected by the decision to parasitise or feed upon a host.

Host-handling strategies

This thesis focuses on the decision how to handle a host once it has been encountered. A parasitoid can reject, feed upon or parasitise an encountered host. This decision can be divided into the decision to reject or accept an encountered host, and the decision to feed upon or parasitise an accepted host. The classical optimal-diet model predicts that predators (or parasitoids) should reject a prey (or host) when its profitability (fitness gain divided by handling time) falls below a certain threshold, independent of the encounter rate with the low-profitability prey (Krebs & McCleery 1984). Other factors that can affect the decision to reject an unparasitised host include for example host-type-dependent mortality risks for the ovipositing parasitoid through host defence or predator attack, egg limitation, host recognition time, and life expectancy of the ovipositing parasitoid (Stephens & Krebs 1986; Godfray 1994). Rejection is thus an important decision in the strategy set when studying host-handling behaviour.

The decision to feed upon or parasitise an accepted host reflects the trade-off between current and future reproduction. Host feeding is the consumption of host hemolymph and body tissue by the adult female parasitoid. Jervis & Kidd (1986) reviewed the taxonomic distribution of host-feeding behaviour in Hymenoptera, discussed the physiology of host feeding, and constructed some analytical and simulation models to explore the effect of host density on the decision between host feeding and oviposition. They found records of host-feeding behaviour in more than 140 hymenopteran parasitoid species belonging to 17 families. The most common type of host-feeding behaviour is non-concurrent (different hosts are used for host feeding and oviposition) and destructive (host feeding kills the host). After emergence, some species cannot oviposit without host feeding first (anautogeny), as opposed to autogenous species that can, using reserves from the larval stage. Flanders (1950) distinguished proovigenic species, whose females mature all eggs before emergence, from synovigenic species, whose females mature most eggs during adult life and depend thereby on adult nutrition, especially host-feeding. Jervis *et al.* (2001) showed that there is a continuum in the fraction of the maximum potential lifetime egg complement that is mature upon emergence.

Heimpel & Collier (1996) expanded on the work by Jervis & Kidd (1986) by updating their review on the physiology of host feeding, considering the costs and benefits of host feeding, and reviewing the theoretical predictions and empirical evidence concerning host-feeding strategies. Rosenheim & Heimpel (1994) also reviewed several sources of inter- and intraspecific variation in host-feeding behaviour of *Aphytis* parasitoids (Hymenoptera, Aphelinidae). Host-feeding meals are rich in protein, vitamins and salts, in contrast to the non-host food source nectar. The nutrients obtained are often essential to mature eggs. Using radioactive labelling, Rivero & Casas (1999b) showed that the host-feeding gain is stored and used gradually throughout the parasitoid's life. In some species, host feeding also increases longevity, although non-host food sources are generally more effective in promoting longevity. Eggs matured from the host-feeding gain can also be reconverted by oosorption (Bell & Bohm 1975), which may prolong longevity. Although the parasitoid can increase fecundity through

Table 1 Review of factors affecting the tendency to host feed, relative to the tendency to oviposit (after Rosenheim & Heimpel 1994 and Heimpel & Collier 1996).

| Factor | Predicted effect | References | Empirical support (<i>parasitoid species</i>) | Empirical contradiction (<i>parasitoid species</i>) |
|--|-----------------------|--|---|--|
| Internal biotic | | | | |
| Genetic variation | | | | |
| Egg load | negative | Chan 1991; Chan & Godfrey 1993; Collier <i>et al.</i> 1994; Heimpel <i>et al.</i> 1994, 1998; Collier 1995b; McGregor 1997 | Chan 1991 (<i>Encarsia formosa</i>); Collier <i>et al.</i> 1994 (<i>Aphytis melinus</i>); Heimpel <i>et al.</i> 1994, 1996 (<i>Aphytis aonidae</i>); Heimpel & Rosenheim 1995 (<i>Aphytis melinus</i>); Ueno 1999a (<i>Agrothereutes lanceolatus</i>) | Rosenheim & Rosen 1992 (<i>Aphytis lingnanensis</i>) |
| Energy level | negative | Jervis & Kidd 1986; Chan 1991; Houston <i>et al.</i> 1992; Chan & Godfrey 1993; Collier <i>et al.</i> 1994; Heimpel <i>et al.</i> 1994, 1998; Collier 1995b; McGregor 1997 | Gulmahamad & DeBach 1978 (<i>Aphytis aonidae</i>); Heimpel & Rosenheim 1995 (<i>Aphytis melinus</i>) | |
| Host-feeding gain | positive | Chan 1991; Collier <i>et al.</i> 1994; Collier 1995b | | |
| Time delay between host feeding and egg maturation | positive | Collier 1995b | | |
| Egg resorption | negative | Collier 1995b | | |
| Parasitoid age ¹ | negative | Chan & Godfrey 1993; Heimpel <i>et al.</i> 1994; McGregor 1997 | Gulmahamad & DeBach 1978 (<i>Aphytis aonidae</i>); Lauzière <i>et al.</i> 1999 (<i>Cephalonomia stephanoderis</i>) | Heimpel <i>et al.</i> 1996 (<i>Aphytis aonidae</i>) |
| Life expectancy | positive | Chan 1991; Houston <i>et al.</i> 1992; Chan & Godfrey 1993; Collier <i>et al.</i> 1994; Collier 1995b; Heimpel <i>et al.</i> 1998 | | Heimpel & Rosenheim 1995 (<i>Aphytis melinus</i>) |
| Handling time | small ² | Chan 1991 | | |
| Previous host encounter | complex ³ | | Chan 1991 (<i>Encarsia formosa</i>); Heimpel & Rosenheim 1995 (<i>Aphytis melinus</i>); Nurindah <i>et al.</i> 1999 (<i>Trichogramma australicum</i>) | |
| External biotic | | | | |
| Host quality | negative | Chan 1991; Kidd & Jervis 1991a; McGregor 1997; Heimpel <i>et al.</i> 1998 | Flanders 1953 (<i>Aphytis chrysomphali</i>); Quechua 1964 (<i>Aphytis lingnanensis</i>); Abdelraouan 1974 (<i>Aphytis melinus</i>); Nell <i>et al.</i> 1976 (<i>Encarsia formosa</i>); Nichols & Tailor 1977 (<i>Encarsia formosa</i>); Arakawa 1982 (<i>Encarsia formosa</i>); Fransen & van Montfort 1987 (<i>Encarsia formosa</i>); Lohr <i>et al.</i> 1988 (<i>Epidinocarsis lopezi</i>); Walde <i>et al.</i> 1989 (<i>Aphytis melinus</i>); Heinz & Parrella 1989 (<i>Diglyphus begini</i>); review Kidd & Jervis 1991a; Chan 1991 (<i>Encarsia formosa</i>); Rosenheim & Rosen 1992 (<i>Aphytis lingnanensis</i>); Enkegaard 1993 (<i>Encarsia formosa</i>); Bokanon <i>et al.</i> 1995 (<i>Anagyrus mangicola</i>); Heimpel & Rosenheim 1995 (<i>Aphytis melinus</i>); McAulane & Nguyen 1996 (<i>Eretmocerus sp.</i>); Heimpel <i>et al.</i> 1996 (<i>Aphytis aonidae</i>); Videll <i>et al.</i> 1997 (<i>Encarsia pergandiella</i>); Ueno 2000 (<i>Agrothereutes lanceolatus</i>); Tran & Takasu 2000 (<i>Diadromus subilicornis</i>); Tran & Takasu 2000 (<i>Diadromus subilicornis</i>); Lauzière <i>et al.</i> 2001 (<i>Cephalonomia stephanoderis</i>); Nakamura & Noda 2001 (<i>Oomyzus sokolowskii</i>); Choi <i>et al.</i> 2001 (<i>Anisopteromathus calandrarum</i>) | van Alphen <i>et al.</i> 1976 (<i>Encarsia formosa</i>); Reeve 1987 (<i>Aphytis melinus</i>) |
| Host availability | negative except below | Jervis & Kidd 1986; Chan & Godfrey 1993; Collier 1995b | DeBach 1943 (<i>Metaphycus helvolus</i>); Bartlett 1964 (<i>Microterys flavus</i>); Sandlan 1979 (<i>Coccysominus turionellae</i>); Collins <i>et al.</i> 1981 (<i>Aphelinus thomsoni</i>); Yano 1987 (<i>Encarsia formosa</i>); Lohr <i>et al.</i> 1988 (<i>Epidinocarsis lopezi</i>); Bai & | |

| | | | |
|--------------------------------|---|--|--|
| critical level ¹ | | | Mackauer 1990 (<i>Aphelinus asychis</i>); Barrett & Brunner 1990 (<i>Prigallo flavipes</i>); Sahragard <i>et al.</i> 1991 (<i>Dicondylus indianus</i>); Avidov <i>et al.</i> 1970 reanalysed by Rosenheim & Heimpel 1994 (<i>Aphytis coheni</i>); Reeve & Murdoch 1985 (<i>Aphytis melinus</i>); Lauzière <i>et al.</i> 1999 (<i>Cephalonomia stephanoderis</i>); Gulnabad & DeBach 1978 (<i>Aphytis aonidiace</i>); Reeve & Murdoch 1985 (<i>Aphytis melinus</i>); Choi <i>et al.</i> 2001 (<i>Anisopteromalus calandrine</i>); DeBach 1943 (<i>Metaphycus helvolus</i>); Gulnabad & DeBach 1978 (<i>Aphytis aonidiace</i>); Yano 1987 (<i>Encarsia formosa</i>); Ueno 1998 (<i>Ioplectis naranyae</i>); Collier & Hunter 2001 (<i>Encarsia sophia</i>); Collier <i>et al.</i> 2002 (<i>Encarsia formosa</i>); Yu <i>et al.</i> 1990 (<i>Encarsia perniciosi</i> and <i>Aphytis melinus</i>); Ueno 1999c (<i>Pimpla nipponica</i> and <i>Ioplectis naranyae</i>); Collier <i>et al.</i> 2002 (<i>Encarsia formosa</i> and <i>E. pergandiella</i>); Urbaneja <i>et al.</i> 2001 (<i>Cirrospilus</i> sp. near <i>lynceus</i>) |
| positive | Collier <i>et al.</i> 1994; Heimpel <i>et al.</i> 1994; McGregor 1997 | | |
| absent | Heimpel <i>et al.</i> 1998 | | |
| positive | Houston <i>et al.</i> 1992 | | |
| positive | | | |
| Variance in time to find hosts | | | |
| Intraspecific competition | | | |
| Interspecific competition | | | |
| positive | | | |
| domed | | | |
| Abiotic | | | |
| Temperature | | | |
| Relative humidity | | | |
| Light | | | |

¹ The absolute number of host feedings can increase (Fisher 1952 according to Rosenheim & Heimpel 1994; Lauzière *et al.* 1999), remain constant (Quednau 1967; Sugimoto & Ishii 1979), decrease (Quednau 1964; Gulnabad & DeBach 1978; Arakawa 1982; Ridgeway & Mahr 1990) or peak (Videll *et al.* 1997) with age.

² The effect of increasing handling time is that hosts of increasingly high quality are rejected.

³ There is a positive effect of previous rejection, a negative effect of previous host feeding and no effect (Chan 1991) or a negative effect (Nurindah *et al.* 1999) of previous oviposition on the current decision to host feed.

⁴ The absolute number of host feedings can be density independent (Reeve & Murdoch 1985; Auerbach 1991) or positively density dependent (Avidov *et al.* 1970; Sahragard *et al.* 1991). All models predict an initial increase in host-feeding tendency at very low host availability. This has not been shown experimentally.

⁵ Interspecific competition had a positive effect when time intervals between attacks were long; multiparasitism occurred when time intervals were short.

host feeding, destructive host feeding results in the loss of an oviposition site and non-destructive host feeding possibly in a reduction of host quality for offspring development. Another cost of host feeding is an increased handling time, which decreases searching time and may increase the risk of predator attack. The decision between oviposition and host feeding therefore reflects a trade-off between current and future reproduction (Heimpel & Rosenheim 1995). Parasitoids realise current reproduction through oviposition but synovigenic species (with at least some immature eggs upon female emergence; Jervis *et al.* 2001) can increase lifetime reproductive output by investing in future reproduction through host feeding. Table 1 summarises theoretical and empirical studies on the effect of several factors on the tendency to host feed (discussed in Rosenheim & Heimpel 1994 and Heimpel & Collier 1996). Furthermore, host feeding can have a stabilising effect (Yano 1987; Yamamura & Yano 1988; Krivan 1997; Murdoch *et al.* 1997), no effect (Kidd & Jervis 1989, 1991b; Briggs *et al.* 1995; Krivan 1997; Murdoch *et al.* 1997) or destabilising effect (Burnett 1967) on host-parasitoid population dynamics (short review by Mills & Getz 1996).

Biological control and the whitefly parasitoid *Encarsia formosa*

Parasitoids are defined by the destructive feeding behaviour of the *larval* stage (Godfray 1994). In destructive host-feeding species (Jervis & Kidd 1986), also the *adult* female parasitoid destructively feeds upon the host, in which capacity it is defined as a predator. The destructive feeding habit of parasitoids is used to apply parasitoids as biological control agents against pest insects. The larval parasitoid *Encarsia formosa* Gahan (Hymenoptera, Aphelinidae) is a famous example of a successful biological control agent against whiteflies (Homoptera, Aleyrodidae) in greenhouse crops (van Lenteren & Woets 1988; van Lenteren *et al.* 1997). Around 1450 species of whiteflies have been named (Martin *et al.* 2000), of which some tens may be considered potential pests (Gerling 1990). Pest species cause crop damage by phloem feeding, honeydew production and virus transmission (Vet *et al.* 1980). In 1926, a crop grower noticed black nymphs of the normally white nymphs of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) on *Cassia tomentosa* (Caesalpiniaceae) in a greenhouse in Britain (Speyer 1927). The parasitoids emerging from similar black nymphs on tomato plants (Solanaceae, *Solanum lycopersicum*) in the same greenhouse were identified as *Encarsia formosa* by Gahan, who had then recently described the species from specimens collected in Idaho, USA (Gahan 1924). Within a few years, *E. formosa* was commercially exploited. After World War II, crop protection became strongly chemically oriented in the 1950s, but interest in natural enemies revived after development of resistance against pesticides (van Lenteren 2000).

Although Speyer (1927) described some biology and life history of *E. formosa*, schemes on how and when to introduce the natural enemy were initially found by trial and error. Vet *et al.* (1980) reviewed some introduction methods that were developed in the 1970s. To develop sustainable pest management and more reliable introduction schemes, research became more fundamentally oriented on the biology and life history

of *E. formosa* (reviews by Viggiani 1984; Noldus & van Lenteren 1990; van Roermund & van Lenteren 1992b; Hoddle *et al.* 1998) and whitefly (reviews by Gerling 1990; Byrne & Bellows 1991; van Roermund & van Lenteren 1992a; Gerling & Mayer 1996).

In the 1980s, a long-term project was started that aimed at obtaining a quantitative understanding of the tritrophic system of crop, whitefly and *E. formosa* in order to explain success or failure of biological control (van Lenteren & van Roermund 1999). Biological control of greenhouse whitefly with *E. formosa* was very reliable in tomato, sweet pepper and gherkin, but not in egg plant and cucumber (van Lenteren *et al.* 1977; Woets & van Lenteren 1976). Van Roermund (1995) filled important gaps in the extended knowledge of the foraging behaviour of *E. formosa* (van Roermund & van Lenteren 1995a,b; van Roermund *et al.* 1994), reviewed life-history parameters of both greenhouse whitefly and *E. formosa* (van Roermund & van Lenteren 1992a,b), and incorporated the detailed studies into simulation models to understand the main causal factors determining the success or failure of biological control of whitefly pests in greenhouses with *E. formosa* (van Roermund *et al.* 1996, 1997a,b,c).

Aim and outline

The mechanistic models by van Roermund *et al.* (1996, 1997a,b,c) explain how *E. formosa* parasitoids realise the observed level of parasitism in greenhouses in terms of searching efficiency, host handling and available eggs. They do not explain, however, why this behaviour has evolved in evolutionary terms of selection pressures acting upon behaviour. *E. formosa*'s foraging behaviour under natural conditions has never been studied, nor the question under which conditions this behaviour is adaptive. Functional explanations for the parasitoid's behaviour can improve population dynamical models that aim to understand parasitoid-host interactions. Because both host feeding and oviposition behaviour result in whitefly mortality but only oviposition results in direct parasitoid offspring, host-handling decisions can have important implications for population dynamics and the final outcome of biological control. This thesis therefore deals with the functional explanation of *E. formosa*'s host-handling behaviour, expanding on the mechanistic explanations given by van Roermund (1995).

Aim of this thesis is to further behavioural ecology, in particular the evolution of host-handling strategies in the whitefly parasitoid *Encarsia formosa*. For testing evolutionary hypotheses, the focus on a well-known species has the advantage that realistic constraints can be incorporated into optimal-foraging models and that realistic, detailed and quantitative predictions can be made and validated. Modelling foraging decisions of *E. formosa* is simplified by a number of additional advantages. *E. formosa* is a solitary endoparasitoid, i.e. it lays only one egg in a host. Thus, it does not make clutch size decisions. In addition, most insect parasitoids have a haplodiploid sex determination system, where males develop from unfertilised eggs and females develop from fertilised eggs (arrhenotokous parthenogenesis). In many sexual Aphelinidae, the two sexes are obligatory restricted to developing on different types of hosts, e.g. males developing in or on females of their own or different species of parasitoid

(heteronomous hyperparasitism) (Godfray 1994; Hunter & Wooley 2001). The aphelinid *E. formosa*, on the other hand, reproduces asexually, i.e. virgin females produce female offspring from unfertilised eggs. This so-called thelytokous parthenogenesis is induced by *Wolbachia* bacteria (Stouthamer 1997). Thus, *E. formosa* also does not make sex ratio decisions. Finally, parameterisation of a model is facilitated by the detailed laboratory studies on the parasitoid's biology, life history and foraging behaviour.

In general, previous studies already showed that nutrients obtained by host feeding are used to mature eggs and that non-host food sources such as nectar and honey do not contain the essential nutrients (mainly amino acids) in sufficient amounts. Searching for nectar has the additional disadvantage that it reduces the time available to search for hosts. Honey is not even available to a parasitoid in the field because it is produced and stored by bees. Honeydew, on the other hand, can be rich in amino acids and directly imbibed from the host's anus. Parasitoids attacking honeydew-producing hosts, such as *E. formosa*, do not lose host searching time nor energy when searching for honeydew. Natural selection would strongly act against individuals that would destroy limited opportunities to reproduce when an available non-destructive food source could be an alternative. In **Chapters 2 and 3**, I therefore sought for an evolutionary advantage of destructive host feeding over non-destructive feeding on honeydew in terms of fecundity, longevity and egg quality. These laboratory experiments also expand on previous studies, which were unable to explain the function of host-feeding behaviour in *E. formosa*.

The natural conditions under which *E. formosa* has evolved before it was commercially produced is the starting point of a functional explanation of the parasitoid's behaviour. No data were available on these conditions. In **Chapter 4**, a quantitative description was made of natural whitefly densities and distributions from fieldwork in the presumed area of origin of *E. formosa*. Whitefly densities and distributions may be an important factor determining the trade-off between current and future reproduction. Whitefly densities were expected to be considerably lower in the field than in artificial cropping systems, because in the field whiteflies experience non-host plants, bad weather, pathogens, predators and parasitoids. Although it is uncertain where *E. formosa* originates from, I decided to do the fieldwork in Costa Rica, because (1) morphology and nucleotide sequence data place *E. formosa* in the *luteola* group, which originates from the American continent, (2) the intrinsic rate of increase peaks at 30 °C and walking and flight activity are hardly observed below 20 °C, which suggest a (sub)tropical origin, and (3) *E. formosa* was relatively abundant in a field survey in Florida, the Caribbean and Latin America. During two field seasons in 1999 and 2001, leaves were collected at different spatial scales in different areas, and checked for whitefly nymphs. Spatial levels of sampling were incorporated as random effects in generalised linear mixed models. These models allow the simulation of the number of hosts on a leaflet that a randomly-searching parasitoid such as *E. formosa* encounters. Geostatistics were used to quantify the degree and scale of spatial dependence of hosts along transects and within plants.

In **Chapter 5** a dynamic state variable (DSV) model was developed to predict optimal host-handling decisions using knowledge from previous chapters and available literature. I assumed that the function of host feeding is to gain nutrients that can be matured into eggs, that oogenesis is continuous and egg load dependent, that parasitoid survival is exponentially distributed, and that parasitoids encounter hosts randomly, are autogenous and have unlimited access to non-host food sources to obtain energy for maintenance and activity. First, the optimal decision whether to reject, host feed or oviposit was calculated using stochastic dynamic programming, given the parasitoid's state (egg load and energy level), its age and the host type it encounters. The optimal decision is the decision that results in the highest expected reproductive output. Second, the life of a cohort of parasitoids was simulated using a Monte Carlo approach to make testable predictions, such as the optimal fraction of hosts that should be fed upon per day. Host-handling decisions were studied under six scenarios using host density and parasitoid's life expectancy. These parameters were expected to have an important impact on the value of future reproduction and their estimates differed considerably between laboratory and field conditions.

In **Chapter 6**, I tested the prediction from the DSV model (Chapter 5) that host feeding is maladaptive under natural conditions of low host density and short life expectancy. In addition, I tested whether host aggregation had a positive effect on host-feeding behaviour, because this spatial variation found in the field was ignored in the DSV model. Thirdly, I quantified the effects of host distribution and parasitoid experience on patch-leaving behaviour and tested whether parasitoids learn to leave sooner after host encounter when host distribution is uniform than when host distribution is aggregated. Such flexible behaviour can be adaptive when living in a habitat as described in Chapter 4, where host density varies in a predictable way through host aggregation. Parasitoids were observed individually in a three-dimensional set-up of 21 leaflets for 10 hours per day during 6 days. In one treatment, each leaflet contained 1 host, corresponding to the average field host density. In the other treatment, leaflets contained on average 1 host, but natural variation was incorporated by simulating the exact number using the statistical model developed in Chapter 4.

Chapter 7 is the general discussion, where I will briefly summarise the most important results, expand the discussion, draw the main conclusion and give suggestions for future research.

The value of future reproduction and thus optimal host-handling strategies strongly depend on the life expectancy of the parasitoid. Data were available on longevity of *E. formosa* under laboratory conditions, but not under natural field conditions. Predators, bad weather or food deprivation were expected to considerably reduce a parasitoid's life expectancy in the field compared with that in the relatively safe laboratory. In the **Appendix**, several methods were tested to determine the age of a field-caught parasitoid in order to quantify life expectancy of *E. formosa* and other whitefly parasitoids in the field and to test the hypothesis that life expectancy is shorter in the field than in the laboratory.

2

Host feeding in insect parasitoids: why destructively feed upon a host that excretes an alternative?

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Abstract

Many species of insect parasitoids can increase their fecundity and longevity through host feeding. Because host feeding usually destroys the host for oviposition, we studied the function of feeding on honeydew as an alternative to host feeding in the whitefly parasitoid *Encarsia formosa* (Hymenoptera, Aphelinidae). Parasitoids were allowed to oviposit in greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera, Aleyrodidae) until host feeding was attempted. Host feeding was either prevented or allowed. Parasitoids had access to sucrose solution with or without additional access to host-derived honeydew. In contrast to our expectation, parasitoids that were allowed to feed upon a host did not have a significantly higher egg load 20 hours or 48 hours after host feeding than parasitoids prevented from host feeding. Host feeding did not increase the estimated number of eggs matured within these periods, neither did the time spent host feeding positively affect any of these response variables. On the other hand, presence of honeydew did have a positive effect on egg load 20 hours and 48 hours after host feeding compared with parasitoids deprived of honeydew. Parasitoids with access to honeydew were estimated to mature more eggs within these periods than honeydew-deprived parasitoids. Host feeding did have a positive effect on life expectancy, but this effect was nullified when honeydew was supplied after the host-feeding attempt. In conclusion, feeding on honeydew could be an advantageous alternative to host feeding, especially in homopterid parasitoids where honeydew is produced by the host itself. Secondly, the function of host feeding in *E. formosa* remains unknown. We hypothesise

that host feeding is unavoidable to be able to produce anhydrotic eggs, which may have an advantage over hydropic eggs in the parasitoid's natural environment.

Introduction

Consumption of host tissue by adult female parasitoids has been observed in more than 140 species belonging to 17 hymenopteran families (Jervis & Kidd 1986). Since host feeding is destructive to the host or at least reduces its quality for oviposition (Jervis & Kidd 1986; Ueno 1999b), evolutionary benefits are expected to counteract these costs. It has been known for a long time that host feeding supplies nutrients needed for egg maturation (Doten 1911 according to Flanders 1953). In numerous studies host feeding has been shown to increase fecundity; in some species it also increases longevity (see Heimpel & Collier 1996 for a review). However, it has not been clarified yet what the advantage of host feeding is over the consumption of alternative food sources such as nectar, fermenting fruit, pollen and honeydew (Jervis, Kidd & Walton 1992, Jervis *et al.* 1993, Jervis, Kidd & Heimpel 1996; Jervis 1998).

In most previous studies on the function of host feeding, honey was offered as an alternative food source (e.g. Heimpel, Rosenheim & Adams 1994; Heimpel, Rosenheim & Kattari 1997a; Collier 1995a; Heimpel & Collier 1996). However, honey is unavailable to most insects as it is produced by bees, which store it in their nests (Maurizio 1975). Furthermore, the chemical composition and sugar concentration of honey differs from available sugar sources (Crane & Walker 1986a,b), due to partial dehydration and addition of enzymes by bees (Maurizio 1975). True investigation of the additional value of host feeding requires a comparison of the effect of host feeding in the presence of versus the absence of realistic alternatives mentioned above.

Nectar is produced in flowers and extrafloral nectaries and commonly exploited by insect parasitoids (Jervis & Kidd 1999). The predominant components in nectar are the saccharides sucrose, fructose and glucose (Maurizio 1975; Baker & Baker 1983). Access to sugar solution can increase longevity tremendously (e.g. van Lenteren *et al.* 1987; Wäckers 2001). Non-host food sources are even more effective in promoting longevity than host hemolymph (Jervis & Kidd 1986). However, substances other than sugars, e.g. amino acids, inorganic salts and vitamins (Bracken 1965, 1966), are necessary to sustain oogenesis. In nectar, these are present in relatively small amounts (Maurizio 1975; Baker & Baker 1983). Fermenting fruits could be used to overcome this potential deficiency. However, they cannot overcome another deficiency. Searching for nectar or fermenting fruits substantially reduces time that can be spent on searching for hosts (Wäckers 1994; Takasu & Lewis 1995). This is considered an important cost to time-limited parasitoids (Jervis, Kidd & Sahragard 1987). Feeding on nectar or fruit reinforces the trade-off between searching for food and searching for hosts (e.g. Sirot & Bernstein 1996; Krivan & Sirot 1997; Lewis *et al.* 1998).

Pollen, as a third potential alternative to host hemolymph, has a high nutritional value (Stanley & Linskens 1974; Harborne 1993) and is commonly used by beetles and bees

(Stanley & Linskens 1974). However, cell walls of pollen are resistant to decay and chemical treatment (Jervis & Kidd 1996) and thus difficult to grind for ingestion. Some parasitoid flies possibly consume the contents of pollen grains using saliva or piercing mouthparts (Gilbert & Jervis 1998). For minute parasitoid wasps, however, pollen is considered a non-exploitable resource (Gilbert & Jervis 1998; Jervis & Kidd 1999).

Another potential alternative to host feeding is feeding on honeydew. Honeydew is excreted by phloem feeding Homoptera such as aphids and whiteflies, as a result of an excessive carbon-to-nitrogen ratio of plants compared with the bodies of the phloem feeders. Like nectar, honeydew is commonly exploited by parasitoids (Wäckers 2000). Homopterid parasitoids drink honeydew directly from their host's anus (Jervis *et al.* 1996; Zoebelein 1955; Vos 1995), thus avoiding the impeded intake of crystalline honeydew deposits. Honeydew can have a positive effect on survival, egg production and fat reserves (Zoebelein 1955; Idoine & Ferro 1988; England & Evans 1997; Eijs, Ellers & van Duinen 1998). Until 1952 honeydew was mainly seen as a sugar solution. Host feeding was considered unavoidable to meet the high protein demands needed for egg maturation (Bartlett 1964; Gerling 1966; Jervis & Kidd 1986), since lack of nitrogenous compounds in the insect diet will terminate oogenesis (Bell & Bohm 1975) and host hemolymph contains amino acids (e.g. Florkin & Jeuniaux 1964; Fisher & Ganesalingam 1970; Morgan & Chippendale 1983; Mullins 1985). However, honeydew has been shown to contain amino acids in significant amounts (Auclair 1963; Maurizio 1975; van Vianen 1982, 1987; Cochran 1985; Crane & Walker 1986b; Byrne & Miller 1990). Furthermore, contact with honeydew provokes arrestment behaviour in *E. formosa* (van Roermund *et al.* 1994; van Roermund & van Lenteren 1995a,b; Romeis & Zebitz 1997; van Vianen 1987; van Vianen & van de Veire 1988). The use of honeydew as both food source and kairomone to find hosts implies that searching for food can be complementary to searching for hosts. Moreover, parasitoids of honeydew-producing hosts, such as the whitefly parasitoid *Encarsia formosa*, may lose neither host searching time nor energy at all when searching for honeydew because honeydew can be imbibed directly from the host anus (Jervis *et al.* 1996; Zoebelein 1955; Vos 1995). This cancels out the trade-off between searching for food and searching for hosts.

Gast & Kortenhoff (1983) and van Lenteren *et al.* (1987) found that *E. formosa* females that were unable to host feed did not differ in the number of ovipositions from females that were able to host feed. In addition, females allowed to feed on hosts and honeydew did not live longer than females allowed to feed only on honeydew. They suggested that parasitoids that do not feed on hosts obtain the nitrogenous compounds from the honeydew of the whiteflies. In a population-dynamical simulation model, van Roermund *et al.* (1997c) therefore assumed that host feeding does not affect egg maturation. Van Vianen & van Lenteren (1986b) still found mature eggs in *E. formosa* after 30 days on a carbohydrate source without host-feeding possibilities. From these findings the functional question arises why these parasitoids destructively feed upon their hosts.

In the experiments by Gast & Kortenhoff (1983) and van Lenteren *et al.* (1987) no comparison was made of egg load dynamics between females allowed to feed on

honeydew only and females allowed to feed on honeydew and hosts. Furthermore, parasitoids that were allowed to host feed were offered small first instars to prevent *E. formosa* from oviposition, since oviposition is preferred in larger instar hosts (Nell *et al.* 1976). Unfortunately, in a no-choice situation *E. formosa* will oviposit in first instars (pers. obs.). Thus, a host-feeding effect was confounded with a possible oviposition effect.

In conclusion, the advantage of host feeding over feeding on honeydew has not been shown for parasitoids of honeydew-producing hosts, whereas the disadvantage is clearly the destruction of a possibility to oviposit. Natural selection would strongly act against individuals that would destroy limited opportunities to reproduce without an evolutionary benefit, especially in insect parasitoids, where foraging decisions are directly linked to reproductive success. Hence, we will address the question here of why homopterid parasitoids show host-feeding behaviour. More specifically, we question what the benefit of destructive host feeding is in terms of fecundity and longevity compared with non-destructive honeydew feeding. We experimentally investigate this question using the whitefly parasitoid *E. formosa*, a destructive host feeder and one of the "confusing exceptions" (Heimpel & Collier 1996) to the conventional wisdom that says the adaptive value of host-feeding behaviour is that it enables females to achieve greater lifetime reproductive success.

Materials & Methods

Life materials

Tomato plants (Solanaceae, *Solanum lycopersicum* L. [*Lycopersicon esculentum* Miller] cv. Moneymaker) and bean plants (Fabaceae, *Phaseolus vulgaris* L. cv. Prelude) were reared by Unifarm, Wageningen UR, the Netherlands, at 21°C, 70% R.H. and L:D=16:8 hours. Nymphs of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) were obtained from the whitefly culture on tomato at the Laboratory of Entomology, Wageningen UR, the Netherlands, either directly or after one generation on bean plants. Pupae of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) parasitoids were obtained from Koppert Biological Systems Ltd., Berkel en Rodenrijs, the Netherlands.

Experimental set-up

Females were assigned to four treatments. In each they had access to sucrose solution and were allowed to oviposit during a certain observation period. In the control treatment (S) females were not allowed to host feed and did not have additional access to honeydew. In the other three treatments parasitoids were additionally allowed to feed on one host (SH), had additional access to honeydew (SD), or both (SDH). The procedure of how these diets were offered is described below for each experiment. Sucrose solution

Table 1 Procedure of fecundity experiments I and II. S: *ad libitum* sucrose solution, D: *ad libitum* honeydew, H: host feeding.

| Day | Event | S | SH | SD | SDH |
|-----|--|--------------------|--------------------|---------------------|---------------------|
| 0 | Emerging | S | S | SD | SD |
| 1-3 | Ageing | S | S | SD | SD |
| 4 | Oviposition allowed up to first H attempt | H prevented → S | 1 H allowed → S | H prevented → SD | 1 H allowed → SD |
| 5 | Dissection 20 h (I) or 48 h (II) after H attempt | S | S | SD | SD |

was continuously provided to meet basic metabolic requirements so that only the additive effects of hemolymph and honeydew were studied. Experiments were carried out at 25 °C and L:D=16:8 hours.

Fecundity experiment I

The procedure of the first and second fecundity experiment is summarised in Table 1. On the first day (day 0), cards containing *E. formosa* pupae were placed in Petri dishes, each containing a little ball of cotton wool drenched in a sucrose solution of 0.1 g sucrose per 1 g water. Honeydew was supplemented to treatments SD and SDH. To collect honeydew, a whitefly infested tomato or bean leaf was inverted on a piece of moist cotton wool in the bottom part of a Petri dish, which was then covered by parafilm. After one day, the parafilm containing droplets of honeydew was cut into pieces of about 5 cm² and offered to the parasitoids. On day 1, cards were removed leaving emerged parasitoids of 0 to 1 day old. Sucrose solution was replenished daily, cotton wool was replaced once every two to three days to prevent fungal growth. Honeydew, if supplemented, was replaced daily. On day 4, parasitoids were allowed to forage individually on a whitefly-infested patch. This patch consisted of an inverted disc of bean leaf (Ø 52 mm) on a piece of moist cotton wool. The leaf disc contained 25 fourth instar whitefly nymphs, which is more than *E. formosa*'s egg storage capacity, in a grid of 5×5. Nymphs had been carefully removed from their original feeding site and transferred to the experimental arena. In all treatments, parasitoids were allowed to oviposit until host feeding was attempted. Individuals in the control treatment (S) and treatment SD were removed immediately after the parasitoid attempted host feeding and were thus prevented from doing so. Individuals in treatments SH and SDH were removed directly after they had freely fed upon one host. Parasitoids were then transferred back to their Petri dish containing sucrose solution and, in case of treatments SD and SDH, honeydew. Twenty hours after the observation had been terminated parasitoids were dissected to measure their mature egg load and hind tibia length at 400 power. The 20-hour interval was chosen because daily egg maturation can make up for oviposition rates (Hoddle *et al.* 1998). Usually one parasitoid per treatment per day was

observed. To derive the initial egg load at the beginning of the experiment, one parasitoid from the S treatment and one from the SD treatment were dissected prior to each observation, and their egg load and hind tibia length were measured. Hind tibia length is a reliable measure of parasitoid size (Hora 1994; Roskam & Wessels 1995) and size positively correlates with ovariole number (van Vianen & van Lenteren 1982, 1986a). Experiments were conducted between September and November 1999.

Fecundity experiment II

Since it turned out that in the previously described experiment host feeding had no effect on egg maturation (see results), we hypothesised that parasitoids needed more time to convert the host-feeding gain into eggs. A second experiment was therefore conducted in which the time between host feeding and dissection was increased from 20 hours to 48 hours (Table 1). In addition, two control parasitoids were dissected every observation day at 12:00 and two at 18:00 to estimate the egg loads at the beginning of the observation more accurately. Other revisions included the use of an intact young tomato leaflet as a patch; a slightly more concentrated sucrose solution (10 % w/w), which was offered on a flat, non-fleecing piece of make-up pad; and the season in which experiments were carried out, i.e. June and July 2000.

To verify whether honeydew had a direct nutritional effect on egg production or merely an indirect effect through increased patch quality assessment by the parasitoids, parasitoids were kept on a 10 % sucrose solution with (SD) or without (S) additional honeydew (see above) and dissected at day 6, 10 and 20 for egg load measurement. The absence of a direct nutritional effect is expected to cancel out or even invert in the long term an (initially positive) indirect effect of honeydew on oogenesis.

Longevity experiment

The procedure of the longevity experiment (Table 2) differed from the first fecundity experiment regarding three aspects. First, whitefly nymphs were offered when parasitoids were 13 to 14 days old. This was expected to increase the detectability of a potential diet effect, since average life expectancy is 22 days on a glucose solution (van Lenteren *et al.* 1987). Second, honeydew was not provided in any treatment before parasitoids had contact with hosts on day 13, to prevent large quantitative differences between honeydew availability and gain from a single host feeding. Third, instead of dissection after 20 hours, survival was scored during the consecutive days until the parasitoid died. After death, parasitoids were dissected to check their mature egg load. The last day the parasitoid was seen alive was scored as its longevity.

Parasitoids were observed individually until host feeding because less laborious alternatives appeared unsuitable. Offering small whitefly nymphs does not prevent parasitoids from laying eggs. Puncturing or blending nymphs will alter the hemolymph composition through oxidation and increases the artificiality of the experiment. Camera

Table 2 Procedure of longevity experiment. S: *ad libitum* sucrose solution, D: *ad libitum* honeydew, H: host feeding.

| Day | Event | S | SH | SD | SDH |
|----------|---|--------------------|--------------------|---------------------|---------------------|
| 0 | Emerging | S | S | S | S |
| 1-13 | Ageing | S | S | S | S |
| 14 | Oviposition allowed up to first H attempt | H prevented → S | 1 H allowed → S | H prevented → SD | 1 H allowed → SD |
| 15-death | Survival | S | S | SD | SD |

recording makes manipulation of parasitoids, like preventing them from host feeding, impossible.

Statistical analysis

Fecundity experiments

Parasitoids that did not attempt host feeding during the observation were excluded from the analysis, as well as parasitoids that died before the end of the experiment. First, a generalised linear model was applied on the egg load of the control group (*elcontrol*), using hind tibia length (*htl* in mm) as a (quantitative) predictor variable and presence of honeydew (*d*) as a (qualitative) indicator variable. In fecundity experiment II time of dissection (*t*) was additionally included. This model was used to estimate the egg load of observed parasitoids at the beginning of the observation (*elbegin*). Egg load of observed parasitoids after the observation (*elend*) was estimated by subtracting the number of ovipositions during observation (*nov*) from *elbegin*. If this resulted in a negative value, *elend* was set to 0. The number of eggs matured between the end of the observation and dissection (*nmat*) was estimated by subtracting *elend* from the egg load measured by dissection 20 hours (*el20h*) or 48 hours (*el48h*) after the end of the observation. When *nmat* resulted in a negative estimate, the parasitoid had probably resorbed eggs, which allows the adult female to reinvest egg nutrients into her own maintenance and activity. Observation time minus host-feeding time (*hft*) was taken as foraging time (*fort*). Host-feeding time was measured as duration of contact between parasitoid's mouthparts and whitefly nymph after ovipositorial attack. Egg load at day 20 (*el20d*) was used to test the nutritional value of honeydew. A generalised linear model was built to test the effect of presence of honeydew (*d*), allowance of one host feeding (*h*) or host-feeding time (*hft*) on these variables.

Table 3 summarises for each response variable the assumed distribution, the applied linear predictor, the explanatory and indicator variables investigated, and whether correction for overdispersion was applied (option DSCALE, SAS version 8.00). For each response variable a model was built using partial F tests (when Normal distribution assumed) or partial deviance tests (when Poisson or Gamma distribution assumed)

Table 3 Details of generalised linear models ($\eta = \beta X$) applied to different response variables in fecundity experiments: *elcontrol*: egg load of control group at day 4; *htl*: hind tibia length (mm); *fort*: foraging time until host-feeding attempt (h); *nov*: number of ovipositions until host-feeding attempt; *elend*: estimated egg load after observation; *el###*: egg load ## hours after observation; *nmat*: estimated number of eggs matured or resorbed between end of observation and dissection; *el20d*: egg load at day 20; *t*: time of dissection at day 4; *d*: presence of honeydew; *h*: allowance of one host feeding; *hft*: host-feeding time (min).

| Response variable μ | Assumed distribution | Linear predictor η | Predictor variables | Indicator variables (0/1) | Correction for overdispersion |
|-------------------------------|----------------------|-------------------------|----------------------------|---------------------------|-------------------------------|
| <i>elcontrol</i> | Poisson | $\log(\mu)$ | <i>htl</i> , <i>t</i> (II) | <i>d</i> | no (I), yes (II) |
| <i>htl</i> | Normal | μ | | <i>d</i> , <i>h</i> | no |
| <i>fort</i> | Gamma | μ^{-1} | | <i>d</i> | no |
| <i>nov</i> | Poisson | $\log(\mu)$ | | <i>d</i> | yes |
| <i>elend</i> | Poisson | $\log(\mu)$ | | <i>d</i> | yes |
| <i>el###</i> | Poisson | $\log(\mu)$ | | <i>d</i> , <i>h</i> | no (I), yes (II) |
| <i>nmat</i> | Normal | μ | | <i>d</i> , <i>h</i> | yes |
| <i>hft</i> (if <i>h</i> =1) | Gamma | μ^{-1} | | <i>d</i> | no |
| <i>el###</i> (if <i>h</i> =1) | Poisson | $\log(\mu)$ | <i>hft</i> | <i>d</i> | no (I), yes (II) |
| <i>nmat</i> (if <i>h</i> =1) | Normal | μ | <i>hft</i> | <i>d</i> | yes |
| <i>el20d</i> | Poisson | $\log(\mu)$ | <i>htl</i> | <i>d</i> | yes |

(Neter *et al.* 1996). A model with 1 parameter (the intercept) was tested against all possible models with 2 parameters. The model that gave the highest test statistic (F or partial deviance) was tested against all possible models with 3 parameters, if its test statistic exceeded the critical value $F(0.05;1;n-p)$ or $\chi^2(0.95;1)$. This was repeated until the test statistic became smaller than or equal to the critical value, or the full model was reached. If the test statistic became smaller than or equal to the critical value, the selected model with p parameters was tested against all possible models with more than $p+1$ parameters. If this resulted in a test statistic larger than the critical value, the analysis was continued using the better model with more than $p+1$ parameters. Models with interaction terms were only considered if associated main effects were also included.

Longevity experiment

Per treatment a survival curve was constructed from longevity scores. The effect of host feeding on survival was tested using Kaplan-Meier survival analysis (SPSS version 10.0.5), when honeydew was absent (S vs. SH), and when honeydew was present (SD vs. SDH). The effect of indicator variables host feeding (*h*) and honeydew (*d*) on egg load (*el*) and hind tibia length (*htl*) of died parasitoids was tested using generalised linear modelling (see statistical analysis of fecundity experiments; assumed distribution:

Table 4 Results from generalised linear modelling applied to response variables of fecundity experiments, showing most parsimonious models and decisive P-values from partial F tests (when Normal distribution assumed) or partial deviance tests (when Poisson or Gamma distribution assumed). Refer to header of Table 3 for meaning of response variable names.

| Response variable μ | First fecundity experiment (## = 20) | | Second fecundity experiment (## = 48) | |
|--------------------------|--------------------------------------|---------|--|---------|
| | Most parsimonious model | P-value | Most parsimonious model | P-value |
| <i>elcontrol</i> | $\log(\mu) = 0.3002 + 9.8489*hit$ | 0.0001 | $\log(\mu) = -0.4348 + 8.9387*hit + 0.6626*d$ | 0.0040 |
| <i>hit</i> | $\mu = 0.2280$ | 0.3892 | $\mu = 0.2305$ | 0.2513 |
| <i>fort</i> | $\mu^1 = 1.3484$ | 0.9634 | $\mu^1 = 1.1062 - 0.5682*d$ | 0.0007 |
| <i>nov</i> | $\log(\mu) = 0.6768 + 0.7285*d$ | 0.0001 | $\log(\mu) = 0.6827 + 1.0768*d$ | 0.0001 |
| <i>elend</i> | $\log(\mu) = 2.3885 - 0.2222*d$ | 0.0092 | $\log(\mu) = 1.2200 + 0.2298*d$ | 0.0337 |
| <i>el###</i> | $\log(\mu) = 2.2731 + 0.3181*d$ | 0.0001 | $\log(\mu) = 1.8293 + 0.6869*d$ | 0.0001 |
| <i>nmat</i> | $\mu = -1.1879 + 5.8080*d$ | 0.0001 | $\mu = 2.8419 + 5.2766*d$ | 0.0001 |
| <i>hft</i> (if $h=1$) | $\mu^1 = 0.1950$ | 0.3959 | $\mu^1 = 0.0436$ | 0.1229 |
| <i>el###</i> (if $h=1$) | $\log(\mu) = 2.2548 + 0.3564*d$ | 0.0015 | $\log(\mu) = 1.9157 + 0.6529*d$ | 0.0001 |
| <i>nmat</i> (if $h=1$) | $\mu = -1.2112 + 6.6004*d$ | 0.0013 | $\mu = 3.1871 + 5.3597*d$ | 0.0050 |
| <i>el20d</i> | - | - | $\log(\mu) = -4.3426 + 15.7512*hit + 2.5220*d$ | 0.0090 |

Poisson, linear predictor: $\log(\mu)$, correction for overdispersion only for response variable egg load).

Results

Fecundity experiment I

Results from the first fecundity experiment are shown in Figure 1 (data) and Table 4 (statistics). None of the interaction terms was significant, indicating that each effect was independent. Hind tibia length was a weak but significant predictor of the egg load at the beginning of the observation ($P = 0.0001$), whereas presence of honeydew was not ($P = 0.9153$) (Fig. 1a). Based on this correlation, and the fact that observed parasitoids did not differ significantly in hind tibia length ($P = 0.3892$; Fig. 1b), observed parasitoids were estimated to have almost 13 eggs at the beginning of the observation (Fig. 1c). Parasitoids foraged, i.e. searched for hosts, on average three quarters of an hour, independent of previous diet ($P = 0.9634$; Fig. 1d). On the other hand, on average only 2 eggs were laid during the observation when honeydew had been absent, whereas twice as many were laid when honeydew had been present ($P < 0.001$; Fig. 1e). As a consequence, parasitoids that had access to honeydew were estimated to have about 2

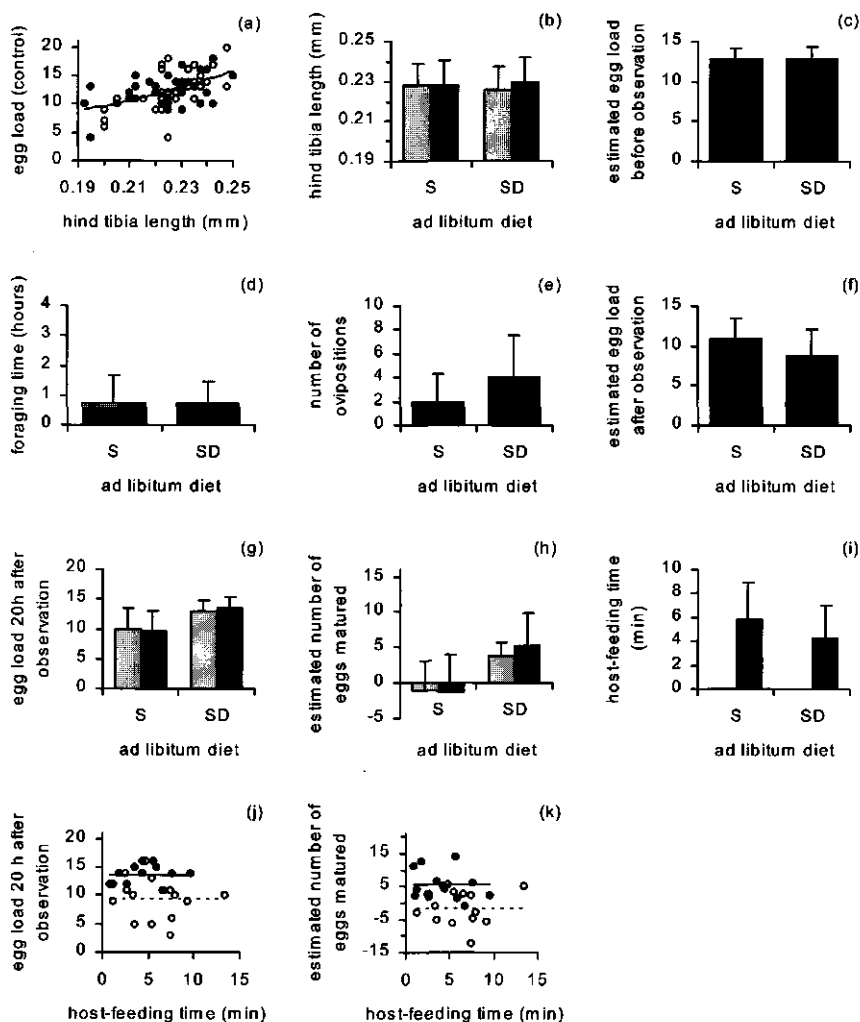


Fig. 1 Results from first fecundity experiment. (a) Effect of hind tibia length on egg load of control parasitoids at day 4 when honeydew had been absent (open circles, $n_S = 32$) or present (closed circles, $n_{SD} = 34$) in addition to sucrose solution. Solid line represents generalised linear model (see text). Mean + sd of (b) hind tibia length, (c) estimated egg load before observation, (d) foraging time until host-feeding attempt, (e) number of ovipositions until host-feeding attempt, (f) estimated egg load after observation, (g) egg load 20 h after observation, (h) estimated number of eggs matured within 20 h, (i) host-feeding time, when honeydew was absent (S) or present (SD) in addition to sucrose solution, and when host feeding was still ahead (dark grey bars, $n_S = 31$, $n_{SD} = 26$), prevented (light grey bars, $n_S = 16$, $n_{SD} = 13$) or allowed (black bars, $n_{SH} = 15$, $n_{SDH} = 13$). Effect of host-feeding time on (j) egg load 20 h after observation and (k) estimated number of eggs matured within 20 h, when honeydew had been absent (open circles, dotted line) or present (closed circles, solid line) in addition to sucrose solution.

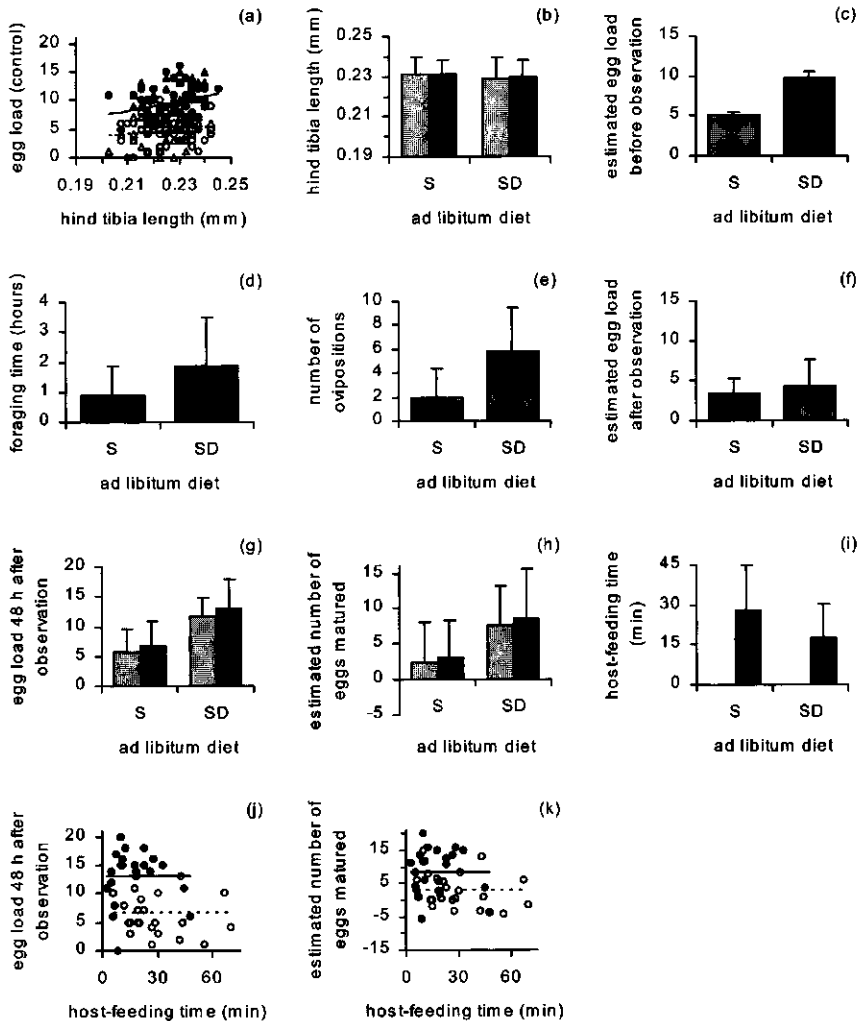


Fig. 2 Results from second fecundity experiment. (a) Effect of hind tibia length on egg load of control parasitoids at 12:00 (circles, $n_S = n_{SD} = 54$) and 18:00 (triangles, $n_S = n_{SD} = 36$) of day 4 when honeydew had been absent (open symbols, dotted line) or present (closed symbols, solid line) in addition to sucrose solution. Mean + sd of (b) hind tibia length, (c) estimated egg load before observation, (d) foraging time until host-feeding attempt, (e) number of ovipositions until host-feeding attempt, (f) estimated egg load after observation, (g) egg load 20 h after observation, (h) estimated number of eggs matured within 20 h, (i) host-feeding time, when honeydew was absent (S) or present (SD) in addition to sucrose solution, and when host feeding was still ahead (**dark grey bars**, $n_S = 48$, $n_{SD} = 42$), prevented (**light grey bars**, $n_S = 24$, $n_{SD} = 21$) or allowed (**black bars**, $n_{SH} = 24$, $n_{SDH} = 21$). Effect of host-feeding time on (j) egg load 20 h after observation and (k) estimated number of eggs matured within 20 h, when honeydew had been absent (open circles, dotted line) or present (closed circles, solid line) in addition to sucrose solution.

eggs less at the end of the observation than parasitoids deprived of honeydew ($P = 0.0092$; Fig. 1f).

In contrast to our hypotheses, parasitoids that were allowed to feed on a host did not have a higher egg load after 20 hours ($P = 0.9459$; Fig. 1g) and were estimated not to mature more eggs within this period ($P = 0.5420$; Fig. 1h) than parasitoids that were prevented from host feeding. On the other hand, 20 hours after the observation, parasitoids had about 3.6 eggs (37 %) more when honeydew had been present compared with parasitoids that had no access to honeydew ($P < 0.0001$; Fig. 1g). They were estimated to mature almost 5 eggs within this period, whereas parasitoids deprived of honeydew were estimated to resorb about 1 egg ($P < 0.0001$; Fig. 1h).

Presence of honeydew did not significantly reduce host-feeding times of parasitoids allowed to feed ($P = 0.3959$; Fig. 1i). Host-feeding time had no effect on egg load at dissection ($P = 0.9232$; Fig. 1j) or estimated number of eggs matured ($P_{\text{hft}} = 0.5672$; Fig. 1k). Note, however, that host-feeding times averaged only about 5 minutes (Fig. 1i), whereas normally about 15 minutes are used for host feeding (van Roermund & van Lenteren 1995b; van Lenteren, Nell & Sevenster-van der Lelie 1980).

Fecundity experiment II

Allowing parasitoids to convert nutrients from host feeding into eggs over a longer time span did not change the most important results in a qualitative sense (Fig. 2, Table 4). Again, host feeding had neither a significant effect on egg load at time of dissection ($P = 0.0545$, Fig. 2g), nor on the estimated number of eggs matured within 48 hours ($P = 0.5296$, Fig. 2h). Presence of honeydew again did have a strong, positive effect on both variables ($P < 0.0001$).

Strikingly, the results of the two fecundity experiments did differ qualitatively in the part where the approach was still the same. In the second experiment, not only hind tibia length ($P = 0.0040$) but also (and especially) presence of honeydew ($P < 0.0001$) was a significant predictor of egg load at the beginning of the observation (Fig. 2a). The beginning time of the observation did not significantly contribute to the explanatory power ($P > 0.2087$). Parasitoids deprived of honeydew in the second experiment (Fig. 2c) were estimated to have about half as many eggs before the observation as parasitoids in the first experiment (Fig. 1c). Like in the first experiment, parasitoids that had access to honeydew laid more eggs before attempting to host feed than honeydew-deprived parasitoids ($P < 0.0001$; Fig. 2e). Unlike the first experiment, however, also their foraging time significantly exceeded that of honeydew-deprived parasitoids ($P = 0.0007$; Fig. 2d). In the second experiment, parasitoids that had access to honeydew spent almost 2 hours laying on average almost 6 eggs, compared with less than 1 hour laying only 2 eggs by honeydew-deprived parasitoids. As a result, parasitoids that had access to honeydew were estimated to have slightly more eggs at the end of the observation than parasitoids deprived of honeydew ($P = 0.0337$; Fig. 2f), considerably fewer compared with the first experiment, i.e. about 4 compared with almost 11 (S) and 9 (SD).

If allowed, parasitoids additionally supplied with honeydew did not feed significantly shorter upon the host (17.4 ± 12.8 min) than honeydew-deprived parasitoids (27.7 ± 17.5 min) ($P = 0.1229$; Fig. 2i). Host-feeding time again did not affect egg load at dissection ($P = 0.0939$; Fig. 2j) or estimated number of eggs matured ($P = 0.2373$; Fig. 2k). None of the interactions contributed significantly to the explanatory power of any statistical model.

Differences between fecundity experiments in the part where the approach was still the same might be explained by a difference in the time until non-active parasitoids were discarded. Figures 1d and 2d suggest that in the second experiment parasitoids were given more time to forage until host feeding and that more "slow" parasitoids were included. The number of parasitoids that did not attempt host feeding during the second experiment, and therefore excluded from the analysis, was 0 (S), 3 (SH), 13 (SD) and 7

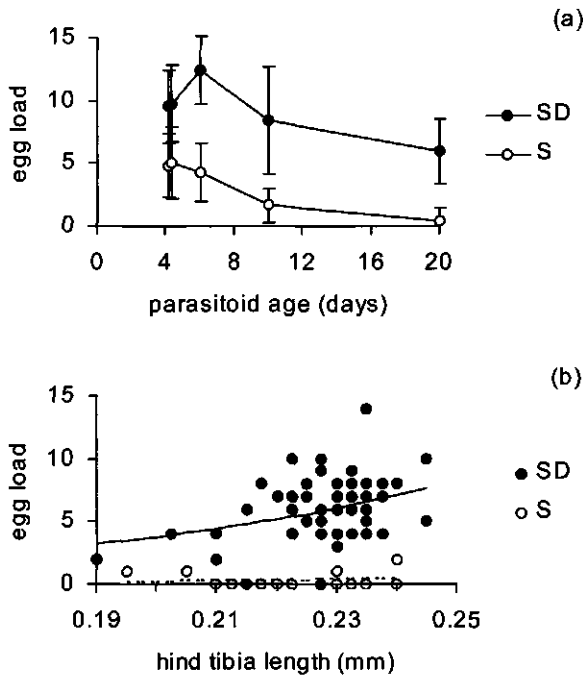


Fig. 3 (a) Egg load dynamics (mean \pm sd) of parasitoids that had access to sucrose solution only (open circles, $n_{4(12:00),4(18:00),6,10,20} = 54, 36, 30, 16, 20$) and with additional access to honeydew (closed circles, $n_{4(12:00),4(18:00),6,10,20} = 54, 36, 30, 16, 50$). (b) Egg load of 19-20 days-old parasitoids that had access to sucrose solution only (open circles, dotted line) and with additional access to honeydew (closed circles, solid line), corrected for hind tibia length.

(SDH). These discarded parasitoids were observed for 4.5 ± 1.3 hours. (Similar data are not available for the first experiment.) Furthermore, more replicates were achieved in the second experiment; in the first fecundity experiment honeydew was obtained from whiteflies reared on two different host plant species; and seasonal effects might have played a role.

Figure 3a shows egg loads between day 4 and 20 of parasitoids with and without access to honeydew in addition to sucrose solution. The first two data points in time, i.e. 12:00 and 18:00 of day 4, correspond to the control dissections of fecundity experiment II (Fig. 2a). The egg load of parasitoids that only had access to sucrose solution showed a gradual decrease over the time span examined, i.e. the rate of oosorption exceeded the rate of oogenesis. Parasitoids that had additional access to honeydew both delayed oosorption and maintained a higher egg load than parasitoids without additional access to honeydew. At day 20, parasitoids with access to honeydew still had a higher egg load than honeydew-deprived parasitoids ($P < 0.0001$; Table 4; Fig. 3b), and survived in larger numbers until that day ($n_S = 20$, $n_{SD} = 50$).

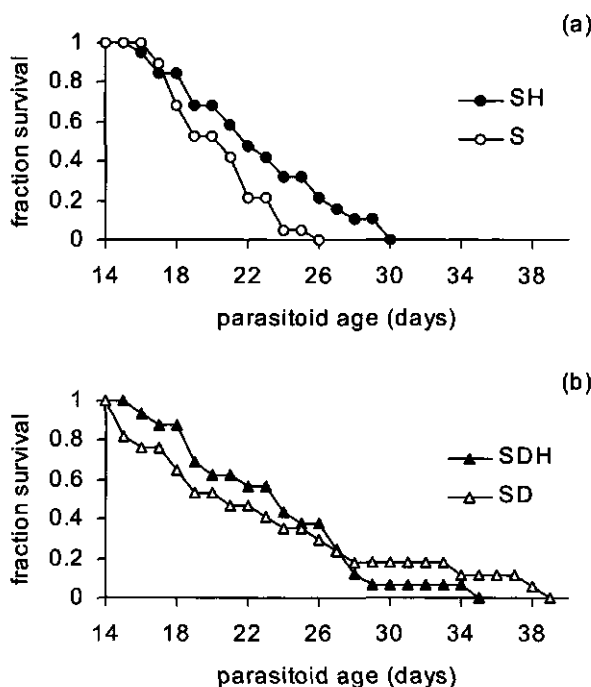


Fig. 4 Survival curves of parasitoids (a) when only sucrose solution was supplied (circles, $n_S = n_{SH} = 19$) and (b) when, in addition to sucrose solution, honeydew was present from the host-feeding attempt onwards (triangles, $n_{SD} = 17$, $n_{SDH} = 16$). At day 14, parasitoids were allowed to oviposit until host feeding was attempted. Host feeding was prevented (open symbols) or allowed (closed symbols).

Table 5 Mean \pm sd of, and results from generalised linear modelling applied to egg load (*el*) and hind tibia length (*htl*) at death of parasitoids in longevity experiment, showing most parsimonious models and decisive P-values from partial F tests (normal distribution assumed for *htl*) or partial deviance tests (Poisson distribution assumed for *el*). S: *ad libitum* sucrose solution, D: *ad libitum* honeydew, H: host feeding allowed.

| μ | Mean \pm sd | | | | Most parsimonious model | P-value |
|------------|-------------------|--------------------|--------------------|---------------------|--|---------|
| | S (<i>n</i> =19) | SH (<i>n</i> =19) | SD (<i>n</i> =17) | SDH (<i>n</i> =16) | | |
| <i>el</i> | 0.16 \pm 0.37 | 0.26 \pm 0.73 | 2.53 \pm 2.03 | 0.81 \pm 1.33 | $\log(\mu) = -1.8541 + 1.1274*d - 1.1356*h + 1.6465*\min(d+h,1)$ | 0.0001 |
| <i>htl</i> | 91.47 \pm 3.76 | 89.63 \pm 5.05 | 92.06 \pm 2.66 | 91.25 \pm 3.13 | $\mu = 0.2277$ | 0.0720 |

Longevity experiment

Parasitoids allowed to host feed had a higher life expectancy than parasitoids prevented from host feeding, when only sucrose solution was supplied (logrank statistic = 3.97, $P = 0.0462$; Fig. 4a). The incremental effect of the host feeding increased with parasitoid's age. However, when parasitoids had additional access to honeydew after the observation, host feeding did not increase life expectancy (logrank statistic = 0.00, $P = 0.9698$; Fig. 4b).

During the observation on day 14, most parasitoids immediately attempted host feeding without ovipositing (mean \pm sd of number of ovipositions: 0.10 ± 0.34 [$n=71$]). If allowed, parasitoids host-fed on average 12.60 ± 10.86 ($n=35$) minutes. No censored data occurred, i.e. all parasitoids were observed until death. The average egg load at death was usually less than 1 (Table 5), except for parasitoids in the SD treatment where it was on average about 2.5 ($P < 0.0001$; Table 5). Hind tibia length did not differ significantly between treatments ($P = 0.0720$; Table 5).

Discussion

The whitefly parasitoid *Encarsia formosa* has been characterised as one of the "confusing exceptions" to host-feeding theory (Heimpel & Collier 1996). Previous studies found no effect of host feeding on number of ovipositions and longevity (Gast & Kortenhoff 1983; van Lenteren *et al.* 1987). Although our approach more directly investigates the effect of host feeding on fecundity and longevity, the results confirm previous conclusions. Host feeding in *E. formosa* neither significantly increased the egg load after 20 hours, nor after 48 hours. It did not significantly increase the estimated number of eggs matured within these periods, neither did the time spent host feeding positively affect any of these response variables. Host feeding did have a positive effect

on life expectancy, but this effect was nullified when honeydew was supplied between host feeding and death.

Endoparasitoids like *E. formosa* oviposit and develop internally, i.e. inside the host. They presumably evolved from ectoparasitoids, which oviposit, and usually also develop, externally, i.e. on the host. This evolution to internal development might have been a strategy to reduce the risk of being dislodged by an exposed and, in case of koinobionts, active host (Godfray 1994). It was made possible by association with viruses that could suppress encapsulation by the host's immune system (Price 1997). In ectoparasitoids, the developing parasitoid only starts feeding from the host after the parasitoid's egg has hatched. The mother should therefore provide all nutrients necessary for egg development. To meet the high nutrient demand for oogenesis and to produce large, so-called anhydropic eggs that contain protein inclusions, the adult female usually needs to host feed (Jervis & Kidd 1986; Le Ralec 1995; Jervis *et al.* 2001). Once internal oviposition had evolved, absorption of host nutrients by the parasitoid's egg allowed for embryogenesis after oviposition. So-called hydropic eggs can swell hundreds or even thousands of times in volume between oviposition and hatching (e.g. Goldson *et al.* 1995). This uptake of host resources by the egg stage possibly reduces the need of the adult female to destructively host feed. Indeed, species with hydropic eggs never host feed (Jervis & Kidd 1986; Quicke 1997). Furthermore, hydropic eggs can be stored in higher numbers and for longer periods than anhydropic eggs (Jervis & Kidd 1986; Jervis *et al.* 2001), and can be laid in less than a second (e.g. Völkl & Mackauer 2000), whereas laying an anhydropic egg usually requires several minutes (e.g. van Roermund & van Lenteren 1995b).

Encarsia formosa (Agekyan 1981) and many other endoparasitoids (Jervis & Kidd 1986; Le Ralec 1995; Jervis *et al.* 2001) produce large, anhydropic eggs, despite the suggested advantages of hydropy. Blackburn (1991a,b) showed that there is a trade-off between fecundity (the number of eggs) and egg size. Generally, there is a positive relationship between adult size and fitness (Visser 1994; Ellers, van Alphen & Sevenster 1998), which may also apply to egg size and fitness (e.g. Mayhew & Heitmans 2000; Donnell & Hunter 2002). Since resources are limited in a discrete host, anhydropic eggs have a lead over hydropic eggs. Possibly, anhydropic eggs are also less susceptible to encapsulation by the host's immune system, although this has not been studied directly. Furthermore, whereas hydropic eggs are stored in the lateral oviducts, anhydropic eggs remain surrounded by follicle cells in the ovariole. This enables oosorption of eggs, a strategy to overcome periods of food or host deprivation and to maintain egg viability (Bell & Bohm 1975). *E. formosa* does have the ability of oosorption (van Vianen & van Lenteren 1986b; this study, Fig. 3, 1h). Thus, not only the parasitoid embryo but also the adult female can benefit from producing anhydropic eggs.

Clearly, both hydropic and anhydropic eggs have advantages and disadvantages. Evolution towards either one of the two egg types depends among others on environmental conditions. Since hydropic species can store many eggs without the ability to resorb them, it seems to be an adaptation to aggregated, highly abundant or exposed hosts or to ephemeral habitats, whereas anhydropy is likely to be advantageous

when hosts are highly dispersed, scarce or concealed or when the ecosystem is stable (Jervis & Kidd 1986; Price 1975; Dowell 1978). Field estimates of natural whitefly densities (Chapter 4) show rather low averages and suggest that egg viability and the ability of oosorption might be more important in whitefly parasitoids than the ability to store many eggs. In conclusion, the function of host feeding in *E. formosa* might not be to increase the number of eggs, but to produce high-quality anhydropic eggs. If this hypothesis is true, an effect of host feeding on egg maturation and longevity is not expected. To test the new hypothesis, egg-to-adult survival need to be compared of eggs laid by parasitoids prevented from host feeding and parasitoids allowed to host feed.

Remains the possibility that we did not find a positive effect of host feeding on egg load or egg maturation with our experimental set-up. One host feeding might have been insufficient to detect an effect, especially after several days of host deprivation (factor *h* was excluded from the model explaining *el48h* on the basis of the close to significant P-value of 0.0545). The egg maturation delay might have been different than assessed beforehand. Rivero & Casas (1999b) used radioactive labelling to show that the host-feeding gain is not a discrete event but is spread over the lifetime of a parasitoid. Ideally, lifetime egg load dynamics or oviposition rates are measured of parasitoids continuously prevented from or allowed to host feed in the absence and the presence of honeydew. This would also correct for the unbalanced availability of honeydew over hemolymph. This is very difficult, however, because natural conditions regarding probabilities of host encounter and survival need to be simulated but are practically unknown. The absence of an effect of host-feeding time could be explained by the fact that parasitoids were freely allowed to host feed until saturation. Experimental manipulation of host-feeding time might result in a positive effect. In addition, host feeding possibly promotes other traits not included in our study, e.g. walking speed and thus host encounter probability, or resistance against diseases.

The most striking result of the present study is undoubtedly the fact that honeydew did have a clear positive effect on fecundity and egg maturation initially expected from host feeding. Even after 20 days, honeydew had a positive effect on egg load compared with sucrose solution only, suggesting a direct nutritional effect. Because parasitoids that had access to honeydew also had higher egg loads, honeydew could also increase survival indirectly through oosorption (Fig. 3b). Previous studies showed little or even negative effects of honeydew on adult fecundity and survival (Leius 1961; Avidov, Balshin & Gerson 1970). This could be attributed to toxic effects of secondary plant compounds or low suitability of insect-synthesised sugars like melezitose (Wäckers 2000; Zoebelein 1955) or raffinose (Wäckers 2001). However, in the honeydew of the silverleaf whitefly *Bemisia argentifolii* the most abundant sugar is the unusual disaccharide trehalulose (Byrne & Miller 1990; Tarczynski, Byrne & Miller 1992; Hendrix, Wei & Leggett 1992; Hendrix & Wei 1994; Yee *et al.* 1996), probably produced by obligate symbiotic microorganisms (Davidson *et al.* 1994). Possibly, the trehalulose component accounts for the positive honeydew effect found in our experiments. Trehalulose excretion is affected by several factors, e.g. host plant species and whitefly stage (Costa *et al.* 1999), dietary sucrose concentration (Salvucci, Wolfe &

Hendrix 1997), ambient temperature (Salvucci, Hendrix & Wolfe 1999), and water-stress of the host plant (Isaacs, Byrne & Hendrix 1998). Consequently, the net suitability of honeydew as an advantageous alternative to hemolymph will depend on a complex set of environmental conditions.

In conclusion, the positive effects of honeydew call for a reconsideration of previous studies of host-feeding benefits, especially in parasitoids of honeydew-producing hosts. Second, the function of host feeding in *E. formosa* remains unsolved and the species remains a confusing exception to host-feeding theory. Possibly, destructive host feeding is unavoidable to be able to produce anhydrotic eggs, which might have an advantage over hydrotic eggs in the parasitoid's natural environment.

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3

An evolutionary advantage of destructive host feeding over non-destructive feeding on honeydew

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Abstract

Host feeding is the consumption of host blood and tissue by the adult female parasitoid. Although it enhances future reproduction or survival, it kills the host and therefore destroys an opportunity to oviposit. Natural selection would strongly act against destructive host feeding when feeding on honeydew could be an advantageous non-destructive alternative. In this paper we therefore seek for an evolutionary advantage of host feeding over feeding on honeydew in terms of fecundity, longevity and egg volume. During 5 days, *Encarsia formosa* parasitoids (Hymenoptera, Aphelinidae) were daily allowed to oviposit in fourth instar nymphs of the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera, Aleyrodidae) until host feeding was attempted, or until 3 hours were spent foraging. Host feedings were either prevented or allowed. Parasitoids had *ad libitum* access to honeydew between foraging bouts. After 5 days, parasitoids were put on an *ad libitum* diet of sucrose solution only and their survival was scored. Additionally, we studied effects of non-host and host-derived food sources water, sucrose solution and honeydew on egg load dynamics. Even in the presence of honeydew, parasitoids allowed to host feed laid significantly more eggs per hour of foraging per host-feeding attempt (1.25 ± 0.38) than parasitoids prevented from host feeding (0.84 ± 0.43). Although parasitoids allowed to host feed laid more eggs during the experiment than parasitoids prevented from host feeding (27.57 ± 7.59 vs. 19.44 ± 5.68), they did not differ in survival probability (median longevity 31 vs. 29 days), nor in the change in estimated egg volume over time ($-3E3 \pm 6E3$ vs. $-1E3 \pm 8E3 \mu m^3$ per day). Parasitoids with only access to water quickly started resorbing eggs and

all died within 6 days. The average egg load of parasitoids with access to food initially increased to about 10 (sucrose), 12 (honeydew) and 14 (sucrose and honeydew) eggs at day 2. Between day 2 and 10 net oosorption was about 0.42 eggs per day. We conclude that destructive host feeding can have an advantage over non-destructive feeding on honeydew, especially for egg-limited parasitoids. At low host densities, however, feeding on non-host or host-derived food sources could supply enough nutrients to prevent egg limitation. Time- or host-limited parasitoids may benefit from host feeding by resorbing the extra eggs to increase life-span and thus searching time, or by using the extra eggs to exploit rare host aggregates.

Introduction

Life-history theory deals with trade-offs that arise when change in a trait that increases fitness changes another trait that decreases fitness (Stearns 1992; Daan & Tinbergen 1997). If the trade-off is genetically variable, selection can occur in the population towards an optimal allocation of limited resources between two or more traits. This optimal allocation can be constrained e.g. by physical, physiological, genetic, or phylogenetic restrictions. By analysing the phenotypic value of different life-history traits, life-history theory helps to explain phenotypic variation and adaptation.

Two of the most studied trade-offs between life-history traits are the ones between current and future reproduction, and between reproduction and survival. In some Hymenoptera, such trade-offs are reflected in the decision to parasitise or feed upon a host by the adult female parasitoid (e.g. Heimpel & Rosenheim 1995). An adult female parasitoid that parasitises a host through oviposition invests in current reproduction, which is the most direct way to realise fitness. However, oviposition results in fewer eggs that can be used for future reproduction or survival through egg resorption. Host feeding is the consumption of host blood and tissue by the adult female parasitoid and provides nutrients that can be used to mature eggs (Jervis & Kidd 1986; Heimpel & Collier 1996). Indeed, host-feeding species tend to have a lower fraction of their potential egg production matured upon emergence than non-host-feeding species (Jervis & Kidd, 1986; Jervis *et al.* 2001). One host feeding often yields more future eggs than can currently be laid in one host (Collier 1995a; Heimpel *et al.* 1994, 1997a; Rivero & Casas 1999b). In some species, host feeding also increases longevity (Heimpel & Collier 1996). Thus, an adult female parasitoid that feeds upon a host invests in future reproduction and survival. Trade-offs arise because a single host cannot be used for both oviposition and host feeding. The adult female usually kills the host by feeding upon it (Jervis & Kidd 1986; Ueno 1999b). If not, it still reduces the already limited amount of resources available to the parasitoid's offspring. The decision to parasitise or host feed therefore represents trade-offs between current and future reproduction and between reproduction and survival.

In Chapter 2, we addressed the question what the evolutionary advantage is of destructive host feeding over non-destructive feeding on honeydew. Honeydew is

exploited by insect parasitoids (Wäckers 2000; Jervis *et al.* 1996). It contains amino acids in significant amounts (Auclair 1963; Maurizio 1975; van Vianen 1982, 1987; Cochran 1985; Crane & Walker 1986b; Byrne & Miller 1990), needed to sustain oogenesis. Thirdly, searching for alternative food sources usually reduces time that can be spent on searching for hosts (Wäckers 1994; Takasu & Lewis 1995). In case of homopterid parasitoids, however, feeding on honeydew does not reduce host-searching time because it is produced by the host and can be imbibed directly from the host's anus (Jervis *et al.* 1996; Zoebelein 1955; Vos 1995).

Encarsia formosa (Hymenoptera, Aphelinidae) is a homopterid parasitoid attacking at least 13 species of whitefly (Homoptera, Aleyrodidae) (Schauff *et al.* 1996; Polaszek *et al.* 1992). It is successfully applied worldwide to control whitefly pests in greenhouses (van Lenteren *et al.* 1996). Honeydew excretion is one of the reasons why whitefly have a pest status. Honeydew enhances development of moulds that reduce leaf photosynthesis, and residues on fruits and ornamentals cause economic damage (van Lenteren & Noldus 1990). Application of *E. formosa* as biological control agent has revealed a lot of information on its biology and life-history parameters (reviewed by Viggiani 1984; Noldus & van Lenteren 1990; van Roermund & van Lenteren 1992b; Hoddle *et al.* 1998). *E. formosa* is a host-feeding, larval endoparasitoid with a very low ovigeny index (Jervis *et al.* 2001; van Lenteren *et al.* 1987), yolk-rich (anhydropic) eggs (Agekyan 1981), and the ability to resorb them (van Vianen & van Lenteren 1986b). Nevertheless, although tested, the function of host feeding was not empirically shown in *E. formosa* (Chapter 2; van Lenteren *et al.* 1987; Gast & Kortenhoff 1983).

Using manipulation experiments, we found in Chapter 2 that *E. formosa* parasitoids allowed to host feed neither had higher egg loads nor matured more eggs than parasitoids prevented from host feeding. Parasitoids with access to honeydew, on the other hand, both had higher egg loads and matured more eggs than parasitoids without access to honeydew. In the presence of honeydew, host feeding did not have a positive effect on survival. Feeding on honeydew could therefore be an advantageous alternative to destructive host feeding.

The results obtained in Chapter 2 might be explained by quantitative differences between host-feeding gain and honeydew availability. Moreover, other traits like egg volume could benefit more from host feeding than from feeding on honeydew. Aim of this paper was therefore to investigate whether several host feedings can have an advantage over honeydew-feeding regarding quantity and quality of eggs produced. Since parasitoids can use eggs not only for reproduction but also for survival through oosorption (Bell & Bohm 1975), a positive effect on egg production might be counteracted by a negative effect on survival. Therefore, the effect of host feeding on survival was also measured.

Materials & Methods

Life materials

Tomato plants (Solanaceae, *Solanum lycopersicum* L. cv. Moneymaker) were reared by Unifarm, the Netherlands, at 21°C, 70% R.H. and L:D=16:8 hours. Nymphs of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) were obtained from the whitefly culture on tomato at the Laboratory of Entomology, Wageningen, the Netherlands. Pupae of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) parasitoids were obtained from Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands.

Experimental set-up

Effect of host feeding on oviposition, survival and egg quality

E. formosa pupae were allowed to emerge over a period of 24 hours in a Petri dish containing honeydew. To collect honeydew, a whitefly infested tomato leaflet was inverted on a piece of moist cotton wool in the bottom part of a Petri dish, which was then covered by parafilm. After one day, the parafilm containing droplets of honeydew was cut into pieces of about 5 cm² and offered to the parasitoids. After the cards had been removed, 0 to 1-day-old parasitoids were allowed to forage individually on a whitefly-infested patch up to a maximum of 3 hours. The patch consisted of an inverted tomato leaflet on a piece of moist cotton wool, and contained 25 fourth instar whitefly nymphs in a grid of 5×5. Nymphs had been carefully removed from their original feeding site and transferred to the experimental arena. In both treatments parasitoids were allowed to oviposit until host feeding was attempted. Individuals in one treatment (P) were prevented from host feeding by removing them as soon as host feeding was attempted. Individuals in the other treatment (A) were allowed to feed upon one host and removed directly thereafter. Parasitoids were then transferred back to their Petri dish containing honeydew. Each parasitoid was allowed to forage in this way for five consecutive days. Parasitoids in treatment P could thus be prevented from host feeding up to five times, whereas parasitoids in treatment A had five opportunities to host feed. Within each observation period, we determined the number of ovipositions by nymphal dissection and scored foraging time (host-feeding time excluded), whether or not host feeding was attempted, and host-feeding time. As a measure of egg quality, egg length and width were measured in phosphate-buffered saline at 400 power of each egg laid from replicate 15 onwards. After the fifth observation period, parasitoids were transferred to a Petri dish containing a piece of cotton wool pad drenched in a 10 % w/w sucrose solution, instead of honeydew. The sucrose solution was replaced daily and the parasitoids were kept in this Petri dish until they died. Experiments were conducted at 25 °C and L:D=16:8 hours.

Effect of hemolymph alternatives on egg load dynamics

E. formosa pupae were allowed to emerge over a period of 24 hours in a Petri dish containing water only (C), 10 % w/w sucrose solution (S), honeydew (D), or 10% w/w sucrose solution and honeydew (SD). Each diet was sprayed as droplets on a piece of parafilm to equalise accessibility. Sucrose solution was replaced daily; water and honeydew were replaced twice a day because of higher rates of evaporation or crystallisation. Honeydew was collected as described above. Parasitoids were dissected and egg loads were measured before feeding, and 2, 4, 6, 8 and 10 days after emergence.

Statistical analysis*Effect of host feeding on oviposition, survival and egg quality*

Of each parasitoid, repeated measures were taken over time, and the daily number of ovipositions could co-vary with daily foraging time, i.e. the time spent on the leaflet excluding host-feeding time, and with whether or not host feeding was attempted within the 3-hour period. To overcome these difficulties, the total number of ovipositions per parasitoid was divided by the total foraging time and the total number of host-feeding attempts. Only 1 parasitoid had to be excluded from this analysis because it never attempted host feeding during the 5 days of foraging. The effect of allowance of host feeding (indicator variable $h = 0/1 = \text{prevented/allowed}$) was tested on this particular response variable, i.e. number of ovipositions per hour per attempt, in a general linear model ($E\{y_i\} = \beta_0 + \beta_1*htl_i + \beta_2*h_i$). Hind tibia length (htl) was used as covariate because it is a reliable measure of parasitoid size, which may reduce the error variance.

The effect of allowance of host feeding (h) on survival after 5 days of foraging was tested using Kaplan-Meier survival analysis (SPSS version 10.0.5).

Egg volume v (μm^3) was estimated using the equation for a prolate spheroid, $v = 4/3*\pi*(l/2)*(w/2)^2$ (Otto & Mackauer 1998), where w is egg width (μm) and l egg length (μm). Because for each parasitoid repeated measures were taken over time, a regression coefficient (bl_j) was estimated per parasitoid by regressing the estimated volume of egg i of parasitoid j to time t ($E\{v_{ij}\} = \beta_{0j} + \beta_{1j}*t_{ij}$). The effect of allowance of host feeding (h) on the estimated regression coefficients bl_j (μm^3 per day) was tested in a general linear model again using hind tibia length (htl) as covariate ($E\{bl_j\} = \beta_0 + \beta_1*htl_j + \beta_2*h_j$).

Effect of hemolymph alternatives on egg load dynamics

In this experiment, measurements over time were independent. A generalised linear model with Poisson distribution and log link function was applied using egg load (el) as response variable, time (t) as predictor variable and presence of sucrose solution ($s = 0/1 = \text{absent/present}$) and honeydew ($d = 0/1 = \text{absent/present}$) as indicator variables ($\log(E\{el_i\}) = \beta_0 + \beta_1*t_i + \beta_2*s_i + \beta_3*d_i + \beta_4*t_i*s_i + \beta_5*t_i*d_i + \beta_6*s_i*d_i + \beta_7*t_i*s_i*d_i$).

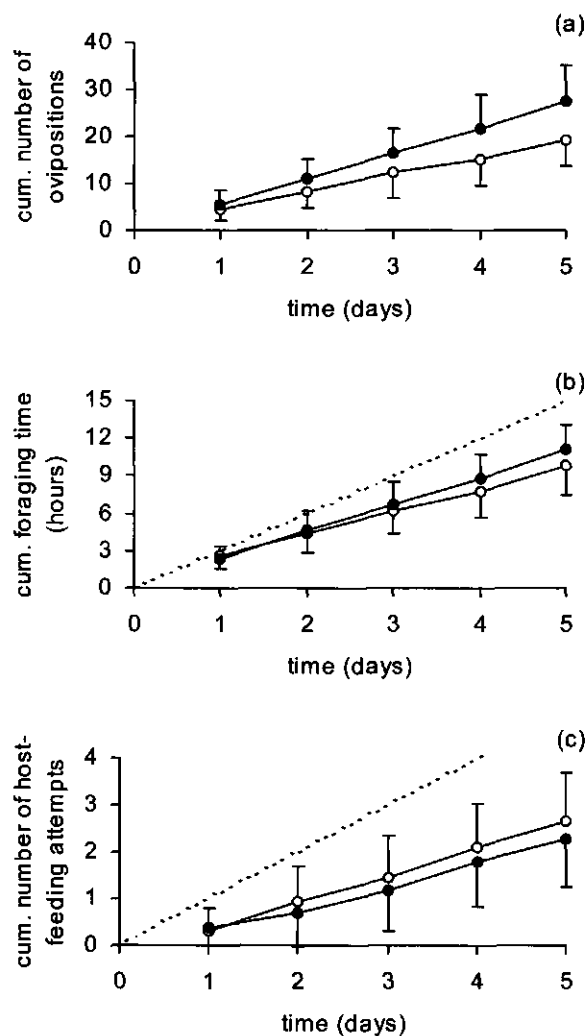


Fig. 1 Foraging behaviour of *E. formosa* parasitoids that were daily allowed to oviposit until host feeding was attempted, or until 3 hours were spent foraging, during 5 days. Host feedings were either prevented (open symbols, $n_{\text{day}1,2,3,4,5} = 28, 28, 27, 27, 25$ parasitoids) or allowed (closed symbols, $n_{\text{day}1,2,3,4,5} = 30, 29, 28, 28, 28$ parasitoids). Mean \pm sd of (a) cumulative number of ovipositions, (b) cumulative foraging time (hours), (c) cumulative number of host-feeding attempts. Dotted lines show maximum values imposed by the experimental procedure.

Model building: selection of predictor variables, indicator variables and covariates

To test which parameters differed significantly from 0, partial F tests (when Normal distribution assumed) or partial deviance tests (when Poisson distribution assumed) were used (Neter *et al.* 1996). A model with 1 parameter (the intercept) was tested against all possible models with 2 parameters. The model that gave the highest test statistic (F or partial deviance) was tested against all possible models with 3 parameters, if its test statistic exceeded the critical value $F(0.05;1;n-p)$ or $\chi^2(0.95;1)$. This was repeated until the test statistic became smaller than or equal to the critical value, or the full model was reached. If the test statistic became smaller than or equal to the critical value, the selected model with p parameters was tested against all possible models with more than $p+1$ parameters. If this resulted in a test statistic larger than the critical value, the analysis was continued using the better model with more than $p+1$ parameters. Models with interaction terms were only considered if associated main effects and lower-order interactions were also included.

Results

Effect of host feeding on oviposition, survival and egg quality

In Fig. 1, the mean (\pm sd) cumulative number of ovipositions, foraging time and number of host-feeding attempts in the course of time are compared between parasitoids allowed to host feed and parasitoids prevented from host feeding. Parasitoids allowed to host feed laid on average 5.41 ± 1.61 eggs per day, 1.43 times the average daily oviposition rate of parasitoids prevented from host feeding (3.79 ± 1.38). After 5 days,

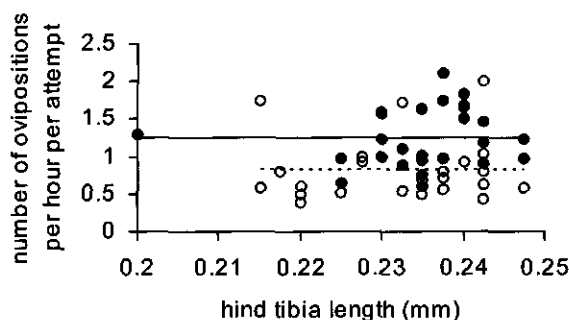


Fig. 2 Number of ovipositions per hour of foraging per host-feeding attempt plotted against hind tibia length (mm) when host feedings were either prevented (open symbols, broken line, $h = 0$) or allowed (closed symbols, solid line, $h = 1$). Lines represent most parsimonious general linear model: $y = 0.8405 + 0.4107 \cdot h$.

the former had laid 27.57 ± 7.59 eggs, the latter 19.44 ± 5.68 . Parasitoids allowed to host feed, however, also foraged on average 1.13 times longer than parasitoids prevented from host feeding (2.09 ± 0.57 vs. 1.84 ± 0.58 hours per day). After 5 days, the former had spent 11.09 ± 1.95 hours foraging on the patch, the latter 9.87 ± 2.36 hours. In addition, parasitoids allowed to host feed attempted to do so slightly less often (64 of 143 cases, i.e. 45 %) than parasitoids prevented from host feeding (66 of 135 cases, i.e. 49 %). The treatment effect was most pronounced at day 2 (Fig. 1c). After 5 days, parasitoids allowed to host feed had attempted 2.29 ± 1.05 times to do so compared with 2.64 ± 1.04 attempts by parasitoids prevented from host feeding. If allowed, parasitoids host-fed during 10.9 ± 13.7 min.

Thus, host feeding had a positive effect on the oviposition rate, but also affected foraging time and the number of host-feeding attempts. Fortunately, we were able to disentangle the effect of host feeding from the effect of removing parasitoids after host feeding. Fig. 2 shows that even per hour of foraging and per host-feeding attempt, parasitoids allowed to host feed laid significantly more eggs (1.25 ± 0.38) than parasitoids prevented from host feeding (0.84 ± 0.43) ($P = 0.0006$). Hind tibia length did not significantly contribute to the explanatory power of the model ($P = 0.9020$) and was therefore not useful as a covariate ($y = 0.8405 + 0.4107 \cdot h$).

After 5 days of foraging in a rich environment with hosts and honeydew, parasitoids were transferred to a diet of sucrose only. Although parasitoids allowed to host feed laid more eggs per hour per attempt (Fig. 2), their survival was not significantly lower than parasitoids prevented from host feeding (Fig. 3, Kaplan-Meier: logrank = 0.68, $P = 0.4079$). Median longevity of parasitoids was 29 (host feeding prevented) and 31 (host feeding allowed) days. Thus, the increased oviposition rate in parasitoids allowed to host feed was not offset by a decreased longevity.

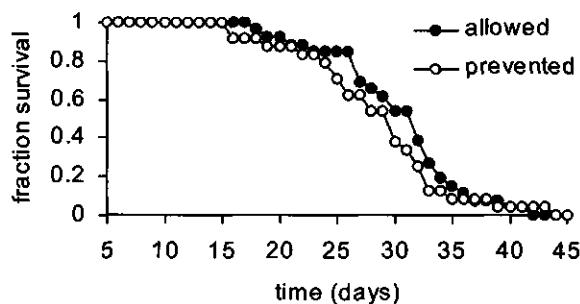


Fig. 3 Survival of parasitoids after the first 5 days of life. During the first 5 days parasitoids were daily allowed to oviposit until host feeding was attempted, or until 3 hours were spent foraging. Host feedings were either prevented (open symbols, $n = 25$ parasitoids including 1 censored) or allowed (closed symbols, $n = 28$ parasitoids including 2 censored). After the first 5 days parasitoids had access to sucrose solution only.

Fig. 4a shows mean \pm sd of estimated egg volumes per day. Eggs laid by parasitoids allowed to host feed had an average estimated volume of $110\text{E}3 \pm 37\text{E}3 \mu\text{m}^3$ ($n = 426$ eggs by 18 parasitoids), compared with $108\text{E}3 \pm 41\text{E}3 \mu\text{m}^3$ by parasitoids prevented from host feeding ($n = 265$ eggs by 15 parasitoids). The change over time per parasitoid in estimated egg volume was not significantly different between parasitoids allowed to host feed (on average $-3\text{E}3 \pm 6\text{E}3 \mu\text{m}^3$ per day) and parasitoids prevented from host feeding (on average $-1\text{E}3 \pm 8\text{E}3 \mu\text{m}^3$ per day) (Fig. 4b). Neither a model including allowance of host feeding ($P = 0.4223$), nor one including hind tibia length ($P = 0.2878$), nor one including both ($P = 0.4113$) performed better than the overall mean. Thus, the increased oviposition rate in parasitoids allowed to host feed was also not offset by a decrease in egg volume.

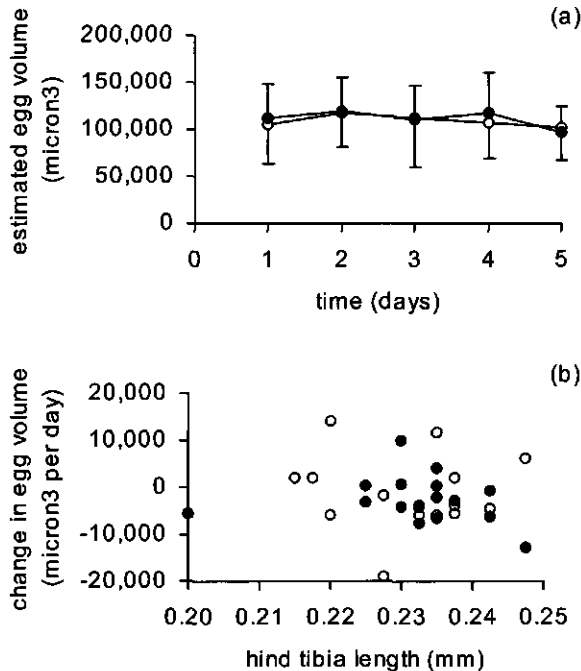


Fig. 4 (a) Mean \pm sd of estimated egg volume (μm^3) over time. Parasitoids were daily allowed to oviposit until host feeding was attempted, or until 3 hours were spent foraging. Host feedings were either prevented (open symbols; $n_{\text{day}1,2,3,4,5} = 68, 46, 44, 45, 62$ eggs, respectively; {fraction of parasitoids ovipositing} $\}_{\text{day}1,2,3,4,5} = {}^{15}/_{15}, {}^{13}/_{15}, {}^{11}/_{15}, {}^{11}/_{15}, {}^{15}/_{15}$, respectively) or allowed (closed symbols; $n_{\text{day}1,2,3,4,5} = 84, 84, 83, 79, 96$ eggs, respectively; {fraction of parasitoids ovipositing} $\}_{\text{day}1,2,3,4,5} = {}^{17}/_{18}, {}^{17}/_{18}, {}^{16}/_{18}, {}^{17}/_{18}, {}^{18}/_{18}$, respectively). (b) Change per parasitoid in egg volume (μm^3 per day) plotted against hind tibia length when host feedings were either prevented (open symbols, $n = 15$ parasitoids) or allowed (closed symbols, $n = 18$ parasitoids).

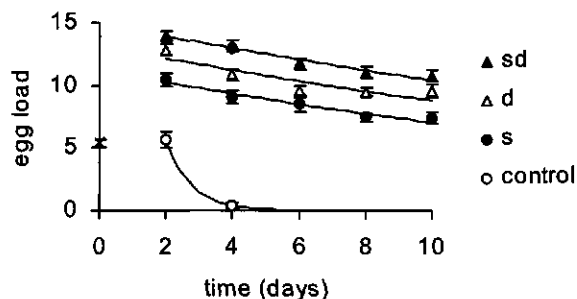


Fig. 5 Egg load dynamics (mean \pm se) of parasitoids without (open symbols, $s = 0$) and with (closed symbols, $s = 1$) *ad libitum* access to sucrose solution, and without (circles, $d = 0$) and with (triangles, $d = 1$) *ad libitum* access to honeydew. Lines represent generalised linear model with Poisson distribution and log link applied on data from day 2 onwards: $\log(y) = 4.4178 - 1.3406 \cdot x + 0.1261 \cdot s + 0.2839 \cdot d + 0.0045 \cdot x \cdot s + 0.011 \cdot x \cdot d - 2.1225 \cdot \min(s+d, 1) + 1.2891 \cdot x \cdot \min(s+d, 1)$. Number of observations per treatment were 28 (day 2); control: 18, rest: 27 (day 4); 27 (day 6); 20 (day 8) and 17 (day 10). Cross indicates mean \pm se of egg load immediately after emerging and before access to food ($n = 26$).

Effect of hemolymph alternatives on egg load dynamics

Fig. 5 shows egg load dynamics of parasitoids with access to water only (control), sucrose solution, honeydew, or both. The full model with all interaction terms performed significantly better than any simpler model ($P < 0.0001$; $\log(y) = 4.4178 - 1.3406 \cdot x + 0.1261 \cdot s + 0.2839 \cdot d + 0.0045 \cdot x \cdot s + 0.0110 \cdot x \cdot d - 2.1225 \cdot \min(s+d, 1) + 1.2891 \cdot x \cdot \min(s+d, 1)$). As a consequence, $\exp(\beta_i)$ can no longer be interpreted as the change in y with 1 unit increase in x . Nevertheless, access to sucrose solution, honeydew or both had a strong positive effect on egg load and survival compared with access to water only. Parasitoids with only access to water quickly started resorbing eggs. Most of them had died after 4 days and none of them survived for 6 days. The average egg load of parasitoids with access to food initially increased to about 10 (sucrose), 12 (honeydew) and 14 (sucrose and honeydew) eggs at day 2. Between day 2 and 10 net oosorption was about 0.42 eggs per day.

Discussion

Even in the presence of honeydew, *E. formosa* parasitoids allowed to host feed laid significantly more eggs per hour of foraging per host-feeding attempt than parasitoids prevented from host feeding. Although the former laid more eggs during the experiment, they did not differ in survival probability, nor in the change in egg volume, from

parasitoids prevented from host feeding. Thus, the higher oviposition rate and realised fecundity were actually "paid" by acquisition of nutrients from the host, rather than by going to the expense of the other two traits. Estimated egg volumes correspond well with the value of $109\text{E}3 \mu\text{m}^3$ estimated from measurements by Agekyan (1981). Since parasitoids had *ad libitum* access to honeydew as a potential alternative to hemolymph during the 5-day period of foraging, results show that host feeding, although destroying an opportunity to oviposit, can have an evolutionary advantage over non-destructive feeding on honeydew.

Previously, we found that non-destructive feeding on honeydew could be an alternative to destructive host feeding (Chapter 2). Destructive host-feeding behaviour could have evolved if the advantage of feeding upon hosts over feeding on honeydew alone, i.e. an increased oviposition rate, could offset the loss of oviposition sites. An increased fecundity e.g. through host feeding is only necessary when hosts are abundant and parasitoids run the risk of becoming (temporarily) egg limited. In the experimental set-up used in this chapter, host density and foraging time allowed parasitoids to become egg limited. Under these conditions host feeding in addition to feeding on honeydew did have an advantage over feeding on honeydew alone. On the other hand, the decision to oviposit or host feed only becomes a trade-off between current and future reproduction when hosts rather than eggs are limiting. Destroying an opportunity to oviposit through host feeding is only costly at low host densities. A striking conclusion from our work is that at low host densities, parasitoids could maintain a sufficiently number of viable eggs by feeding on alternative food sources like nectar and honeydew (Fig. 5; van Vianen & van Lenteren 1986b; van Lenteren *et al.* 1987; Chapter 2).

In our experiment, parasitoids used the host-feeding gain to increase oviposition rate. If parasitoids are limited in the available searching time, e.g. when hosts are scarce or bad weather enforces parasitoids to interrupt foraging (Fink & Völkl 1995), the extra eggs from host feeding could instead be used to increase life-span through egg resorption. This could only be adaptive when instant mortality is not the main determinant of life expectancy. Predation, however, can have a considerable effect on instant mortality (Heimpel *et al.* 1997b). Another possibility is that stochasticity in host encounter rate, e.g. by an aggregated host distribution, can lead to optimal egg loads exceeding the expected number of hosts encountered, not only in proovigenic parasitoids (Sevenster *et al.* 1998; Ellers *et al.* 2000) but also in synovigenic parasitoids (Ellers *et al.* 2000). Thus, not only egg-limited parasitoids but also host- or time-limited parasitoids may benefit from host feeding by resorbing the extra eggs to increase life-span and thus searching time or by using the extra eggs to exploit rare host aggregates.

To analyse the trade-off between current and future reproduction and to predict optimal host-feeding decisions (e.g. Houston *et al.* 1992; Chan & Godfray 1993; Collier *et al.* 1994; Collier 1995b; Heimpel *et al.* 1994, 1998; McGregor 1997), the costs and benefits, especially the host-feeding gain, need to be quantified. In *Aphytis melinus*, females allowed to feed on honey and one host had about 1.5 eggs more after 24 h than females that were allowed to feed on honey alone (Collier 1995a). In *Aphytis lingnanensis*, females allowed to oviposit and to feed on one host matured on average

2.3 eggs more in two days than females allowed to oviposit only (Heimpel *et al.* 1994). In *E. formosa*, females laid 1.8 eggs per host feeding per day on the greenhouse whitefly *Trialeurodes vaporariorum* and 2.6 on the silverleaf whitefly *Bemisia tabaci* (Szabo *et al.* 1993). We found an increase in oviposition rate of 0.41 eggs per hour of foraging per host-feeding attempt. Because in our experiment parasitoids spent on average about 10 hours on foraging during the 5-day period, our estimated host-feeding gain would be at least 4.1 eggs. Alternatively, parasitoids allowed to host feed laid on average 8.13 eggs more than parasitoids prevented from host feeding during the 5-day period. This corresponds to about 3.6 eggs per host feeding, since on average 2.29 hosts were fed upon during the 5-day period. This estimate is based on eggs laid, assuming that parasitoids in both treatments emptied their ovaries down to the same value. Furthermore, it is merely a minimum 5-day gain, because the later host-feeding meals were probably not fully converted into eggs yet. Our estimates are higher than found in most references because materials obtained from host feeding can be stored for oogenesis later in life (Heimpel *et al.* 1997a; Rivero & Casas 1999b).

In conclusion, destructive host feeding can have an advantage over non-destructive feeding on honeydew, especially for egg-limited parasitoids. At low host densities, however, feeding on non-host or host-derived food sources could supply enough nutrients to prevent egg limitation. Time- or host-limited parasitoids may benefit from host feeding by resorbing the extra eggs to increase life-span and thus searching time, or by using the extra eggs to exploit rare host aggregates. Whether the host-feeding gain, estimated at 4 eggs minimum, offsets the loss of opportunities to oviposit depends on host density and distribution and exploitation by the parasitoid of alternative food sources in the field. No field data are available yet on the ecological conditions under which host-feeding behaviour of *E. formosa* has evolved before it was commercially produced. Although we did not find an effect of host feeding on egg volume, there could still be other effects of host feeding e.g. on egg viability. Finally, the evolutionary advantage of host feeding over feeding on honeydew remains to be studied in homopterid parasitoids other than *E. formosa*.

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4

Natural history of whitefly in Costa Rica: an evolutionary starting point

J.M.S. Burger, G. Gort, J.C. van Lenteren & L.E.M. Vet

Abstract

To understand evolution of foraging behaviour in the whitefly parasitoid *Encarsia formosa* (Hymenoptera, Aphelinidae), we quantified natural densities and distributions of whitefly (Homoptera, Aleyrodidae) in *E. formosa*'s presumed area of origin, the Neotropics. Leaves were collected in Costa Rican nature areas in 1999 and 2001 along long transects (2 to 4 km), short transects (100 m), within three-dimensional plots (50 dm³ to 2.3 m³) and once along suspension bridges within the canopy, and checked for presence of whitefly nymphs. At most sites, an average of 0.5 to 2 whitefly nymphs per leaflet was found. Of the randomly collected leaflets, 71 % was empty, whereas some carried up to several dozens of nymphs. Usually over 95 % of the whitefly nymphs was on the lower side, and 20 to 90 % was in a developing stage. In the canopy (21 to 37 m above ground), on average only 0.06 nymphs per leaflet were found, compared with 1.3 at the forest floor (0 to 3 m) in the same area. Generalised linear mixed modelling revealed that the number of hosts on the lower side of a leaflet of an average plant within an average spot of an average transect could be described by a Poisson distribution with mean and variance equal to 0.241, in a ratio of I1:I2:I3:I4 = 0.14:0.23:0.26:0.37. The Poisson mean was largely affected by the plant ($\sigma_{plant}^2 = 2.485$) and less by the spot ($\sigma_{spot}^2 = 0.197$) or transect ($\sigma_{trsect}^2 = 0.522$). Variation between plants could hardly be explained by variation in leaf area ($\sigma_{plant}^2 = 3.605$ when only plant was incorporated as random effect and $\sigma_{plant}^2 = 2.698$ when leaf area was added). Based on the shape of the opening in vacated puparia, the probability that a whitefly became eventually parasitised was 0.12 on an average leaflet. Parasitism was more patchily distributed among spots ($\sigma_{spot}^2 = 1.006$) but more evenly distributed among plants within spots ($\sigma_{plant}^2 = 0.952$) than whitefly nymphs. Semivariance analysis was used to quantify the degree and scale

of spatial dependence of whitefly nymphs. In 1 of 3 short transects, the numbers of whiteflies on leaves were spatially dependent. This was not a result of patchiness in host plants because it was in 1 of the 2 transects where leaves were collected from a continuous understorey of a single host plant genus (*Piper* sp., Piperaceae). In 4 of 7 three-dimensional plots at least one level of spatial dependence could be detected; 3 of 4 within a single host plant species. In one plot, patches of about 16 cm were nested within patches of about 37 cm. In the other three, similar patch sizes of 41, 20 and 15 cm were found in seclusion. Results are discussed in the context of understanding evolution of foraging behaviour by *E. formosa*.

Introduction

To understand the relationships of organisms to each other and to their *oikos*, as ecologists aim, it makes sense to first study the *oikos*, Greek for house or home. Home is the natural environment where selection pressures establish fitness differences between individuals with different genotypes (Ricklefs 1990). A genotype that gives rise to a higher fitness than another genotype will be selected for. The fitness value of a genotype depends, however, on the environment. If we are to interpret form, function and behaviour of an organism as an adaptation to its natural environment, a description of the natural environment (the natural history) is indispensable. The natural environment defines the conditions under which form, function and behaviour of an organism have evolved and are evolving.

Behaviour of the biological control agent and whitefly parasitoid *Encarsia formosa* (Hymenoptera, Aphelinidae) has been studied extensively in the laboratory and greenhouse (Noldus & van Lenteren 1990; Hoddle *et al.* 1998). Many aspects on biology and life-history parameters are known (van Roermund & van Lenteren 1992b) and have been incorporated into simulation models on foraging behaviour (van Roermund *et al.* 1996, 1997a,b) and population dynamics (van Roermund *et al.* 1997c). This has resulted in a thorough understanding of how biological control of greenhouse whitefly with *E. formosa* works (van Lenteren *et al.* 1996). Much less is known, however, about *E. formosa*'s behaviour from an evolutionary point of view and its ecology in nature. We are developing among others an optimal foraging model to study the effects of environmental factors on the foraging and reproductive behaviour of *E. formosa*, in particular the decision to reject, host feed or parasitise a host (for reviews on the subject see Jervis & Kidd 1986; Heimpel & Collier 1996). Because host feeding results in whitefly mortality but not in parasitoid offspring, understanding host-handling decisions not only satisfies and stimulates curiosity, it is also essential to develop sustainable pest management (van Lenteren 1999). This requires information on the conditions under which host-handling decisions by *E. formosa* have evolved before it was commercially produced. This information is lacking.

Aim of our field survey was therefore to quantify the natural environment of the successful biological control agent *E. formosa* in its natural area of origin. We focussed

on describing natural host densities and distributions on different spatial scales. First, these parameters are expected to have a profound impact on the trade-off between current and future reproduction. Investment in future reproduction is only adaptive when parasitoids run the risk of egg limitation, which depends on host availability. Second, host availability in the natural environment is expected to differ considerably from artificial conditions like greenhouses. Presently, about 1450 species of whitefly have been named (Martin *et al.* 2000). Only *Bemisia tabaci*, *Trialeurodes vaporariorum*, and a dozen others cause damage in cropping systems because they are extremely polyphagous, showing flexible host selection behaviour and a wide degree of host suitability (Byrne *et al.* 1990). In the natural environment, pest densities are less likely because most whitefly species are oligophagous, colonise woody host plants (Mound & Halsey 1978), live in a heterogeneous habitat (Stewart *et al.* 2000) and have to deal with bad weather, competition, pathogens, predators and parasitoids (Ricklefs 1990).

The geographical region of the natural environment where *E. formosa* has evolved is uncertain. *E. formosa* was first described from specimens collected in Idaho, USA (Gahan 1924). Thus, the extension *formosa* does not refer to the former name of Taiwan in East Asia; instead, it is merely derived from the Latin *formosus* meaning "beautiful" (Brown 1956). *E. formosa* is placed phylogenetically in the *luteola* group, based on morphological characters (Polaszek *et al.* 1992; Gahan 1924), recently supported by analysis of nucleotide sequence data (Babcock *et al.* 2001). Studies on the *luteola* group by Polaszek *et al.* (1992) suggest "beyond reasonable doubt" that the *luteola* group originates from the American continent. The intrinsic rate of increase r_m peaks at 30 °C (van Lenteren *et al.* 1996) and walking and flight activity are hardly observed below 20 °C (van Roermund & van Lenteren 1995a), which further suggests a (sub)tropical origin (Vet *et al.* 1980; Speyer 1927). We therefore assumed that *E. formosa* is native to the Neotropics.

We decided to carry out our fieldwork in Neotropical Costa Rica for a number of reasons. In a survey of parasitoids attacking *Bemisia* whiteflies in Florida, the Caribbean and Latin America, Schuster *et al.* (1998) found that *E. formosa* was relatively abundant in Costa Rica. From extensive Malaise trap sampling throughout Costa Rica, Hanson & Gould (1995) found that the genus *Encarsia* is generally common. Furthermore, as the name implies, the "Rich Coast" is famous for its large variety of tropical habitats (Janzen 1983), which are protected by a relatively well-developed national park system. This allowed us to make generalised estimates of natural whitefly availability. Although Costa Rica has a dry season from December to April, the Caribbean and southern Pacific coasts tend to be wet year round, which creates flexibility within the season of sampling.

Materials & Methods

General

Natural whitefly density and distribution was quantified by sampling leaves in different habitats (Fig. 1) at different spatial levels (Table 1 & 2) in 1999 and 2001. Sampling methods were adjusted to local conditions. First, leaves were sampled along “long transects” of several kilometres to obtain a general description of whitefly availability in the field and to study what factors determine its variation. Second, although parasitoids may disperse by wind over kilometres, they will usually forage actively within decimetres to several metres through leaf hopping (van Roermund *et al.* 1997c). To study the spatial dependence and scale of patchiness of whitefly availability

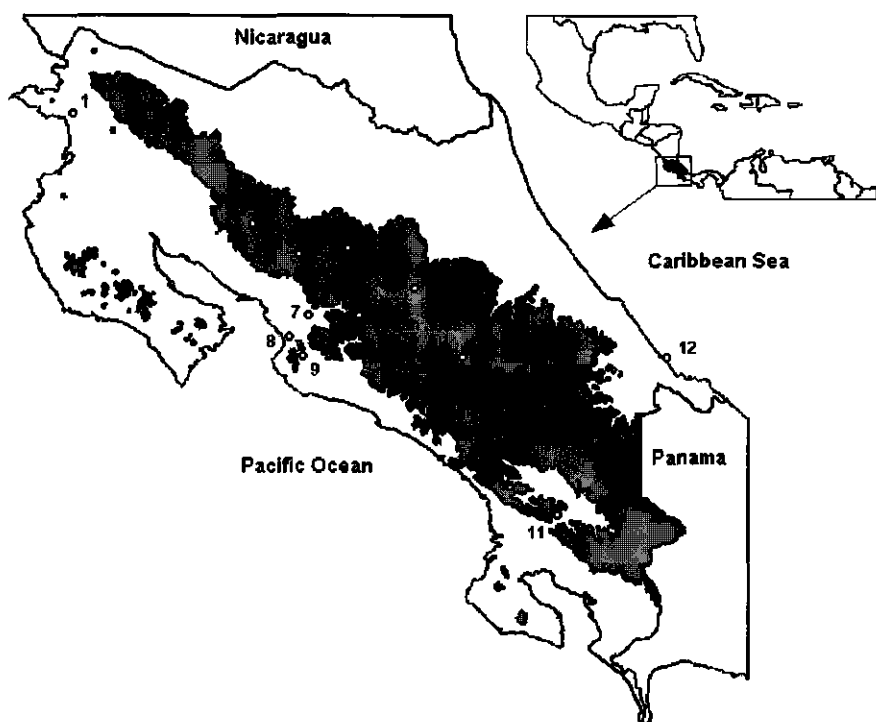


Fig. 1 Map of Costa Rica (8-11° N, 82-86° W) with 500 m and 2000 m contour lines, showing sampling sites: 1 Santa Rosa National Park; 2 Santa Elena Forest Reserve and “sky walk”; 3 Los Alpes Refuge; 4 Juan Castro Blanco National Park; 5 Braulio Carrillo National Park; 6 El Rodeo Protected Area; 7 Finca CUP, Orotina; 8 Carara Biological Reserve; 9 El Sur; 10 Tapanti National Park; 11 Boruca Indian Reserve; 12 Cahuita National Park.

at such scales, leaves were sampled along "short transects" of 100 metres and within "3-D plots" of several decimetres. Third, species richness of arthropods reach very high levels in canopies (Stork *et al.* 1997). To compare whitefly availability between the cool, dark forest floor and the light, airy forest canopy where most of the direct sunlight is intercepted, leaves were sampled at these two strata ("forest floor vs. canopy"). This provided information on to what extent our samples at the forest floor could be an underestimate of whitefly availability.

Long transects

The first long transect was laid out in Carara Biological Reserve, a tropical wet forest at the pacific coast about 90 km west of the capital San José (Fig. 1; Table 1). Leaves were sampled on 18 and 19 May 1999 along the 4-km *Laguna Meandrica* trail parallel to the Tárcoles river. Every 250 m up 3 leaves per plant were collected from 2 plants left and 2 plants right of the trail at a height of 0 to 3 m.

The second long transect was laid out in Tapantí National Park, a tropical premontane wet forest on the northern slopes of the Cordillera de Talamanca, 12 km south-east of Orosi (Fig. 1; Table 1). Leaves were sampled from 15 to 17 June 1999 along the *Sendero Natural Arboles Caídos* ("nature trail of fallen trees"). The first 1050 m the trail went up from an elevation of about 1200 m to about 1600 m in roughly eastern direction (compass angle $106^\circ \pm 35^\circ$, $n=9$). From 1050 m the trail went down again in almost opposite direction (compass angle $246^\circ \pm 48^\circ$, $n=8$). Every 125 m 5 leaves per plant were collected from 2 plants left and 2 plants right of the trail at a height of 0 to 3 m. Flowering plants were preferred over non-flowering plants to facilitate plant identification.

The third long transect was laid out in Santa Rosa National Park, a tropical dry forest at the Pacific coast near the border of Nicaragua (Fig. 1; Table 1). Leaves were sampled on 7 and 8 July 1999 along a trail system in south-western direction (compass angle $217^\circ \pm 43^\circ$, $n=19$). The first 800 m (trail *m* and *s* on GPS map) ran through 200 years old cove forest, and connected to trail *l*, *h*, *g*, *c* and *a* in 80 to 120-year-old tropical dry forest (E. Olson, pers. comm.). Every 125 m, 5 leaves per plant were collected from 2 plants left and 2 plants right of the trail at a height of 0 to 3 m.

Short transects

The first short transect was laid out in the tropical lower montane wet forest of Juan Castro Blanco National Park (Fig. 1; Table 1). The transect ran from the southern edge of the park near the village of Bajo del Toro, 18 km north of Sarchí, straight into the (steep) forest. Every meter, two leaves of one plant species were collected along 100 m on 10 April 2001.

The second and third short transects were laid out along the coastal trail from Kelly Creek to Puerto Vargas in Cahuita National Park, a tropical moist forest at the Caribbean coast near the border of Panama (Fig. 1; Table 1). Here the almost continuous

Table 1 Sites, Holdridge Life Zones (Janzen 1983), dates and methods of transect sampling.

| Method | Site | Holdridge Life Zone ^(a) | Sampling date | Transect length | Sampling distance | # plants per spot | # leaves per plant |
|--------------------------------|------|------------------------------------|-----------------|-----------------|-------------------|-------------------|--------------------|
| Long transects | | | | | | | |
| Carara | | Tropical wet forest | 18-19 May 1999 | 4000 m | 250 m | 4 | 3 |
| Tapanti | | Tropical premontane wet forest | 15-17 June 1999 | 2000 m | 125 m | 4 | 5 |
| Santa Rosa | | Tropical dry forest | 7-8 July 1999 | 2250 m | 125 m | 4 | 5 |
| Short transects | | | | | | | |
| Juan Castro Blanco | | Tropical lower montane wet forest | 10 April 2001 | 100 m | 1 m | 1 | 2 |
| Cahuita I | | Tropical moist forest | 24 April 2001 | 100 m | 1 m | 1 | 1 |
| Cahuita II | | Tropical moist forest | 24 April 2001 | 100 m | 1 m | 1 | 1 |
| Forest floor vs. canopy | | | | | | | |
| Santa Elena Forest Reserve | | Tropical lower montane wet forest | 18 April 2001 | 2500 m | 500 m | 5 | 5 |
| Santa Elena "sky walk" | | Tropical lower montane wet forest | 19 April 2001 | 1800 m | 1 bridge | 5 | 5 |

^(a) Latitudinal region: tropical; altitudinal gradient: sea level, premontane, lower montane, montane, subalpine; precipitation gradient: dry, moist, wet, rain.

Table 2 Sites, Holdridge Life Zones (Janzen 1983), dates, plant species and plot volumes of 3-D sampling.

| Site | Holdridge Life Zone ^(a) | Sampling date | Host plant species (family) | Plot volume (m ³) |
|------------------|------------------------------------|-----------------|--------------------------------------|-------------------------------|
| El Sur | Tropical wet forest | 24-26 May 1999 | <i>Piper</i> sp. (Piperaceae) | 1.74 |
| El Rodeo | Tropical premontane wet forest | 8 June 1999 | <i>Esterhus</i> sp. (Asteraceae) | 0.08 |
| Santa Rosa | Tropical dry forest | 9, 11 July 1999 | <i>Croton niveus</i> (Euphorbiaceae) | 1.42 |
| Los Alpes | Tropical premontane wet forest | 7 May 2001 | 1 unidentified species | 0.05 |
| Braulio Carrillo | Tropical premontane rain forest | 15 May 2001 | 3 unidentified species | 0.10 |
| Orotina | Tropical moist forest | 23 May 2001 | <i>Piper</i> sp. (Piperaceae) | 0.69 |
| Bonuca | Tropical wet forest | 12 July 2001 | several unidentified species | 2.28 |

^(a) Latitudinal region: tropical; altitudinal gradient: sea level, premontane, lower montane, montane, subalpine; precipitation gradient: dry, moist, wet, rain.

understorey was dominated by *Piper* sp. (Piperaceae), a host plant of whitefly. This allowed us to exclude patchiness in host plant distribution as an underlying cause of patchiness in whitefly distribution. Every meter, one leaf of a *Piper* sp. was collected if present along 100 m on 24 April 2001. This was done twice along the trail.

3-D plots

For the three-dimensional plots (Fig. 1; Table 2), whitefly-infested plants were selected, preferably free-growing and monospecific. The spatial co-ordinates of each leaf were measured before leaves were collected. A stick was vertically placed in the centre of the plot. From the centre of each leaf, we measured the distance to the stick, the distance to the ground, and the clockwise angle between the north and the line from the central axis to the centre of the leaf. Alternatively, in two plots (Orotina and Boruca), two of three leaf co-ordinates were measured in 5-cm intervals using auxiliary ropes stretched across the plot as gridlines.

The first plant sampled was a *Piper* sp. (Piperaceae) along a rural trail, about 500 m north of El Sur, a small village south-east of Carara Biological Reserve (see section *Long transects*). Leaves were measured on 24 and 25 May and collected on 26 May 1999. The second plant was an *Esterasus* sp. (Compositae) sampled on 8 June 1999 along *Sendero natural interpretado Las Pacayas* in El Rodeo Protected Area, a tropical premontane wet forest about 10 km south-west of Ciudad Colón. The third plant was a *Croton niveus* (Euphorbiaceae) along trail 1 in Santa Rosa National Park (see section *Long transects*). Leaves were measured on 9 July and collected on 11 July 1999. The fourth plant was an unidentified species sampled on 7 May 2001 in Los Alpes Refuge, a tropical premontane wet forest about 10 km north of San Ramón. The fifth plot consisted of three unidentified plant species sampled on 15 May 2001 in tropical premontane rain forest, about 200 m off the highway from San José to Guápiles just before the entrance of Braulio Carrillo National Park. The sixth plant was a *Piper* sp. (Piperaceae) sampled on 23 May 2001 along an unpaved road at a *finca* owned by *Colegio Universitario de Puntarenas* (CUP) near Orotina, about 40 km east of Puntarenas. The seventh plot consisted of several unidentified plant species sampled on 12 July 2001 at Boruca Indian Reserve, a tropical wet forest about 20 km south of Buenos Aires.

Forest floor vs. canopy

A so-called "sky walk" has been built including five suspension bridges in primary tropical lower montane wet forest halfway between Santa Elena village and Santa Elena Forest Reserve, the northern part of Monteverde Biological Reserve. This enabled us to sample the canopy and thus to study the vertical distribution of whitefly. As a control, the forest floor was sampled along the *Encantado* trail in Santa Elena Forest Reserve. On 18 April 2001, we sampled five locations within the reserve, 500 m apart. The next day we were able to sample four locations along the "sky walk", i.e. bridge 2 (97 m long, 21 m high), 3 (117 m long, 31 m high), 4 (102 m long, 23 m high) and 5 (120 m long,

37 m high). At each location we collected five leaves per plant of five plants (Fig. 1; Table 1).

Leaf handling

Leaves were stored in plastic bags or aluminium foil and kept in a refrigerator if possible. In a laboratory both upper and lower leaf sides were checked for presence of whitefly under a dissecting microscope at 9 (1999) or 5 (2001) power. Leaflets were checked in random order as much as possible to exclude a possible effect of time between collecting and checking on whitefly detectability to the observer. Whitefly nymphs were measured at 27 (1999) or 15 (2001) power, and classified either as first (I1), second (I2), third (I3) or fourth (I4) instar; as "T", based on the T-shaped moulting sutures in a vacated puparium indicating a whitefly adult had emerged (Martin *et al.* 2000); as "P", based on a round hole in the vacated puparium indicating a parasitoid had emerged; or as undefinable. Nymphs in a developing stage (I1-4) were considered available for host feeding or oviposition, although they may have been dead or parasitised. We did not discriminate between a convex fourth instar and elevated (pre)pupal stages, nor did we define parasitism by melanisation of the nymphal cuticle like in *Trialeurodes vaporariorum*, because some whitefly species remain convex and are also melanised in unparasitised form (Gill 1990; Caballero 1994). Puparia including some carrier material were transferred into micro tubes and stored in alcohol 90%. A sample of 50 whitefly nymphs was slide-mounted using Martin (1987) for identification. To determine leaf area a (cm²), each leaflet was drawn on overhead sheet, the sheet was copied, the copy was scanned at 72, 75 or 100 dots per inch dpi (inch⁻¹), and the number of dots d was counted per leaflet using software developed by R. van Zoest. Thus, $a = d * (2.54 \text{ cm inch}^{-1} / dpi)^2$.

Data analysis

Effect of sampling scale on whitefly occurrence

Leaflets of compound leaves were treated as separate leaves, because the leaflet edge is more likely to be an important patch determinant to a parasitoid than the rather taxonomic distinction between leaflet and leaf. The numbers of whitefly nymphs on a leaflet were discrete counts with large outcomes being rare events (see results), which could be described by a Poisson distribution with mean and variance equal to λ . It appeared, however, that the variance to mean ratio (s^2/m) in our data set largely exceeded unity (see results), indicating aggregation. We therefore incorporated the spatial scales of sampling to study the spatial scale at which whitefly nymphs aggregated and to be able to simulate the natural environment of a whitefly parasitoid. Since upper leaflet sides appeared virtually empty (see results), we only modelled the number of whitefly nymphs on the lower leaflet side. We also excluded vacated puparia and undefined stages from this analysis because only developing stages (I1-4) are available

to the parasitoid for host feeding or oviposition. Thus, we assumed that the number of available whitefly nymphs on the lower side of a leaflet (w) is Poisson distributed with parameter λ , and modelled the (random) effects of three spatial scales of sampling on λ using a generalised linear mixed model with log link (Littell *et al.* 1996; SAS v. 8.00, macro GLIMMIX):

$$\log(\lambda) = \text{intercept} + \underline{tsct}_i + \underline{spot}_{j(i)} + \underline{plant}_{k(ij)}, \quad (1)$$

where \underline{tsct}_i is the effect of the i th randomly selected transect, which is assumed independent and normally distributed with mean 0 and variance σ_{tsct}^2 , $N(0, \sigma_{tsct}^2)$; $\underline{spot}_{j(i)}$ is the effect of the j th sampling spot randomly selected along the i th transect and assumed independent $N(0, \sigma_{spot}^2)$; and $\underline{plant}_{k(ij)}$ is the effect of the k th plant randomly selected within the j th sampling spot along the i th transect and assumed independent $N(0, \sigma_{plant}^2)$.

Analogously, the state of a vacated puparium (v) has two possible outcomes: either an adult whitefly (T-shaped opening) or a parasitoid (round hole) emerged. State v can thus be described by a binomial distribution with n vacated puparia, and a probability p that it was parasitised. The spatial scales of sampling were incorporated again as random effects in a generalised linear mixed model with logit link:

$$\log\left(\frac{p}{1-p}\right) = \text{intercept} + \underline{tsct}_i + \underline{spot}_{j(i)} + \underline{plant}_{k(ij)}, \quad (2)$$

where \underline{tsct}_i , $\underline{spot}_{j(i)}$, $\underline{plant}_{k(ij)}$ were defined above.

The stage of a whitefly nymph potentially available for host feeding or oviposition has four rather than two possible outcomes, because four nymphal stages were discerned. To model the population structure, we first modelled whether or not a whitefly nymph was a first instar (g_1). We therefore assumed that g_1 was binomially distributed with n_1 the number of first, second, third and fourth instars, p_1 the probability that it was a first instar, $1-p_1$ the probability that it was not, and

$$\log\left(\frac{p_1}{1-p_1}\right) = \text{intercept} + \underline{tsct}_i + \underline{spot}_{j(i)} + \underline{plant}_{k(ij)}. \quad (3a)$$

We then excluded the first instars from the data set and modelled whether or not a second, third or fourth instar was a second instar (g_2). Again, we assumed that g_2 was binomially distributed with n_2 the number of second, third and fourth instars, p_2 the probability that a second, third or fourth instar was a second instar, $1-p_2$ the probability that it was not, and

$$\log\left(\frac{p_2}{1-p_2}\right) = \text{intercept} + \underline{tsct}_i + \underline{spot}_{j(i)} + \underline{plant}_{k(ij)}. \quad (3b)$$

We then also excluded the second instars from the data set and modelled whether or not a third or fourth instar was a third instar (g_3). We assumed again that g_3 was binomially distributed with n_3 the number of third and fourth instars, p_3 the probability that a third or fourth instar was a third instar, $1-p_3$ the probability that it was not, and

$$\log\left(\frac{p_3}{1-p_3}\right) = \text{intercept} + \text{tscf}_{i(j)} + \text{spot}_{j(i)} + \text{plant}_{k(ij)} \quad (3c)$$

The probability that a third or fourth instar was a fourth instar then equals $1-p_3$. Thus, the probability that a whitefly nymph is a first instar equals p_1 , that it is a second instar equals $p_2*(1-p_1)$, that it is a third instar equals $p_3*(1-p_2)*(1-p_1)$, and that it is a fourth instar equals $(1-p_3)*(1-p_2)*(1-p_1)$.

For the third long transect (Santa Rosa), we measured leaf areas of both infested and empty leaflets. This allowed us to study to what extent the effect of a randomly selected plant could be explained by variation in leaf area. Again, we assumed that the number of whitefly nymphs on the lower side of a leaflet was Poisson distributed with mean and variance equal to λ . First we modelled the effect of a randomly selected plant on λ while ignoring leaf area:

$$\log(\lambda) = \text{intercept} + \text{plant}_i, \quad (4a)$$

where plant_i is the effect of the i th randomly selected plant and assumed independent $N(0, \sigma_{\text{plant}}^2)$. The nested structure with transect and spot effects was ignored to simplify the analysis. We then studied to what extent σ_{plant}^2 decreases when leaf area was incorporated by assuming a proportional relationship between λ and leaf area a ($\log(a)$ included as OFFSET, i.e. regressor with fixed regression coefficient 1).

$$\log(\lambda) = \text{intercept} + \text{plant}_i + \log(a), \quad (4b)$$

Degree and scale of spatial dependence

Geostatistical semivariance analysis (e.g. Clark 1979; Olea 1977; Burrough 1995) was applied to investigate the degree and scale of spatial dependence of whitefly nymphs in the short transects and 3-D plots. Semivariance γ for a certain distance interval h is defined as half the average squared difference between observations that are distance interval h apart:

$$\gamma(h) = \frac{1}{2 * n(h)} \sum_{i=1}^{n(h)} \left[\sqrt{y(x_i) + 0.5} - \sqrt{y(x_i + h) + 0.5} \right]^2, \quad (5)$$

where $n(h)$ is the total number of pairs that are separated by distance interval h , and $y(x_i+h)$ is the total number of whitefly nymphs on a leaflet (I1-4, T, P, and undefined on

upper and lower side) at a distance interval h from location x_i . Square-root transformation was applied on numbers to meet the normality assumption (Sokal & Rohlf 1995). For the three-dimensional plots, the distance between two leaves 1 and 2 with coordinates (a_1, b_1, c_1) and (a_2, b_2, c_2) equals the square root of $(a_1 - a_2)^2 + (b_1 - b_2)^2 + (c_1 - c_2)^2$, rounded to whole units of measurement. Note that we assume here that the numbers of whitefly nymphs per leaflet are punctual observations, that leaf area is spatially continuous, and that data are isotropic, i.e. independent of direction. By definition, the semivariogram passes through the origin because observations do not differ from themselves. The difference between observations, and thus the semivariance γ , initially increases linearly with distance interval h but levels off when observations become spatially independent.

To quantify the degree and scale of spatial dependence, a number of models was tested (Clark 1979; Rietkerk *et al.* 2000):

Linear model:

$$E\{\gamma\} = C_0 + C_1 * h \quad \text{for } h > 0; \quad (6)$$

Spherical model:

$$E\{\gamma\} = \begin{cases} C_0 + C_1 * \left[\frac{3}{2} * \frac{h}{A_1} - \frac{1}{2} * \left(\frac{h}{A_1} \right)^3 \right] & \text{for } 0 < h \leq A_1; \\ C_0 + C_1 & \text{for } h > A_1 \end{cases} \quad (7)$$

Exponential model:

$$E\{\gamma\} = C_0 + C_1 * \left[1 - \exp\left(-\frac{h}{A_1}\right) \right] \quad \text{for } h > 0; \quad (8)$$

Gaussian model:

$$E\{\gamma\} = C_0 + C_1 * \left[1 - \exp\left(-\frac{h^2}{A_1^2}\right) \right] \quad \text{for } h > 0. \quad (9)$$

Only for the transect data (Stein 1993), we also tested a linear model with a sill:

$$E\{\gamma\} = \begin{cases} C_0 + \frac{C_1}{A_1} * h & \text{for } 0 < h \leq A_1; \\ C_0 + C_1 & \text{for } h > A_1 \end{cases} \quad (10)$$

For Santa Rosa and Boruca, we also tested a nested Gaussian model (Rietkerk *et al.* 2000):

$$E\{\gamma\} = \begin{cases} C_0 + C_1 * \left[1 - \exp\left(-\frac{h^2}{A_1^2}\right) \right] & \text{for } 0 < h \leq q \\ (C_0 + C_1) + C_2 * \left[1 - \exp\left(-\frac{(h-q)^2}{A_2^2}\right) \right] & \text{for } h > q \end{cases} \quad (11)$$

In all models, γ is the semivariance, h the distance interval, C_0 the error variance, C_1 the structural variance of the first semivariogram, C_0+C_1 the sill of the first semivariogram, q the distance interval where a second semivariogram connects with the first, C_2 the structural variance of the second semivariogram, and $C_0+C_1+C_2$ the sill of the second semivariogram. Parameters A_1 and A_2 are associated with the range within which counts are spatially dependent. For the spherical model and the linear model with a sill, A_1 is similar to the range, but for the exponential and Gaussian models, the range is usually defined as $3*A_1$ (Stein 1993). The range of the second semivariogram in the nested Gaussian model is similarly defined as $3*A_2$. Parameters were estimated using iterative estimation algorithms (SPSS v. 10.0.5, CNLR command syntax). Partial F tests were used to decide whether any model was significantly better than the random model ($\gamma = C_0$ for $h > 0$) and if so which model was the most parsimonious (Neter *et al.* 1996).

Results

General

Table 3 summarises for all the different sampling sites and methods the mean and maximum number of whitefly nymphs per leaflet, the aggregation index, the fraction of leaflets with no whitefly nymphs, the fraction of whitefly nymphs on the lower leaf side, the fraction of whitefly nymphs in a developing stage, the population structure, the fraction of vacated puparia parasitised and the mean \pm sd of leaf area. Usually on average about 0.5 to 2 whitefly nymphs occurred per leaflet. The variance to mean ratio, however, largely exceeded unity, which could be caused by whitefly nymphs not being randomly distributed among leaflets. Instead, on average 71 % of the leaflets collected along the long transects was empty, whereas some leaflets contained up to several dozens of whitefly nymphs. Usually over 95 % of the whitefly nymphs were found on the lower leaf side.

Not all whitefly nymphs present on a leaflet are available to a whitefly parasitoid for host feeding or oviposition. The fraction that was in a developing stage (I1-4) varied between 0.2 and 0.9. Older stages were generally found more often than younger stages. Based on the shape of the opening in vacated puparia, the fraction of whitefly nymphs that was eventually parasitised ranged between 0 and 0.6 but averaged 0.16 on randomly selected leaves. Dividing the number of whitefly nymphs per leaflet by its 2-sided leaf area reveals an average whitefly density of 0.0178 ± 0.055 (sd) cm^{-2} at the long transects. When leaf area of an empty leaflet was not measured, whitefly density was scored 0. For the third long transect (Santa Rosa), we also measured leaf areas of empty leaflets. Dividing the total number of whitefly nymphs by the total leaf area reveals an overall whitefly density in Santa Rosa of 0.0184 cm^{-2} . Dividing the average number of nymphs per leaflet by the average leaf area (64 cm^2) reveals a total whitefly density of 0.020 cm^{-2} along the long transects, if we reasonably assume that leaf area of empty leaflets does not differ from infested leaflets (see below).

Table 3 Occurrence of whitefly nymphs on leaflets collected at different sites in Costa Rica using different methods.

| Method | # | leaflets | mean # nymphs per leaflet | s^2/m ^(b) | fraction of leaflets empty | max. # nymphs per leaflet | fraction on lower side | fraction of 11-4 in 1st stage | fraction of 11-4 in 2nd stage | fraction of 11-4 in 3rd stage | fraction of 11-4 in 4th stage | fraction parasitised (# vacated) ^(d) | Mean \pm sd of 2-sided leaf area (cm ²) |
|--------------------------------|------|----------|---------------------------|------------------------|----------------------------|---------------------------|------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|---|---|
| Long transects | | | | | | | | | | | | | |
| Carara | 37 | | 0.973 | 5.338 | 0.703 | 10 | 0.972 | 0.889 | 0.250 | 0.375 | 0.125 | 0 (2) | 176 \pm 130 ^(e) |
| Tapantí | 397 | | 2.081 | 7.670 | 0.562 | 38 | 0.967 | 0.515 | 0.162 | 0.200 | 0.379 | 0.144 (362) | 110 \pm 73.7 ^(e) |
| Santa Rosa | 787 | | 0.921 | 11.92 | 0.783 | 42 | 0.945 | 0.233 | 0.089 | 0.213 | 0.160 | 0.180 (350) | 51.5 \pm 64.8 |
| Pooled | 1221 | | 1.300 | 9.773 | 0.708 | 42 | 0.957 | 0.394 | 0.147 | 0.206 | 0.238 | 0.161 (714) | 64.3 \pm 72.5 |
| Short transects | | | | | | | | | | | | | |
| Juan Castro Blanco | 205 | | 0.439 | 2.685 | 0.795 | 7 | 0.867 | 0.389 | 0.057 | 0.114 | 0.286 | 0.182 (44) | 104 \pm 79.8 |
| Cahuita I | 93 | | 1.151 | 2.115 | 0.409 | 11 | 0.981 | 0.720 | 0.052 | 0.312 | 0.247 | 0.360 (25) | 131 \pm 32.8 |
| Cahuita II | 85 | | 1.271 | 2.856 | 0.494 | 11 | 0.972 | 0.843 | 0.011 | 0.176 | 0.264 | 0.059 (17) | 140 \pm 39.4 |
| 3-D plots | | | | | | | | | | | | | |
| El Sur | 240 | | 1.892 | 2.980 | 0.321 | 19 | 0.978 | 0.493 | 0.085 | 0.205 | 0.362 | 0.228 (197) | - |
| El Rodeo | 41 | | 13.78 | 29.97 | 0.244 | 93 | 0.858 | 0.981 | 0.991 | 0.005 | 0 | 0 (9) | - |
| Santa Rosa | 159 | | 1.541 | 2.520 | 0.371 | 11 | 0.959 | 0.592 | 0.076 | 0.124 | 0.303 | 0.041 (65) | - |
| Los Alpes | 41 | | 0.488 | 2.063 | 0.732 | 4 | 1 | 0.450 | 0.111 | 0.111 | 0.333 | 0.143 (4) | 72.2 \pm 39.0 |
| Braulio Carrillo | 47 | | 1.213 | 4.945 | 0.617 | 12 | 1 | 0.175 | 0 | 0.1 | 0.4 | 0.4 (10) | 191 \pm 104 |
| Orotina | 83 | | 0.530 | 2.960 | 0.735 | 7 | 1 | 0.318 | 0 | 0.214 | 0.143 | 0.308 (26) | 90.1 \pm 36.4 |
| Boruca | 173 | | 0.400 | 1.742 | 0.757 | 5 | 1 | 0.638 | 0 | 0.182 | 0.409 | 0.429 (14) | 101 \pm 60.3 |
| Forest floor vs. canopy | | | | | | | | | | | | | |
| Santa Elena Forest Reserve | 115 | | 1.348 | 17.82 | 0.783 | 31 | 1 | 0.910 | 0.362 | 0.135 | 0.191 | 0.167 (12) | 74.6 \pm 59.6 |
| Santa Elena "sky walk" | 110 | | 0.055 | 1.963 | 0.964 | 3 | 1 | 0.833 | 0 | 0.4 | 0 | 1 (1) | 83.7 \pm 71.8 |

^(a) Not always all leaves collected could be checked because of damage, loss or logistical setback. Leaflets of compound leaves were considered separately.

^(b) Aggregation index for number of nymphs per leaflet; $s^2/m < 1$: regular distribution, $s^2/m = 1$: random distribution, $s^2/m > 1$: aggregated distribution.

^(c) Only instar 1 to 4 available to parasitoid for host-feeding or oviposition.

^(d) Based on shape of opening in vacated puparia: T-shaped when adult whitefly emerged, round hole when adult parasitoid emerged.

^(e) Only infested leaves were measured.

The number of whitefly nymphs per leaflet in the tropical dry forest of Santa Rosa was only about half the number of whitefly nymphs per leaflet in the tropical premontane wet forest of Tapantí. In Santa Rosa, however, mean leaflet area was about half the mean leaflet area in Tapantí, because many plants had compound leaves. As a result, whitefly density was similar in both areas.

Leaves sampled along the two short transects in Cahuita and within the 3-dimensional plots were collected from host plants only. In general, the mean number of whitefly nymphs per leaflet was not higher than the mean on leaflets collected randomly, but the fraction of empty leaflets was smaller. The only exception was the *Esterasus* sp. (Asteraceae) sampled in the tropical premontane wet forest of El Rodeo. On average almost 14 whitefly nymphs per leaflet were present with a maximum of 93. Only 1 of 4 leaves was empty. However, 99 % of the nymphs was still a first instar. Although this plant was apparently selected for oviposition by adult whiteflies, the data do not provide much information on host plant suitability.

The mean number of whitefly nymphs was considerably lower on leaves collected in the canopy along the "sky walk" than on leaves collected at the forest floor in the tropical lower montane forest of Santa Elena. The difference between these two strata was much larger than the variation between sampling sites or years. Fortunately, sampling at the forest floor may therefore not result in an underestimation of whitefly abundance.

The 1221 leaflets collected along the long transects and checked for whitefly were picked from 157 plants. Local plant taxonomists identified 155 of these plants to family level, which revealed 53 different plant families; 122 plants were identified to genus level, which revealed 76 different plant genera in 47 families; 73 plants were identified to species level, which revealed 57 different plant species in 52 genera of 31 families. One or more whitefly nymphs were found on one or more leaflets in 37 of the 53 plant families, 47 of the 76 plant genera and 32 of the 57 plant species. Two fern species (Pteridophyta) were infested with whitefly: *Adiantum* sp. (Polypodiaceae) and *Salpichlaena volubilis* (Cyatheaceae).

Identification of about 50 whitefly nymphs from the long transects Carara and Tapantí revealed 5 specimens of a probably undescribed genus, several specimens of a probably undescribed *Tetraleurodes* species, 1 probably *Tetraleurodes mori*, 1 *Bemisia tabaci*, 1 related to *Bemisia afer*, 1 *Dialeurodes* sp., 1 probably *Aleuroplatus denticutatus*, some *Aleurodoxus* possibly *rhodae*, some *Aleuroplatus* sp., *Aleurotrachelus* sp., *Tetralicia* sp., *Aleuropleurocelus* sp., and 1 *Aleurotulus* sp. (Bink-Moenen, pers. comm.).

Effect of sampling scale on whitefly occurrence

Table 4 shows the results from modelling the effects of sampling scale on the number of whitefly hosts (I1-4) on the lower side of a leaflet, the population structure and the degree of parasitism for the "long transect" data. Models describe the data reasonably well, because the extra-dispersion scales are close to unity (s^2/m in Table 3 would be the

Table 4 Effects of sampling scale on the number of whitefly hosts, population structure and degree of parasitism on the lower side of leaflets collected along the "long transects". Given are assumed distribution, applied linear predictor and parameter estimates of generalised linear models for the number of whitefly nymphs in a developing stage I1-4 (w) with mean and variance equal to λ ; the stage of a first, second, third or fourth instar nymph (g_1), with n_1 nymphs and probability p_1 that it was a first instar; the stage of a second, third or fourth instar nymph (g_2), with n_2 nymphs and probability p_2 that it was a second instar; the stage of a third or fourth instar nymph (g_3), with n_3 nymphs and probability p_3 that it was a third instar; and the state of a vacated puparium (v), with n vacated puparia and probability p that it was parasitised. Linear predictor is $\eta_{ijk} = intercept + \frac{1}{\lambda} x_{ijk} + \frac{1}{\lambda} x_{ijk} + \frac{1}{\lambda} x_{ijk}$, where x_{ijk} is the effect of the i th transect and assumed independent $N(0, \sigma_{\eta_{ijk}}^2)$; and $\frac{1}{\lambda} x_{ijk}$ the independent $N(0, \sigma_{\eta_{ijk}}^2)$; $\frac{1}{\lambda} x_{ijk}$ the effect of the j th sampling spot randomly selected along the i th transect and assumed independent $N(0, \sigma_{\eta_{ijk}}^2)$; and $\frac{1}{\lambda} x_{ijk}$ the effect of the k th plant randomly selected within the j th sampling spot along the i th transect and assumed independent $N(0, \sigma_{\eta_{ijk}}^2)$. Extra-dispersion scale shows ratio of data variance to model variance; if < 1 : underdispersion; if > 1 : overdispersion. Population mean shows λ ($e^{intercept}$) or p_1 , p_2 , or p_3 ($e^{intercept/(1+e^{intercept})}$) for an average plant ($\frac{1}{\lambda} x_{ijk} = 0$) within an average spot ($\frac{1}{\lambda} x_{ijk} = 0$) along an average transect ($\frac{1}{\lambda} x_{ijk} = 0$).

| Assumed distribution | # whiteflies in I1-4 | | | Stage of I2-4 (g_2) | | Stage of I3-4 (g_3) | | State of vacated puparium (v) | |
|-------------------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------|-------------------------|--|-----------------------------------|--|
| | stage (w) | Stage of I1-4 (g_1) | | Stage of I2-4 (g_2) | | Stage of I3-4 (g_3) | | State of vacated puparium (v) | |
| Linear predictor η_{ijk} | $w \sim \text{Poisson}(\lambda)$ | $g_1 \sim \text{Bin}(n_1, p_1)$ | $g_2 \sim \text{Bin}(n_2, p_2)$ | $g_3 \sim \text{Bin}(n_3, p_3)$ | $v \sim \text{Bin}(n, p)$ | | | | |
| <i>Intercept</i> | $\log(\lambda)$ | $\logit(p_1)$ | $\logit(p_2)$ | $\logit(p_3)$ | $\logit(p)$ | | | | |
| $\sigma_{\eta_{ijk}}^2$ | -1.422 | -1.8027 | -1.0154 | -0.3558 | -1.965 | | | | |
| $\sigma_{\eta_{ijk}}^2$ | 0.522 | 0.516 | 0.000 | 0.378 | 0.000 | | | | |
| $\sigma_{\eta_{ijk}}^2$ | 0.197 | 0.525 | 0.000 | - | 1.006 | | | | |
| $\sigma_{\eta_{ijk}}^2$ | 2.485 | 1.304 | 1.239 | 0.949 | 0.952 | | | | |
| Extra-dispersion scale | 0.854 | 0.676 | 0.918 | 1.079 | 0.854 | | | | |
| Population mean | 0.241 | 0.142 | 0.266 | 0.412 | 0.123 | | | | |

extra-dispersion scale of a Poisson distribution without random effects). If a whitefly parasitoid lands on a leaflet of an average plant within an average spot of an average transect, the number of hosts that it can encounter on the lower side could be described by a Poisson distribution with mean and variance equal to 0.241 (Table 4). If we assume that leaf area is similar for empty and infested leaves (see below), dividing the modelled mean by the mean leaf area of 64 cm² (Table 3) results in a mean host density of 0.0037 per cm² (1 per 267 cm²). These estimates are lower than the raw mean of any site (except "sky walk"; Table 3), because vacated puparia, undefined stages and upper sides were excluded and, more importantly, because the raw mean is more sensitive to outliers than the modelled mean. The number of hosts on the lower side of a leaflet varied mostly between plants within a spot of a transect ($\sigma_{plant}^2 = 2.485$). The variation was considerably less on larger spatial scales ($\sigma_{spot}^2 = 0.197$, $\sigma_{iscf}^2 = 0.522$). Thus, the modelled mean host density that includes the sampling scale is even lower than the raw mean and the plant within a spot along a transect is the main source of variation.

The hosts present on the lower side of a leaflet of an average plant within an average spot of an average transect were structured in a ratio of I1:I2:I3:I4 = 0.14:0.23:0.26:0.37 ($p_1: p_2*(1-p_1): p_3*(1-p_2)*(1-p_1): (1-p_3)*(1-p_2)*(1-p_1)$; Table 4). Thus, instar frequency increased on average with about 7 % per moult, possibly because smaller instars were more difficult to detect by the observer. Parameter estimates for σ_{iscf}^2 , σ_{spot}^2 and σ_{plant}^2 show that again the plant is the main source of variation in the population structure. About 12 % of the vacated puparia present on the lower side of a leaflet of an average plant within an average spot of an average transect was parasitised. In contrast to the previous variables, the spot along the transect determines the state of a vacated puparium in the same order of magnitude ($\sigma_{spot}^2 = 1.006$) as the plant within a spot ($\sigma_{plant}^2 = 0.952$). This suggests that parasitism was more patchily distributed among spots but more evenly distributed among plants within spots than whiteflies ($\sigma_{spot}^2 = 0.197$ and $\sigma_{plant}^2 = 2.485$).

Table 5 Effect of leaf area (a in cm²) on parameter estimates of generalised linear model describing the number of whitefly nymphs on the lower side of a leaflet. Leaflets were collected in 1999 along the "long transect" in Santa Rosa. The number of whitefly nymphs was assumed Poisson distributed with mean and variance equal to λ . The effect of the i th randomly selected plant was incorporated as $plant_i$ and assumed independent $N(0, \sigma_{plant}^2)$. A proportional relationship was assumed between λ and a . Extra-dispersion scale shows ratio of data variance to model variance. Population mean shows λ for an average plant ($plant_i = 0$) when leaf area is ignored ($e^{intercept}$) or included ($a * e^{intercept}$).

| | Leaf area ignored | Leaf area included |
|------------------------|---------------------------------------|---|
| Model | $\log(\lambda) = intercept + plant_i$ | $\log(\lambda) = intercept + plant_i + \log(a)$ |
| Intercept | -2.474 | -5.723 |
| σ_{plant}^2 | 3.605 | 2.698 |
| Extra-dispersion scale | 0.566 | 0.549 |
| Population mean | 0.084 | 0.003*a |

Table 5 shows the effect of leaf area on parameter estimates of the generalised linear model describing the number of whitefly nymphs on the lower side of a leaflet. The degree of underdispersion (extra-dispersion scale < 1 ; Table 5) was somewhat larger in both models including only plant as random effect than the model including $\text{tscf}_{(i)}$, $\text{spot}_{(i)}$ and $\text{plant}_{(ij)}$ (Table 4). Nevertheless, mutual comparison remains possible because the degree of underdispersion is similar between both models including a random plant effect only. When leaf area was ignored, the variance of the random plant effect was about 3.6. When leaf area was included, the variance only slightly decreased to about 2.7. The difference is considerably smaller than the difference between σ_{tscf}^2 , σ_{spot}^2 and σ_{plant}^2 in Table 4. This shows that the relatively large variation between plants in the number of hosts on the lower side of a leaflet is not a result of a large variation in leaf area. The variance between plants is more likely to be a result of other characters associated with host plant species.

Degree and scale of spatial dependence

Fig. 2 shows the distributions of the number of whitefly nymphs per leaflet along the short transects and the accompanying semivariograms. For the whitefly distribution in Juan Castro Blanco, none of the models was significantly better than the random model (Fig. 2a). This implies that the number of whitefly nymphs on a leaflet was spatially independent even from the number of whitefly nymphs on a leaflet 1 m away. Leaves were, however, picked from different plant species and hence the distribution of host plants could interfere with the distribution of whiteflies. In Cahuita we sampled a more or less continuous understorey of a single host plant species. In the first sample, semivariance significantly increased with distance, i.e. the spatial dependence between number of whiteflies on leaves decreased with distance between leaves (Fig. 2b). In the second sample, however, no such spatial dependence could be detected (Fig. 2c).

Figures 3 to 9 show the distributions of the number of whitefly nymphs per leaflet within the three-dimensional plots and the accompanying semivariograms. In 4 of 7 plots, we found at least some degree of spatial dependence. Two levels might be discerned. Within the *Croton niveus* (Euphorbiaceae) at Santa Rosa, whitefly nymphs aggregated within patches of about 16 cm (Fig. 5). These patches aggregated within larger patches of about 37 cm (Fig. 5). Similar scales of spatial dependence were found in seclusion in El Sur (41 cm; Fig. 1), Los Alpes (20 cm; Fig. 6) and Boruca (15 cm; Fig. 9). These were aggregations within a single host plant species, except for the plot in Boruca (Table 2). In Boruca, a second level of spatial dependence could be detected, but the estimated range of 45 cm is unreliable because obtained from extrapolation (Fig. 9).

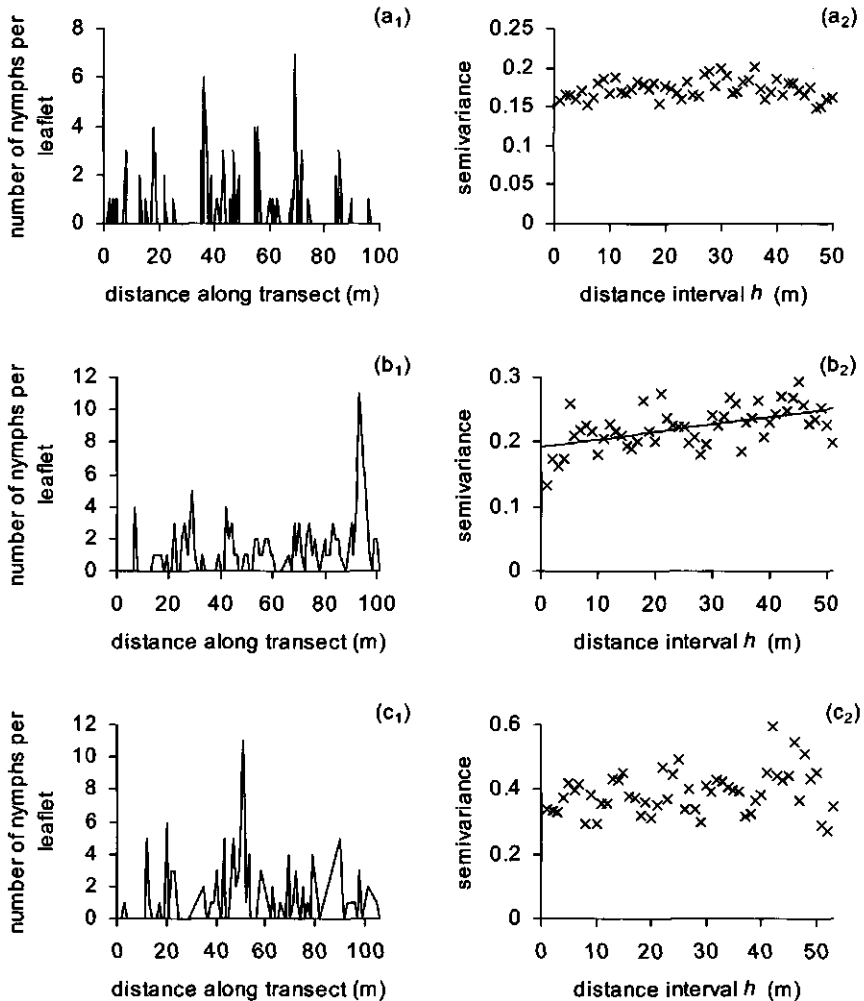


Fig. 2 Whitefly distribution along the short transects sampled in April 2001. Left panels: total number of whitefly nymphs per leaflet against distance along transect (m). Right panels: semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaflet against distance interval between leaflets. (a) Juan Castro Blanco National Park; most parsimonious (linear) model did not perform significantly better than the random model ($R^2 = 0.001$; $P = 0.864$). (b) Cahuita National Park I; most parsimonious (linear) model ($R^2 = 0.284$; $P < 0.001$): $E\{\gamma\} = 0.1922 + 0.0012 \cdot h$. (c) Cahuita National Park II; most parsimonious (linear) model did not perform significantly better than the random model ($R^2 = 0.061$; $P = 0.074$).

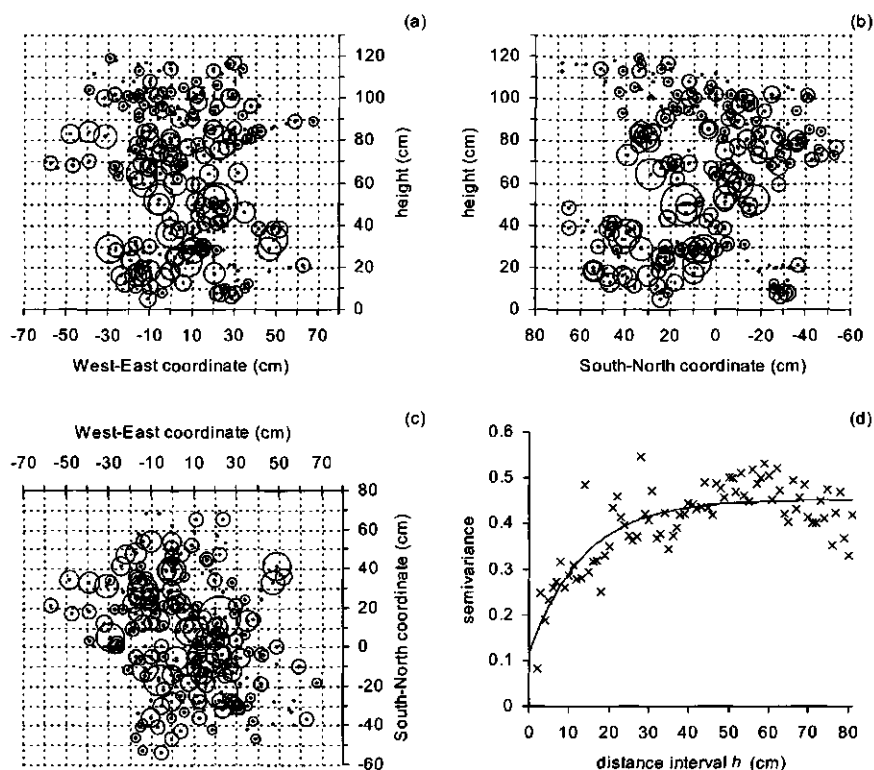


Fig. 3 Whitefly distribution within a *Piper* sp. (Piperaceae) sampled at El Sur, 24-25 May 1999. (a-c) Two-dimensional projections showing position of leaf centre (black dot) and total number of whitefly nymphs on that leaf (bubble area). Bubble size was scaled to 50 % to increase readability. (d) Semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaf against the distance interval between leaves; most parsimonious (exponential) model ($R^2 = 0.668$; $P < 0.001$): $E\{\gamma\} = 0.115 + 0.338 \cdot [1 - \exp(-h/13.6)]$; range defined by $3 \cdot A_1 \approx 41$ cm.

Discussion

Aim of our field survey was to quantify the natural whitefly density and distribution under which the whitefly parasitoid *Encarsia formosa* has evolved to understand its foraging behaviour from a functional point of view. *E. formosa* is an exceptional parasitoid species in that it does not exploit herbivore-induced plant volatiles to locate its hosts. One of the successes in behavioural ecology has been the discovery that parasitoids and predators can exploit herbivore-induced plant volatiles to find their herbivorous hosts or prey (e.g. Turlings *et al.* 1990; Dicke *et al.* 1990). Although these plant odours are less reliable than the host odours, their detectability is much higher

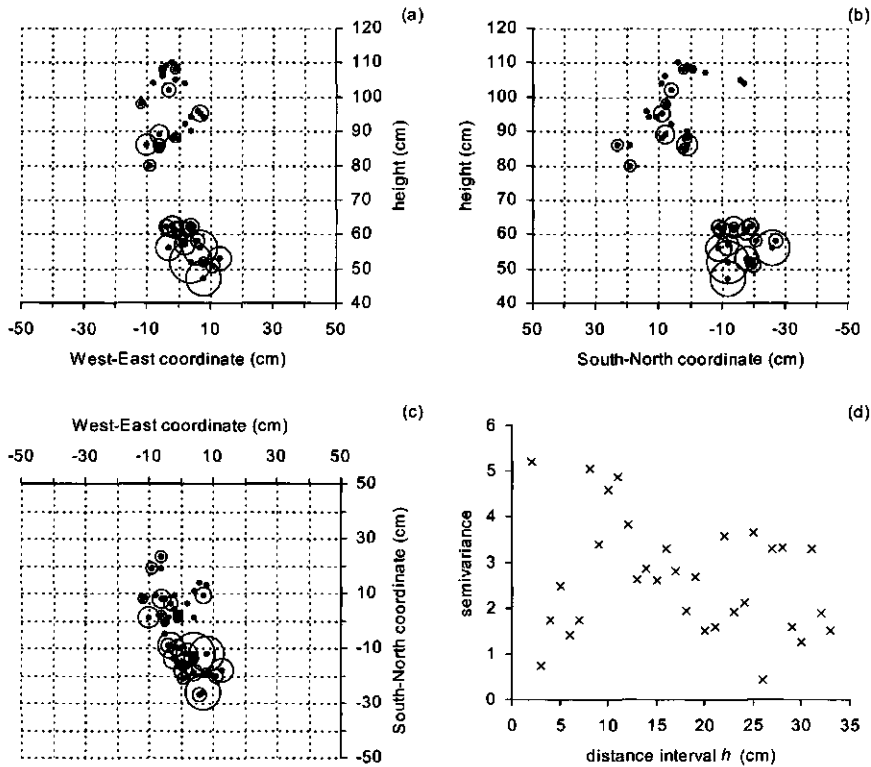


Fig. 4 Whitefly distribution within an *Esterasus* sp. (Asteraceae) sampled at El Rodeo Protected Area, 8 June 1999. (a-c) Two-dimensional projections showing position of leaf centre (black dot) and total number of whitefly nymphs on that leaf (bubble area). Bubble size was scaled to 50 % to increase readability. (d) Semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaf against the distance interval between leaves; most parsimonious (linear) model did not perform significantly better than the random model ($R^2 = 0.000$; $P > 0.999$).

because there is no selection pressure on the plant to chemically hide from an insect parasitoid (Vet *et al.* 1991; Vet & Dicke 1992). On the contrary, attraction of natural enemies of the plant's natural enemies through emission of herbivore-specific plant odours may enhance plant fitness and thus serve as an indirect defence mechanism (Dicke & van Loon 2000). *E. formosa*, on the other hand, seems unable to distinguish whitefly-infested from uninfested plants or leaves before landing (Noldus & van Lenteren 1990; Sütterlin & van Lenteren 2000). After landing, it also does not detect hosts from a short distance (van Roermund & van Lenteren 1995a; van Lenteren *et al.* 1976a). Romeis & Zebitz (1997) showed that *E. formosa* is only attracted visually to

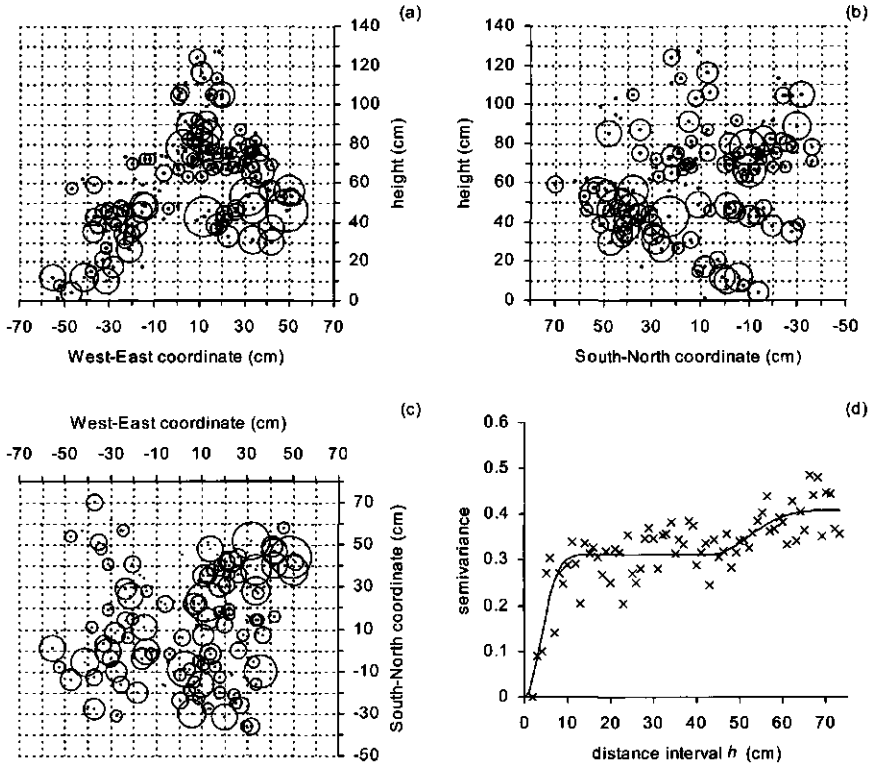


Fig. 5 Whitefly distribution within a *Croton niveus* (Euphorbiaceae) sampled at Santa Rosa National Park, 9, 11 July 1999. (a-c) Two-dimensional projections showing position of leaf centre (black dot) and total number of whitefly nymphs on that leaf (bubble area). Bubble size was scaled to 50 % to increase readability. (d) Semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaf against the distance interval between leaves; most parsimonious (nested Gaussian) model ($R^2 = 0.696$; $P < 0.001$): $E\{\gamma\} = 0 + 0.312 \cdot \{1 - \exp[-(h^2/5.35^2)]\}$ for $0 < h \leq 43.0$ cm and $E\{\gamma\} = (0 + 0.312) + 0.098 \cdot \{1 - \exp[-((h - 43.0)^2/12.5^2)]\}$ for $h > 43.0$ cm; ranges defined by $3 \cdot A_1 \approx 16$ cm (first semivariogram) and $3 \cdot A_2 \approx 37$ cm (second semivariogram).

green light and not to olfactory stimuli. Thus, infested leaves have to be found through random flights and hosts within leaves by random searching.

Hence, our generalised linear mixed models based on random leaf sampling allow one to simulate the natural environment of a randomly searching whitefly parasitoid like *E. formosa*. One could simulate a whitefly parasitoid landing on a leaflet of a random plant within a random spot of a random transect and determine the number of first, second, third and fourth instars on the lower side of the leaflet, using Equations 1 and 3 and the parameter estimates in Table 4. For a leaflet of an average plant within an

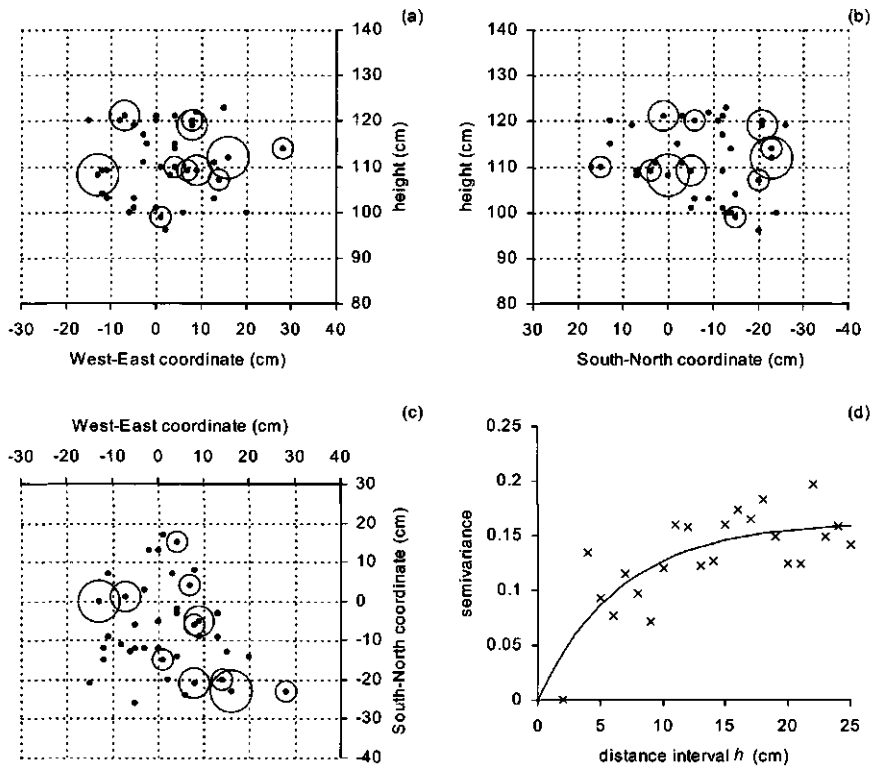


Fig. 6 Whitefly distribution within an unidentified plant species sampled at Los Alpes Refuge, 7 May 2001. (a-c) Two-dimensional projections showing position of leaf centre (black dot) and total number of whitefly nymphs on that leaf (bubble area). Bubble size was scaled to 50 % to increase readability. (d) Semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaf against the distance interval between leaves; most parsimonious (exponential) model ($R^2 = 0.593$; $P = 0.015$): $E\{\gamma\} = 0 + 0.163*[1-\exp(-h/6.65)]$; range defined by $3*A_1 \approx 20$ cm. The spherical model performed equally well ($R^2 = 0.593$; $P = 0.015$) but $C_1/(C_0+C_1)$ was smaller (0.85).

average spot of an average transect, the number of hosts on the lower side would be Poisson distributed with mean and variance equal to 0.241, in a ratio of I1:I2:I3:I4 = 0.14:0.23:0.26:0.37. The increase in frequency with moulting stage could be explained by an increase in detectability to the observer with moulting stage. Another explanation could be that the whitefly population decreases in the wet season. The simulated number of hosts on a leaflet that a parasitoid encounters next depends on its foraging and dispersal behaviour. One simulates that the parasitoids “hops” to another leaflet within the same plant by drawing from the Poisson and binomial distributions with the same parameters. One simulates that the parasitoids flies to another plant by drawing from the

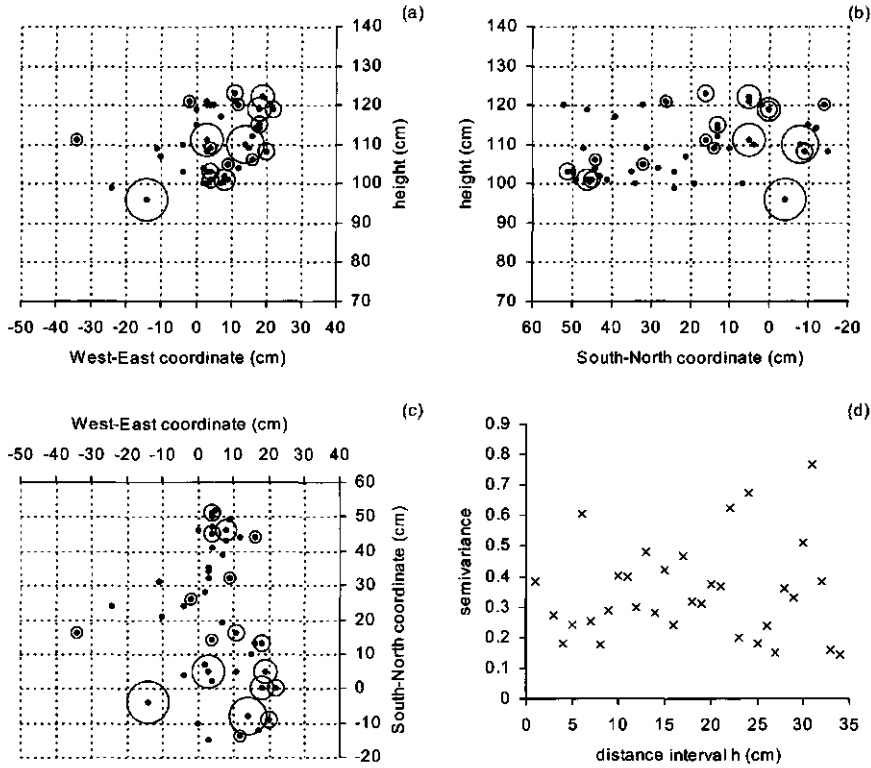


Fig. 7 Whitefly distribution within a plot of three unidentified plant species sampled at Braulio Carrillo National Park, 15 May 2001. (a-c) Two-dimensional projections showing position of leaf centre (black dot) and total number of whitefly nymphs on that leaf (bubble area). Bubble size was scaled to 50 % to increase readability. (d) Semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaf against the distance interval between leaves; most parsimonious (linear) model did not perform significantly better than the random model ($R^2 = 0.006$; $P = 0.666$).

Poisson and binomial distributions with $plant_{k(i)}$ replaced in Equations 1 and 3, where estimates for σ_{plant}^2 are given in Table 4. One simulates that the parasitoids disperses by air currents to another spot by replacing both $spot_{k(i)}$ and $plant_{k(i)}$, or even to another transect by replacing $tsect$ as well as $spot_{k(i)}$ and $plant_{k(i)}$. In that way, realistic host numbers and distributions can be simulated when foraging behaviour of a randomly searching whitefly parasitoid is modelled.

The probability p_e that a randomly searching whitefly parasitoid like *E. formosa* encounters a host during time step dt , can be calculated by $p_e = 1 - \exp(-(sw + dm) * ws * act * dens * dt)$, where sw is the searching width of the parasitoid, dm the diameter of the host, ws the walking speed of the parasitoid, act the fraction of

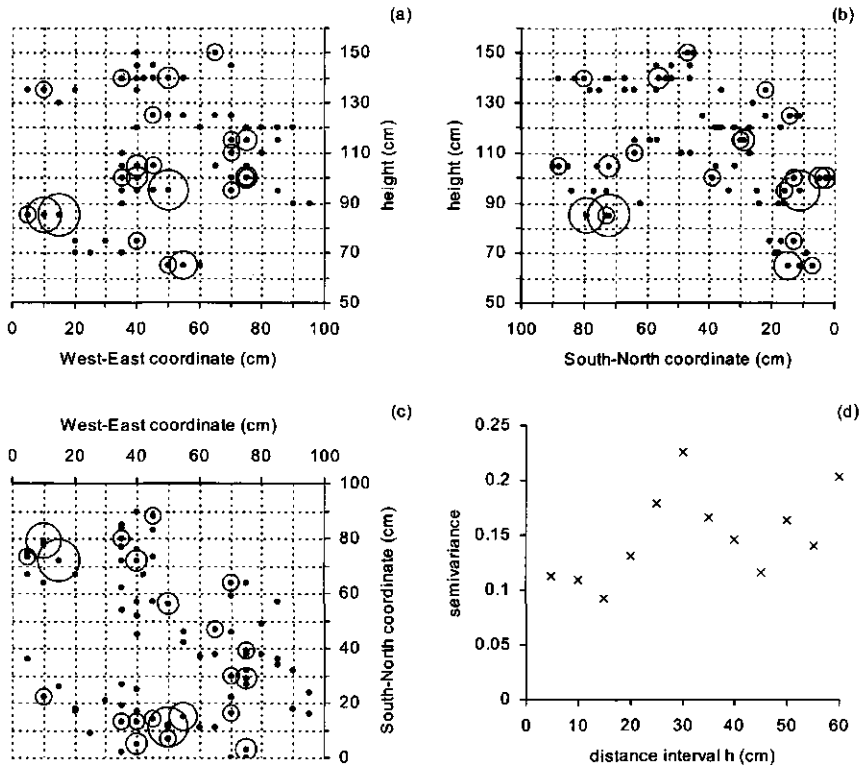


Fig. 8 Whitefly distribution within a *Piper* sp. (Piperaceae) sampled at Orotina, 23 May 2001. (a-c) Two-dimensional projections showing position of leaf centre (black dot) and total number of whitefly nymphs on that leaf (bubble area). Bubble size was scaled to 50 % to increase readability. (d) Semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaf against the distance interval between leaves; most parsimonious (linear) model did not perform significantly better than the random model ($R^2 = 0.233$; $P = 0.112$).

searching time that parasitoid walks actively and *dens* the host density (van Roermund *et al.* 1996). If we assume that $sw = 0.05$ cm, $dm = 0.04$ cm, $ws = 0.04$ cm s⁻¹ (= 144 cm per hour), $act = 0.75$, $dens = 0.241$ hosts/ 64 cm⁻², and $dt = 1$ hour, then p_e equals 0.04 per hour. Investment in future reproduction through host feeding does not seem adaptive at this host encounter rate (see Chapter 5). Some optimisation models have indeed predicted that the critical egg load at which parasitoids should host feed decreases towards very low host encounter rates (Jervis & Kidd 1986; Chan & Godfray 1993; Collier 1995b). Collier (1995b) did not even consider host encounter probabilities below 0.02 per 5 min. Even extremely synovigenic species that have limited egg storage capacity like *E. formosa* (Jervis *et al.* 2001) are unlikely to become egg limited at the

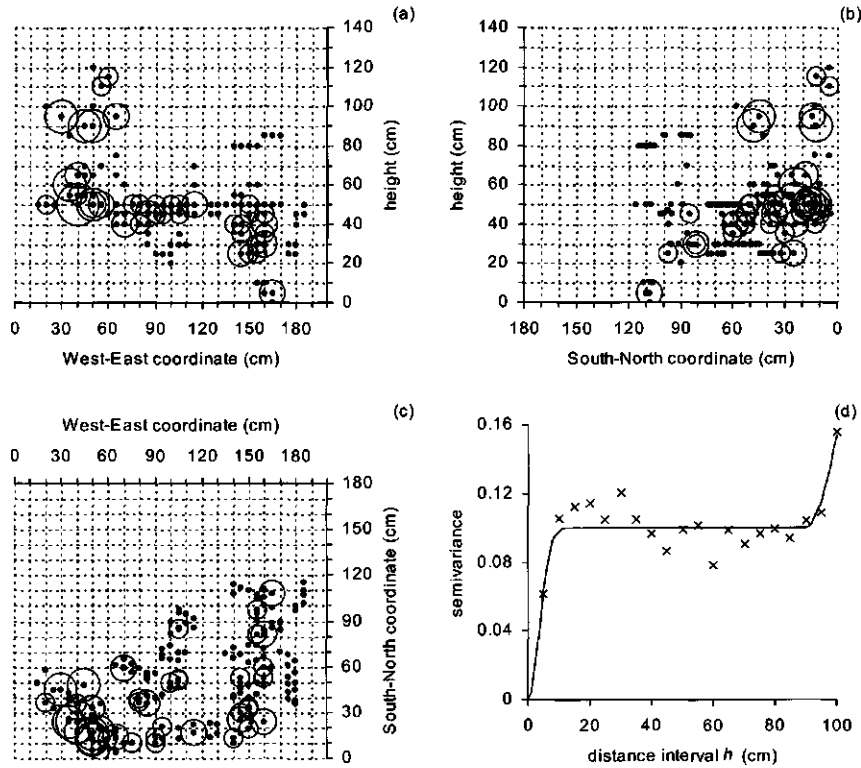


Fig. 9 Whitefly distribution within a plot of several unidentified plant species sampled at Boruca Indian Reserve, 12 July 2001. (a-c) Two-dimensional projections showing position of leaf centre (black dot) and total number of whitefly nymphs on that leaf (bubble area). Bubble size was scaled to 50 % to increase readability. (d) Semivariogram, showing semivariance of square-root-transformed total number of whitefly nymphs per leaf against the distance interval between leaves; most parsimonious (nested Gaussian) model ($R^2 = 0.723$; $P = 0.001$): $E\{\gamma\} = 0 + 0.100 \cdot \{1 - \exp[-(h^2/5.00^2)]\}$ for $0 < h \leq 90.0$ cm and $E\{\gamma\} = (0 + 0.100) + 0.150 \cdot \{1 - \exp[-(h - 90.0)^2/15.0^2]\}$ for $h > 90.0$ cm; range defined by $3 \cdot A_1 \approx 15$ cm (first semivariogram) and $3 \cdot A_2 \approx 45$ cm (second semivariogram).

average host density we found in the field. Heimpel *et al.* (1998) also assumed random host encounter and concluded from dynamic modelling that *Aphytis* parasitoids (Aphelinidae, like *Encarsia*) might become temporarily egg-limited in the field. Their host encounter rate of 0.08 scales per 5 min was, however, based on observations in a deserted citrus orchard (Heimpel *et al.* 1996), rather than in natural vegetation. Thus, our field estimate of natural host density is relatively low and may effect model predictions on optimal host-handling strategies.

The natural host density obtained from our field surveys is more likely to be overestimated than underestimated. First, not all whitefly nymphs in first to fourth instar were alive. The distinction between dead and alive was too uncertain to include in the analysis but at least some died prematurely possibly from desiccation, mutilation or predation. Second, on average 12 % of the hosts was eventually parasitised. Parasitoids might thus encounter already parasitised hosts, which they can distinguish from unparasitised hosts (van Lenteren *et al.* 1976b), and which decreases the number of hosts available for oviposition. It should be noted that the fraction parasitism might be overestimated, because vacated puparia from which a parasitoid emerged tend to stick longer to a leaf than those from which an adult whitefly emerged (A. Loomans, pers. comm.). Third, all whitefly species were pooled, whereas only 14 of the 1450 species of whitefly have been described as host for *E. formosa* (Schauuff *et al.* 1996; Polaszek *et al.* 1992; Gerling 1990). On the other hand, 2 of the 14 host species (*Bemisia tabaci*, *Tetraleurodes mori*) and 5 of the 9 host genera (*Aleurotrachelus*, *Bemisia*, *Dialeurodes*, *Tetraleurodes* and *Tetralicia*) were found in a sample of only 50 whitefly nymphs. Moreover, it is likely that *E. formosa* uses more (economically less-important) whitefly species as a host, although this requires experiments on host acceptance and host suitability. In general, *Encarsia* species have a flexible host range (Gerling 1990). Fourth, the overall estimate was based on samples at the forest floor, whereas densities in the canopy were considerably lower.

Although average whitefly density and average host encounter probability may be low, situations may occur where parasitoids run a risk of becoming egg limited and host feeding becomes adaptive. High variance to mean ratios (Table 3) could be caused by whitefly nymphs not being randomly distributed among leaflets. Although rare, leaflets with dozens of whitefly nymphs were found. Although in El Rodeo (Table 3) these were all still first instar nymphs, there is a relationship between host-plant preference and host-plant suitability for *Trialeurodes vaporariorum* (van Lenteren & Noldus 1990). The probability to encounter a host on a leaflet of 64 cm² with 20 hosts, would be 0.95 per hour. Further analysis of the short transects and 3-D plots revealed at least three levels of spatial dependence (Figs. 2 to 9). *E. formosa* parasitoids cannot detect these patches from a distance, but physical contact with hosts or honeydew and especially oviposition evokes arrestment behaviour through an increase in residence time (van Roermund & van Lenteren 1995a) without a decrease in walking activity (van Roermund & van Lenteren 1995b), and through a decrease in leaving tendency (van Roermund *et al.* 1994). Aggregation of *E. formosa* on infested leaves through arrestment behaviour even caused people initially to think erroneously that *E. formosa* was able to detect hosts from a distance (Ledieu 1976; Hussey *et al.* 1976). Thus, although on average natural whitefly density is much lower than pest density, parasitoid populations may thrive at high host densities within the natural whitefly aggregations.

Arrestment behaviour seems adaptive when hosts are aggregated. Why has *E. formosa* not developed senses to locate hosts or host patches from a distance? This would highly increase searching efficiency because 71 % of the randomly collected leaflets was empty in the field and *E. formosa* has a median residence time of about 20

min on empty leaflets (van Roermund & van Lenteren 1995a). In a natural ecosystem, herbivore-induced plant volatiles only attract parasitoids that attack a small range of oligophagous herbivores (Vet & Dicke 1992). As mentioned above, it is likely that *E. formosa* attacks more than the 14 whitefly species described as its host, and some of its described hosts (e.g. *Trialeurodes vaporariorum*, *Bemisia tabaci*) are highly polyphagous, attacking hundreds of plant species (Mound & Halsey 1978). In addition, when herbivores are aggregated within plants, as in Figs. 3, 5 and 6, systemic induction (see Dicke & van Loon 2000) decreases reliability of herbivore-induced volatiles on a small scale.

The high fractions of whiteflies on the lower leaf side can be explained from an "aleurocentric" point of view by lower exposure to rain and sun (van Lenteren & Noldus 1990). Given this situation, however, it is unclear why *E. formosa* makes no distinction between the upper and lower leaf side during landing and does not search longer on the lower than on the upper side (van Roermund & van Lenteren 1995b). Honeydew as a food source on the upper side is easily washed off and can be obtained from the whiteflies themselves. Maybe commercially produced parasitoids have lost a preference under artificial rearing conditions of extremely high host densities on both leaf sides. Other important uncertainties that need to be resolved to understand *E. formosa*'s foraging behaviour include its area of origin, its life expectancy in the field, and the temporal variation in natural whitefly density and distribution.

Whitefly density and distribution have been quantified in many studies but unfortunately only in artificial cropping systems. Natural history of whitefly as described here differs largely from pest densities that can reach 1000 pupae per leaf (Noldus & van Lenteren 1990). Eggenkamp *et al.* (1982) found that when *Trialeurodes vaporariorum* is under biological control, however, mean density remains below 0.10 pupae per plant in large greenhouses. Further analysis of their data by Noldus *et al.* (1986c) revealed extreme levels of aggregation that could not be described by any theoretical distribution they tested, including the Poisson and negative binomial. They discerned two levels of aggregation, a plant and groups of several or many plants. Aggregation can also occur within leaves and leaflets (Yano 1983, Xu 1985). Kim *et al.* (2001) found that adult *T. vaporariorum* aggregated in patches of 12.5 m. Whereas most of these studies were done to develop sampling schemes for monitoring biological control programmes (Ekbom & Xu 1990), our data provide insight in the natural environment and help to improve our understanding of both biological control and evolutionary ecology. In the next two chapters, we will predict (Chapter 5) and test (Chapter 6) how parasitoids make host-handling decisions when foraging at the host density found in the field. Because whitefly nymphs are scarce in the field, whitefly parasitoids are expected to be under strong natural selection to handle a host optimally, i.e. in a way that maximises their lifetime reproductive success.

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5

Reproduction now or in the future: optimal host-handling strategies in the whitefly parasitoid *Encarsia formosa*

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Abstract

Despite numerous studies on the whitefly parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae) to explain success or failure of biological control in greenhouses, the question to what extent the parasitoid's behaviour has evolved by natural selection as an adaptation to the environment was never studied. A dynamic state variable model was developed to study *E. formosa*'s optimal host-handling strategies, i.e. the decision to reject, host feed or oviposit, to maximise lifetime reproductive output. We focussed on effects of host density and parasitoid's life expectancy because these parameters are expected to have a profound effect on the value of future reproduction through host feeding, and because there is a large difference between laboratory and field estimates for these parameters. We assumed that the function of host feeding is to gain nutrients that can be matured into eggs, that oogenesis is continuous and egg load dependent, that parasitoid survival is exponentially distributed, and that parasitoids encounter hosts randomly, are autogenous and have unlimited access to non-host food sources to obtain energy for maintenance and activity. The most important prediction of our model is that host feeding is maladaptive at natural conditions of low host density (0.015 cm^{-2}) and short life expectancy (mean longevity about 3 days). Nutrients from the immature stage are sufficient to prevent egg limitation. Both host density and parasitoid's life expectancy have indeed a positive effect on the optimal host-feeding ratio. Parasitoids that make random decisions gain on average only 35 % ($0.015 \text{ hosts cm}^{-2}$) to 60 % ($1.5 \text{ hosts cm}^{-2}$) of the lifetime reproductive success of parasitoids that make optimal decisions. Elasticity analyses revealed that the relationship between host density and

acceptance ratio is most sensitive to the relative handling time of host feeding and the temperature at which oogenesis takes place. The relationship between host density and host-feeding ratio is most sensitive to the relative handling time of host feeding and the host-feeding gain from the bad quality host. Parameters that have a large impact on lifetime reproductive success and thus fitness are the parasitoid's life expectancy and the survival probability of deposited eggs (independent of host density), the host encounter probability and length of the active period (when host density is low) and the egg maturation rate and number of host types (when host density is high). Explaining evolution of host-feeding behaviour under natural conditions may require incorporation of variation in host density, incorporation of parasitised host types, or field data showing that life expectancy in the field is not as short as we assumed. Incorporating variation in walking speed, egg resorption, or antennal rejection are not likely to be able to explain the evolution of host-feeding behaviour under natural conditions.

Introduction

Natural selection favours organisms that behave in a way that maximises their fitness. Ultimately, fitness is determined by the contribution of the individual to the gene pool in the next generation (Daan & Tinbergen 1997), but can be expressed as the product of quantity and quality of offspring produced in a lifetime. Insect parasitoids are particularly suitable as a model system to study evolution because behavioural decisions made during foraging directly affect their reproductive output. Examples of such behavioural decisions include the decision to search for food or hosts, to stay in or leave a patch, and choices regarding clutch size, sex ratio, and host handling (Stephens & Krebs 1986; Godfray 1994). Host-handling decisions can be divided into the decision to reject or accept an encountered host, and the decision to parasitise or feed upon an accepted host. These decisions reflect the major life-history trade-off between current and future reproduction, which is the subject of the present paper.

Host feeding has been defined as the consumption of host hemolymph and body tissue by the adult female parasitoid. It provides nutrients that allow parasitoids to mature eggs, to increase longevity or both (Heimpel & Collier 1996; Heimpel *et al.* 1997a). On the other hand, it kills the host or at least reduces the quality of the host for oviposition (Jervis & Kidd 1986; Nell *et al.* 1976; van Lenteren *et al.* 1980). The time needed for host feeding is often longer than that for oviposition (Heimpel & Collier 1996; Heimpel *et al.* 1998; van Roermund & van Lenteren 1995a). This reduces the available searching time, which is an important cost for host-limited parasitoids. An increased handling time may also increase the risk of predation. Thus, when handling an accepted host, the parasitoid chooses between current reproduction through oviposition and future reproduction through host feeding.

We model this life-history trade-off between current and future reproduction in the whitefly parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae). Besides the use of insect parasitoids in developing foraging theory, they are also well studied to understand

why they succeed or fail as biological control agents against pest insects. A famous example is the larval endoparasitoid *E. formosa*, which is successfully applied throughout the world to control whitefly (Homoptera: Aleyrodidae) in greenhouses (van Lenteren & Woets 1988; van Lenteren & van Roermund 1999; van Lenteren 2000). Hundreds of papers have been published on the biology, use, and life-history parameters of *E. formosa*, and its parasitoid-host relationship with the greenhouse whitefly *Trialeurodes vaporariorum* (reviews: Viggiani 1984; Noldus & van Lenteren 1990; van Roermund & van Lenteren 1992b; Hoddle *et al.* 1998). Van Roermund *et al.* (1996, 1997a,b,c) developed simulation models to explain the success or failure of biological control of whitefly with *E. formosa*. In all models, they used probabilities to reject (after antennal contact or ovipositorial insertion), oviposit or host feed, depending on egg load, host stage and host type (unparasitised, self-parasitised or conspecifically parasitised). These were estimated from data by Nell *et al.* (1976), van Lenteren *et al.* (1976a), van Roermund & van Lenteren (1995b), Hulsas-Jordaan (unpublished data) and Sütterlin & van Lenteren (1999). Despite *E. formosa*'s worldwide economical success, the wealth of data on its biology, and the mechanistic explanations for its behaviour, its foraging behaviour under natural conditions was never studied. Especially missing is a functional explanation of its behaviour, i.e. insight in how natural selection has shaped this behaviour as an adaptation to the environment.

In greenhouses with a surface area up to a few hundred square meters, interactions between whitefly and parasitoid are often highly unstable (e.g. Burnett 1967), resulting in extinction of both parasitoid and pest population. Xu *et al.* (1987) found that the higher the ratio of released *Encarsia* to present *Trialeurodes*, the lower the percentage parasitism. They also found that shortly after introduction of the parasitoid in a small greenhouse, many hosts died due to host feeding. Resolving fundamental decisions by *E. formosa* whether to parasitise or host feed can improve our understanding of the extinction process in small greenhouses.

In this paper we present a dynamic state variable model on reproductive decisions in the whitefly parasitoid *E. formosa*, predicting optimal host-handling strategies under different environmental conditions. Focussing on a specific, well-known system has the advantage over a stereotyped "computer parasitoid" in the sense that realistic constraints can be incorporated and more realistic, more detailed and more quantitative predictions can be made and validated. Moreover, *E. formosa* is a solitary endoparasitoid, i.e. it lays only one egg in a host. Thus, it does not make clutch size decisions. In addition, *E. formosa* does not make sex ratio decisions. Virgin females produce female offspring from unfertilised eggs. This so-called thelytokous parthenogenesis is induced by *Wolbachia* bacteria (Stouthamer 1997). Finally, parameterisation of the model is facilitated by the detailed laboratory studies on the parasitoid's biology, life history and foraging behaviour.

We integrate and compile many factors that have previously been studied in seclusion. We incorporate parasitoid's life expectancy, egg load and egg maturation, host quality, host availability, handling time, as well as the decision to reject a host, in addition to the decisions to host feed and oviposit. The optimal decision matrix

parasitoid has to make the decision d to reject ($d=0$), feed upon ($d=1$) or parasitise ($d=2$) the host. Each decision takes a handling time of $t_h(d)$ time steps. The inactive period between the end of a day (t_{max}, a) and the beginning of the next day ($0, a+1$) is modelled as a single event, during which only survival and oogenesis take place. Oogenesis occurs continuously during both the active and the inactive period at a rate of $m(t_m)$ eggs per t_m time steps (see below). For an overview of the used symbols we refer to Table 1.

The model consists of two parts. In the first part, dynamic programming equations are solved using backward iteration. This results in a matrix containing the optimal decision for every possible combination of age a , time step t , egg load x , energy state y and host type h . The second part of the model is a forward iteration, which uses this matrix from the backward iteration to simulate the virtual lives of a cohort of p_{max} computer parasitoids. This results in more testable predictions than generated in the backward iteration.

Backward iteration: the optimal decision

Fitness value

To generate the optimal decision matrix, a so-called fitness value $F(x, y, t, a)$ is calculated. This fitness value is defined as the maximum expected reproductive output between time step t of day a and the death of the optimal foraging parasitoid, given that the parasitoid has an egg load of x eggs and an energy level of y egg equivalents at time step t of day a . It is expressed in number of ovipositions that results in adult offspring, and is thus dimensionless. Fitness values are calculated using two dynamic programming equations, describing the within-day dynamics during the active period and the between-day dynamics during the inactive period. The first equation calculates fitness values at time steps smaller than t_{max} , the second one calculates fitness values at time step t_{max} .

Within-day dynamics

During the active period, the fitness value equals the expected fitness when the parasitoid is interrupted with probability p_{int} , plus the expected fitness when it is not interrupted with probability $1-p_{int}$:

$$F(x, y, t, a) = \left\{ p_{int} * [\text{expected fitness when interrupted}] + (1 - p_{int}) * [\text{expected fitness when not interrupted}] \right\} \quad (1)$$

The expected fitness when interrupted equals the probability to survive the remaining active period $t_{max}-t$ multiplied by the fitness value at time step t_{max} when m egg equivalents are matured into eggs during $t_{max}-t$ time steps:

$$[\text{expected fitness when interrupted}] = e^{-(t_{max}-t)*p_m} * F(x + m(t_{max}-t), y - m(t_{max}-t), t_{max}, a), \quad (2)$$

where we assume that the probability of instant mortality p_m is constant, resulting in an exponential survival distribution. When the parasitoid is not interrupted, it has a probability $1 - \sum p_e(h)$ to gain the expected fitness when no host encountered, and the summation over all host types of a probability $p_e(h)$ to gain the expected fitness when host type h is encountered and handled with the optimal decision (Equation 3).

$$\begin{aligned} &[\text{expected fitness when not interrupted}] = \\ &\left(1 - \sum_{h=1}^{h_{\max}} p_e(h)\right) * [\text{expected fitness when no host encountered}] + \\ &\sum_{h=1}^{h_{\max}} \left(p_e(h) * \left[\begin{array}{l} \text{expected fitness when host type } h \text{ encountered} \\ \text{and handled with optimal decision} \end{array} \right] \right) \end{aligned} \quad (3)$$

When no host is encountered, the expected fitness is the product of the probability that the parasitoid survives the time step, and the fitness value in the next time step when $m(1)$ egg equivalents are matured during one time step (Equation 4).

$$[\text{expected fitness when no host encountered}] = e^{-p_m} * F(x + m(1), y - m(1), t + 1, a). \quad (4)$$

When a host of type h is encountered, the expected fitness equals the fitness increment resulting from the optimal decision. The optimal decision is the decision d that yields the highest sum of current and future expected fitness, provided that $t \leq t_{\max} - t_h(d)$. If the parasitoid rejects the encountered host ($d=0$), the expected fitness equals the product of the probability to survive the handling time needed to reject a host ($t_h(0)$ time steps), and the fitness value at time step $t + t_h(0)$; this fitness value is associated with $m(t_h(0))$ egg equivalents matured into eggs during that period. If the parasitoid would decide to host feed ($d=1$), survival and oogenesis occur over a period $t_h(1)$ time steps needed to feed upon a host, and the fitness value at time step $t + t_h(1)$ is associated with an energy state additionally increased by $g(h)$ egg equivalents to a maximum of y_{\max} . If the parasitoid has at least one egg, it is able to oviposit. In case of oviposition ($d=2$), survival and oogenesis occur over a period of $t_h(2)$ time steps needed to oviposit in a host. The parasitoid gains an immediate fitness increment $W(h)$, which is the egg to adult survival of an oviposition in host type h . This fitness gain is paid for by a reduced egg load in the next time step $t + t_h(2)$. Since solitary parasitoids do not make clutch size decisions, the egg load is simply decreased by 1. Summarising,

$$\begin{aligned} &[\text{expected fitness when host type } h \text{ encountered and handled with optimal decision}] = \\ &\max_d \left[\begin{array}{l} e^{-t_h(0)*p_m} * F(x + m(t_h(0)), y - m(t_h(0)), t + t_h(0), a) \\ e^{-t_h(1)*p_m} * F(x + m(t_h(1)), \min(y - m(t_h(1)) + g(h), y_{\max}), t + t_h(1), a) \\ W(h) + e^{-t_h(2)*p_m} * F(x + m(t_h(2)) - 1, y - m(t_h(2)), t + t_h(2), a) \end{array} \right] \end{aligned} \quad (5)$$

Thus, the first dynamic programming equation describing the within-day dynamics is given by Equation 6 (see also Chan 1991):

$$F(x, y, t, a) = p_{int} * e^{-(t_{max}-t)*P_m} * F(x + m(t_{max}-t), y - m(t_{max}-t), t_{max}, a) +$$

$$(1 - p_{int}) * \left\{ \left(1 - \sum_{h=1}^{h_{max}} p_e(h) \right) * e^{-P_m} * F(x + m(1), y - m(1), t+1, a) + \right.$$

$$\left. \sum_{h=1}^{h_{max}} p_e(h) * \max_d \left[\begin{array}{l} e^{-t_h(0)*P_m} * F(x + m(t_h(0)), y - m(t_h(0)), t + t_h(0), a) \\ e^{-t_h(1)*P_m} * F(x + m(t_h(1)), \min(y - m(t_h(1)) + g(h), y_{max}), t + t_h(1), a) \\ W(h) + e^{-t_h(2)*P_m} * F(x + m(t_h(2)) - 1, y - m(t_h(2)), t + t_h(2), a) \end{array} \right] \right\}. \quad (6)$$

Equation 6 reflects the trade-off between current and future reproduction. Current reproduction, i.e. oviposition, is expressed as the immediate fitness increment $W(h)$, but decreases the egg load and thus the potential to reproduce in the future. Host feeding on the other hand does not have direct fitness benefits but represents an investment in future reproduction through gain of $g(h)$ egg equivalents that can be matured into eggs. Rejection can be an optimal strategy if the time to reject a host is shorter than the handling time of host feeding, which usually is the case. Through rejection, the parasitoid can save time to search for more suitable hosts.

Between-day dynamics

The second dynamic programming equation describes the between-day dynamics. The expected fitness of a parasitoid at the beginning of the inactive period (t_{max}, a) is the probability to survive the inactive period of i time steps, times the fitness value at the beginning of the active period the next day ($0, a+1$) when $m(i)$ egg equivalents are matured into eggs during the inactive period of i time steps (Equation 7).

$$F(x, y, t_{max}, a) = e^{-i*P_m} * F(x + m(i), y - m(i), 0, a+1). \quad (7)$$

Solution

The dynamic programming equations are solved using backward iteration as described in Mangel & Clark (1988). See Appendix 1 for a flow chart of the backward iteration. The starting point is when there is no fitness associated with energy or eggs, i.e. at the end of the maximum reproductive period ($t_{max}+i, a_{max}$). Thus, we set $F(x, y, t_{max}+i, a_{max})=0$ for all combinations of x and y values, which are $(x_{max}+1)*(y_{max}+1)$ zero values. From there the model calculates and stores for each combination of integer egg load and integer energy state the fitness value at $t_{max}-1$, using the first dynamic programming equation (Equation 6). Additionally, the optimal decision is stored for each combination of integer egg load, integer energy state and host type. Third, linear interpolation (see below) in the simulation part of the model requires to store for each

combination of integer egg load, integer energy state, host type and decision, the expected fitness when host of type h is encountered and handled with decision d (the three values in Equation 5). Next, both old and newly acquired fitness values are updated, i.e. they are used in the right hand side of Equation 6 to calculate the optimal decisions and maximum expected fitness values at time step $t_{max}-2$. This iteration process is repeated until fitness values at time step 0 of day a_{max} have been calculated. These are then used in the right hand side of the second programming equation (Equation 7). The resulting fitness values are used again in the right hand side of Equation 6 and the same process repeats for day $a_{max} - 1$ down to 1. The backward iteration ends and the optimal decision matrix is completed when the fitness values at time step 0 of the first day, $F(x,y,0,1)$, have been calculated, which gives the maximum expected lifetime reproductive success for any combination of x and y .

Forward simulation: testable predictions

The optimal decision matrix generated by the first part of the model is used in a Monte Carlo simulation to generate more testable predictions than e.g. the critical egg load below which a parasitoid should host feed. It simulates the virtual lives of a cohort of p_{max} computer parasitoids. See Appendix 2 for a flow chart of the Monte Carlo simulation. Each parasitoid starts to forage alive at time step 0 of the first day (0,1) with x_{init} eggs and y_{init} egg equivalents. The initial egg load x_{init} is randomly chosen within the egg load range. The initial energy level y_{init} is set at $x_{max} - x_{init}$ to a maximum of y_{max} (van Vianen & van Lenteren 1986b; Heimpel *et al.* 1998). A random number generator (RNG) is used to determine whether the parasitoid is interrupted or not between time step $t = 0$ and $t = 1$. If it is, the RNG is used to determine whether or not it will survive the rest of the day. If it survives, the parasitoid matures $m(t_{max}-t)$ egg equivalents (see below: Equation 8) and enters the inactive period t_{max} . If it does not survive, the simulation of the parasitoid ends.

If the parasitoid is not interrupted, the RNG is used to determine whether or not a host is encountered. If no host is encountered, the RNG is used to determine whether or not the parasitoid survives the time step. If it survives, it will enter the next time step with $m(1)$ egg equivalents matured into eggs (see below: Equation 8). If it does not survive, the simulation of the parasitoid ends.

If a host is encountered, the formerly drawn random number is also used to determine the host type. To determine the associated optimal decision, the program reads from the optimal decision matrix the expected fitness associated with the closest integer state values when host of type h is encountered and handled with decision d . Linear interpolation (see below) is used to calculate the optimal decision from these fitness values. In case of a mixed strategy, a decision is randomly selected. After the optimal decision has been determined, the RNG is used once again to determine whether the parasitoid survives the associated time $t_h(d)$ to handle the host. If it survives, it enters the next time step $t+t_h(d)$ with $m(t_h(d))$ egg equivalents matured (see below: Equation 8). In case the parasitoid host feeds, the energy state is additionally increased with $g(h)$ egg

equivalents to a maximum of y_{max} . In case the parasitoid oviposits, its egg load is additionally decreased by 1 and its reproductive output is increased by $W(h)$. If the parasitoid does not survive the $t_h(d)$ time steps, the simulation of the parasitoid ends.

In this way the active period is simulated. When the parasitoid enters the night (t_{max}, a), the RNG is used to determine whether or not the parasitoid survives the inactive period. If it does, it starts foraging at the beginning of the next day ($0, a+1$) with $m(i)$ egg equivalents matured (see below: Equation 8). If it does not, the simulation of the parasitoid ends. The forward procedure ends when the lives of all p_{max} parasitoids have been simulated.

Additional details

Egg maturation

Egg maturation rate declines with egg load and temperature (van Vianen & van Lenteren 1986b; Kajita & van Lenteren 1982). From those data and derivations by van Roermund *et al.* (1996) we model the number of egg equivalents matured into eggs during t_m time steps, $m(t_m)$, as follows. In principle, m increases linearly with decreasing egg load to a maximum of m_{max} eggs per time step when the parasitoid has no eggs. Oogenesis stops, however, when the energy pool y is limiting or when the maximum egg load x_{max} is reached. These conditions are summarised in Equation 8a:

$$m(t_m) = \min \left(\min \left(t_m * m_{max} * \left(1 - \frac{x}{x_{max}} \right), y \right), x_{max} - x \right), \quad (8a)$$

where m_{max} is the maximum rate of oogenesis (per time step), which depends on temperature *temp* (°C) in the following way (after van Roermund *et al.* 1996):

$$m_{max} = \frac{24}{t_{max} + i} * 8.9 * (-0.0205 + 0.004032 * temp). \quad (8b)$$

Linear interpolation

In the optimal decision matrix, only fitness values associated with integer state values are stored. However, fitness values on the right hand side of the dynamic programming equations might be associated with non-integer values of the state variables due to continuous egg maturation. Continuous oogenesis also leads to non-integer state values in the forward simulation. As a consequence, the optimal decision cannot be read directly from the matrix. To determine the fitness value associated with non-integer state values, linear interpolation is applied over stored fitness values associated with integer state values, using Equation 9 (Mangel & Clark 1988, Clark & Mangel 2000):

$$F(x_n, y_n) = \begin{cases} (1 - \Delta x)(1 - \Delta y)F(x, y) + (1 - \Delta x)\Delta y F(x, y + 1) \\ + \Delta x(1 - \Delta y)F(x + 1, y) + \Delta x\Delta y F(x + 1, y + 1) & \text{if } x_n < x_{\max} \wedge y_n < y_{\max} \\ (1 - \Delta y)F(x_{\max}, y) + \Delta y F(x_{\max}, y + 1) & \text{if } x_n = x_{\max} \wedge y_n < y_{\max} \\ (1 - \Delta x)F(x, y_{\max}) + \Delta x F(x + 1, y_{\max}) & \text{if } x_n < x_{\max} \wedge y_n = y_{\max} \\ F(x_{\max}, y_{\max}) & \text{if } x_n = x_{\max} \wedge y_n = y_{\max} \end{cases} \quad (9)$$

where x_n and y_n are non-integer state values, x and y the integer parts of x_n and y_n , and Δx and Δy the non-integer parts of x_n and y_n . Discretisation of states was not applied since differences between fitness values associated with consecutive state values were considered small enough for accurate interpolation.

Mortality

We assume that the probability per time step of instant mortality p_m is constant, resulting in an exponential survival distribution. This implies that the maximum age is theoretically infinite. To limit the time span over which to iterate, we assume that reproduction ceases, i.e. $F(x, y, t_{\max} + i, a_{\max}) = 0$, when survival is 10 %. The probability per time step of instant mortality then becomes:

$$p_m = \frac{-\ln(0.1)}{a_{\max} * (t_{\max} + i)} \quad (10)$$

Parameter Values

a_{\max} , p_{int}

In the laboratory, *E. formosa*'s longevity is affected by temperature, humidity, food, gender (males sometimes occur), host plant species, and host species reared on (van Roermund & van Lenteren 1992b; Hoddle *et al.* 1998). Kajita (1979) found an average longevity of only 1 day at 40°C and 48 days at 15°C. Vet & van Lenteren (1981) even found an average life-span of 99 days at 15.6°C. At 25°C, average longevity was only 2 to 3 days at high and low humidities, and 19 days at intermediate levels (74% R.H.) (Kajita 1979). When parasitoids were able to oviposit and host feed, van Lenteren *et al.* (1987) observed life-spans of on average 52 days. On artificial diets, average life-spans ranged from 37 days on honey, 29 days on honeydew and 22 days on a glucose solution. In absence of food their parasitoids died on average after 3 days. At low temperature, however, Vet & van Lenteren (1981) found that females allowed to oviposit and host feed lived considerably shorter than females who lived on honey alone. Females appeared to live longer than males (Vet & van Lenteren 1981; van Lenteren *et al.* 1987). Females isolated on cucumber had an average life-span of almost 80 days, compared with less than 10 days for females isolated on tobacco (van Lenteren *et al.* 1987). Experiments by Szabo *et al.* (1993) indicated that females reared on *Trialeurodes*

vaporariorum had a longer life-span (11.9 days) than females reared on the smaller *Bemisia tabaci* (8.7 days).

Few data are available on longevity under natural conditions in the field. Predators may reduce survival probabilities considerably compared with laboratory and greenhouse conditions (Rosenheim 1998). Heimpel *et al.* (1997b) observed predation on *Aphytis* parasitoids in a deserted Californian citrus orchard. From these field observations they calculated that the average longevity of *Aphytis* parasitoids should approximate merely 6.7 daylight hours during the predatory season. During these months, *Aphytis*' survival from predators should often not exceed 2 days, and it should rarely exceed 5 days. When considering other seasons as well (Heimpel *et al.* 1998), they still found that overall only 1% of the parasitoids survived 8 days of foraging. These estimates may also apply to *E. formosa*, since *Aphytis* and *Encarsia* belong to the same family, have similar sizes, attack sessile Homoptera, and generally live between 2 and 6 weeks in the laboratory. On the other hand, anecdotal evidence suggests that *E. formosa* can survive up to at least 10 days in the field (J.C. van Lenteren, pers. obs.). A number of age determination methods was tested to quantify life expectancy in the field of *E. formosa* and related whitefly parasitoids, but none proved useful (see thesis' Appendix). Using different scenarios (see below), we studied optimal host-handling decisions and lifetime reproductive success when parasitoid's life expectancy was short ($a_{max} = 7$ days, i.e. mean longevity on average about 3 days) and long ($a_{max} = 70$ days, i.e. mean longevity on average about 30 days).

Parasitoids are able to change their behaviour in response to rain or wind (Fink & Völkl 1995) or to changes in barometric pressure associated with thunderstorms (Roitberg *et al.* 1993). Bad weather might therefore not directly reduce survival probabilities, but can greatly reduce fitness (Weisser *et al.* 1997) by interrupting foraging activity. If we assume parasitoids are interrupted in foraging for the rest of the active period within a day about once every 10 days, and the active period t_{max} consists of 100 time steps (see below), the interruption probability (p_{int}) is to be set at about 0.001 per time step.

t_h, t_{max}, i

Ovipositorial rejection and oviposition take on average 6 minutes (van Roermund & van Lenteren 1995b), although antennal rejection can be much faster (5-35 seconds). Host feeding can take much longer. Van Roermund & van Lenteren (1995b) found an average of 27 min and cite unpublished data by Sevenster-van der Lelie who found a mean of 15 min. Sütterlin & van Lenteren (1999) found that host feeding in *E. formosa* takes about three times longer than oviposition. We set the length of one time step to 6 minutes and the handling times $t_h(d)$ to 1 ($d=0$, rejection), 3 ($d=1$, host feeding) and 1 ($d=2$, oviposition) time steps, i.e. $t_h(d)=(1,3,1)$. In the sensitivity analysis (see below), we compare the output with equal handling times of 1 time step, i.e. $t_h(d)=(1,1,1)$. We set the active period to 10 hours per day, since nocturnal flight is rare (Ekbohm 1982), activity decreases with temperature (van Roermund & van Lenteren 1995a) and

oviposition by *E. formosa* hardly occurs in complete darkness (Hoogcarspel & Jobsen 1984). The number of 6-min time steps within the 10-hour active period (t_{max}) therefore amounts to 100 and within the 14-hour inactive period (i) to 140.

x_{max}, y_{max}

The number of ovarioles in *E. formosa* can range from 6 to 15 with an average of about 9 (van Vianen & van Lenteren 1982, 1986a). Most authors rarely found more than one egg per ovariole (van Keymeulen & Degheele 1977; Kajita & van Lenteren 1982). However, van Vianen & van Lenteren (1986b) found up to 1.7 mature eggs per ovariole and van Lenteren *et al.* (1987) reported that it is able to store up to 3 mature eggs per ovariole at lower temperatures. The egg load capacity (x_{max}) is thus set to 15 eggs. Since *E. formosa* females can lay and mature up to 10 eggs per day (van Vianen & van Lenteren 1986b; van Roermund *et al.* 1996), the energy state capacity y_{max} is set to 10.

h_{max}

Six nymphal stages are distinguished in the hemimetabolous greenhouse whitefly *T. vaporariorum*, which is often treated as holometabolous insect with four larval, a prepupal and pupal stage (Nell *et al.* 1976). The third, fourth and fifth nymphal stage are preferred for oviposition, whereas the second and sixth nymphal stage are preferred for host feeding (Nell *et al.* 1976; Woets & van Lenteren 1976; Nechols & Tauber 1977). In the model, these preferences are translated into two host types ($h_{max}=2$).

$temp, m_{max}$

Temperature $temp$ is set to 25°C, which implies that the maximum rate of egg maturation m_{max} equals about 0.071 eggs per time step (Equation 8b), i.e. about 0.71 eggs per hour.

$p_e(h), r_e(h)$

The probability p_e to encounter host type h within t time steps is derived from the Poisson distribution:

$$p_e(h) = 1 - e^{-r_e(h) \cdot t}, \quad (11a)$$

where $r_e(h)$ is the encounter rate with host type h , i.e. number of hosts of type h encountered during time step t . Equation 11a is based on well-documented observations that *E. formosa* does not distinguish between infested and uninfested plants or leaves (Noldus & van Lenteren 1990; Romeis & Zebitz 1997; Sütterlin & van Lenteren 2000) and has a random walking pattern on leaves (van Lenteren *et al.* 1976a; Li *et al.* 1987;

Table 2 Parameters used to calculate rate $r_e(h)$ and probability per time step $p_e(h)$ to encounter a host of type h .

| Symbol | Description | Unit | Value |
|--------------|---|---------------------------------|------------------|
| <i>act</i> | Fraction of total searching time walking actively | - | 0.75 |
| <i>dens</i> | Density of total host population | cm ⁻² | See scenarios |
| <i>dm(h)</i> | Host diameter | cm | (0.0342, 0.0485) |
| <i>rc(h)</i> | Relative contribution of host type h to total host population | - | (0.5, 0.5) |
| <i>sw</i> | Width of parasitoid's searching path | cm | 0.0546 |
| <i>ws</i> | Walking speed of parasitoid | cm per time step ^(a) | 13 |

^(a) Length of time step corresponds to $1440/(t_{max}+i) = 6$ min.

van Roermund & van Lenteren 1995a; Sütterlin & van Lenteren 1997). Van Roermund *et al.* (1996) used an equation derived by Skellam (1958) for the encounter rate:

$$r_e(h) = (sw + dm(h)) * ws * act * dens * rc(h), \quad (11b)$$

where *sw* is the width of the path the parasitoid covers during searching (cm), *dm(h)* the mean diameter of host type h (cm), *ws* the walking speed of the parasitoid (cm per time step), *act* the walking activity of the parasitoid (expressed as fraction of total time spent searching), *dens* the density of the total host population (cm⁻²) and *rc(h)* the relative contribution of host type h to the total host population (Table 2). If the total probability of host encounter exceeds 1, each probability to encounter host type h is divided by this total probability. In this way, total host encounter probability is set to 1 whereas the ratio between the individual host encounter probabilities is preserved.

sw, dm(h), ws, act

Van Roermund *et al.* (1996) estimated the searching width as twice the head width given by van Vianen and van Lenteren (1986a), and is set here to 0.0546 cm. The mean host diameter is the average of host length and width measured by van Lenteren *et al.* (1976a). The mean host diameters were averaged over first, second and sixth instar, i.e. bad quality nymphs for oviposition, as an estimate for host type 1 (0.0342 cm) and over third, fourth and fifth instar, i.e. good quality nymphs for oviposition, as an estimate for host type 2 (0.0485 cm). Walking speed varies with leaf hairiness and venation (van Lenteren *et al.* 1976a, 1995; Woets & van Lenteren 1976; Hulspsas-Jordaan & van Lenteren 1978; Li *et al.* 1987; Sütterlin & van Lenteren 1997), temperature (van Roermund & van Lenteren 1995b) and egg load (Sütterlin & van Lenteren 1993), and is usually measured per second. Extrapolating from these experiments, we decided to parameterise walking speed at 13 cm per 6-min time step. Walking activity, i.e. walking

while drumming on the leaf surface, is about 75% of the total time on the leaf (van Roermund & van Lenteren 1995a; Sütterlin & van Lenteren 1997, 1999).

dens, rc(h)

Besides the effect of life expectancy, our major focus is on the effect of host density (*dens*) on host-handling decisions. Therefore, we compared natural whitefly densities found in *E. formosa*'s presumed area of origin (Chapter 4) with artificial host densities found during pest outbreaks. Analysis of leaves collected in undisturbed nature areas in Costa Rica indicated that on average merely 0.015 nymphs per cm² leaf area (1 nymph per 67 cm² leaf area) occurred under natural conditions. Since host type 1 and 2 occurred approximately at a 1:1 ratio, *rc(h)* was set to (0.5,0.5). Using these parameter values, the probability of host encounter per 6-min time step equals about 0.0065 for host type 1 and about 0.0075 for host type 2 (Equation 11). On the other hand, yield reduction on tomato has been reported at an average pest density of 22 nymphs cm⁻² and economic damage above 6 nymphs cm⁻² (Hussey *et al.* 1958). On poinsettia plants (*Euphorbia pulcherrima*), 0.3-0.7 nymphs cm⁻² are a commercially acceptable density (Helgesen & Tauber 1974). Thus, if we set the total host density at pest conditions to 1.5 nymphs cm⁻², i.e. a 100-fold higher than at natural conditions, the probability of host encounter per

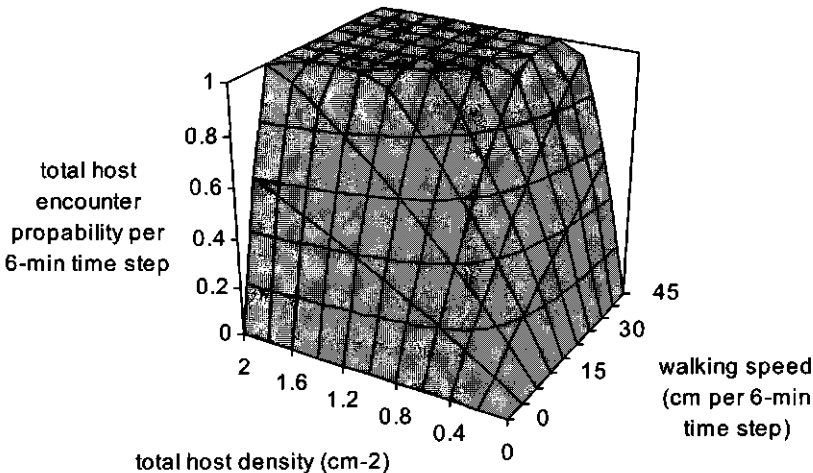


Fig. 1 Total probability per time step $\Sigma p_a(h)$ to encounter a host (per 6-min time step) as determined by total host density *dens* (cm⁻²) and parasitoid's walking speed *ws* (cm per 6-min time step). In the parameter space where total host encounter probability per time step equals 1 per time step, handling time rather than host encounter rate limits the number of hosts that can be handled per day. Other parameter values: *sw* = 0.0546 cm; *dm(h)* = (0.0342, 0.0485) cm; *act* = 0.75; *rc(h)* = (0.5, 0.5).

6-min time step equals 0.47 for host type 1 and 0.53 for host type 2. Fig. 1 shows total host encounter probability $\Sigma p_e(h)$ in relation to the most variable parameters, i.e. total host density $dens$ and walking speed ws . Note that for our parameter values, 1.48 hosts cm^{-2} is about the density above which handling time and below which host density limits host encounter rate.

$g(h)$

Collier (1995a) found that *Aphytis melinus* parasitoids allowed to feed on honey and one host had about 1.5 more eggs after 24 h than females that received only honey. Females that oviposited after host feeding matured about 2.7 eggs within 24 h. Heimpel *et al.* (1994) found that *Aphytis lingnanensis* parasitoids allowed to oviposit and to feed on one third instar host matured within two days on average 2.3 eggs more than parasitoids allowed to oviposit only. For *E. formosa*, Szabo *et al.* (1993) found a ratio of 1.8 ovipositions per host feeding per female per day on *T. vaporariorum*, and 2.6 on *Bemisia tabaci*. These host-feeding gains can be underestimates because materials obtained from host feeding can be stored for oogenesis later in life (Heimpel *et al.* 1997a; Rivero & Casas 1999b). In Chapter 3, 3.6 eggs was a minimum 5-day estimate of the host-feeding gain in *E. formosa*. For *Aphytis melinus*, the host-feeding gain did not differ between a second and a third instar (Collier 1995a). Moreover, host type 1 in our model represents not only the smallest first and second instars but also the largest sixth instar. We therefore set the host-feeding gain $g(h)$ at 4 for both host types.

$W(h)$

We define the direct fitness increment $W(h)$ from oviposition on host type h as the probability that the parasitoid's egg survives to emergence. Arakawa (1982) found no significant differences in survival percentages between the host stages, but scored ovipositions when the time from drumming to withdrawing the ovipositor exceeded 100 seconds, instead of direct verification through nymphal dissection. Nechols & Tauber (1977) found that about a quarter of the parasitoid eggs survived to adult emergence when *E. formosa* females parasitised sessile first instars, whereas the majority (about 90%) survived when third, fourth or fifth instars were parasitised. Since larval development of the parasitoid starts in the fourth nymphal stage of the host (Nechols & Tauber 1977), sixth instars are also less suitable hosts for oviposition. Therefore, the direct fitness increment from oviposition on host type 1 is set to 0.25 and on host type 2 to 0.90.

Scenarios

Host-feeding strategies were investigated under six different scenarios (Table 3) using the parameters host density $dens$ and age horizon a_{max} . In three scenarios,

Table 3 Scenarios, characterised by age horizon a_{max} (days), and total host density $dens$ (cm^{-2}). S stands for short, L for long life expectancy; F for field density, I for intermediate density and P for pest density.

| | | $dens$ (cm^{-2}) | | |
|------------------|----|----------------------|------|-----|
| | | 0.015 | 0.15 | 1.5 |
| a_{max} (days) | 7 | SF | SI | SP |
| | 70 | LF | LI | LP |

parasitoids had a short (S) life expectancy, corresponding to assumed field conditions ($a_{max} = 7$ days). In the other three, parasitoids had a long (L) life expectancy corresponding to greenhouse conditions ($a_{max} = 70$ days). These time horizons correspond to a mean longevity of about 3 (S) and 30 (L) days ($1/p_m$ time steps). Within each of these scenarios, parasitoids were simulated at field (F) host density, corresponding to the average field density of 0.015 cm^{-2} , and at pest (P) host density, corresponding to a pest density of 1.5 cm^{-2} . Additional simulations were run using an intermediate (I) host density of 0.15 cm^{-2} .

Model Predictions

The backward iteration resulted in a matrix containing the optimal decision for every combination of a , t , x , y and h , i.e. 246,400 (short life expectancy) or 2,464,000 (long life expectancy) optimal decisions. The size and five dimensions of the matrix make it difficult to study the effects of a , t , x , y and h on the optimal decision under the different scenarios. Even qualitative predictions are complicated as a result of high-order interactions. Furthermore, possible output like the critical egg load at and below which a parasitoid should host feed, is difficult to test empirically. We therefore present output only from the forward iteration, which used the matrix to simulate the virtual lives of a cohort of parasitoids.

Figure 2 shows the effect of host density and parasitoid's life expectancy on the optimal fraction of host encounters accepted for either host feeding or oviposition ("acceptance ratio"; Fig. 2a), the optimal fraction of accepted hosts fed upon ("host-feeding ratio"; Fig. 2b) and the lifetime reproductive success ("fitness"; Fig. 2c). At low host densities, each host encountered should be accepted, but the acceptance ratio sigmoidally decreases with increasing host density (Fig. 2a). At pest density (scenario P), only about 27 % of the hosts encountered can be accepted due to egg limitation (see below). This percentage is stable for host densities above 1.48 cm^{-2} , because the shortest handling time (1 time step or 6 min) then limits the number of host encounters per day, given the other parameter values in Table 2 (see Fig. 1; $w_s=13\text{ cm}$ per time step). Life expectancy does not importantly affect the acceptance ratio (Fig. 2a).

Under presumable field conditions, i.e. short life expectancy and low host density, none (0.001 ± 0.014) of the accepted hosts should be used for feeding (Fig. 2b).

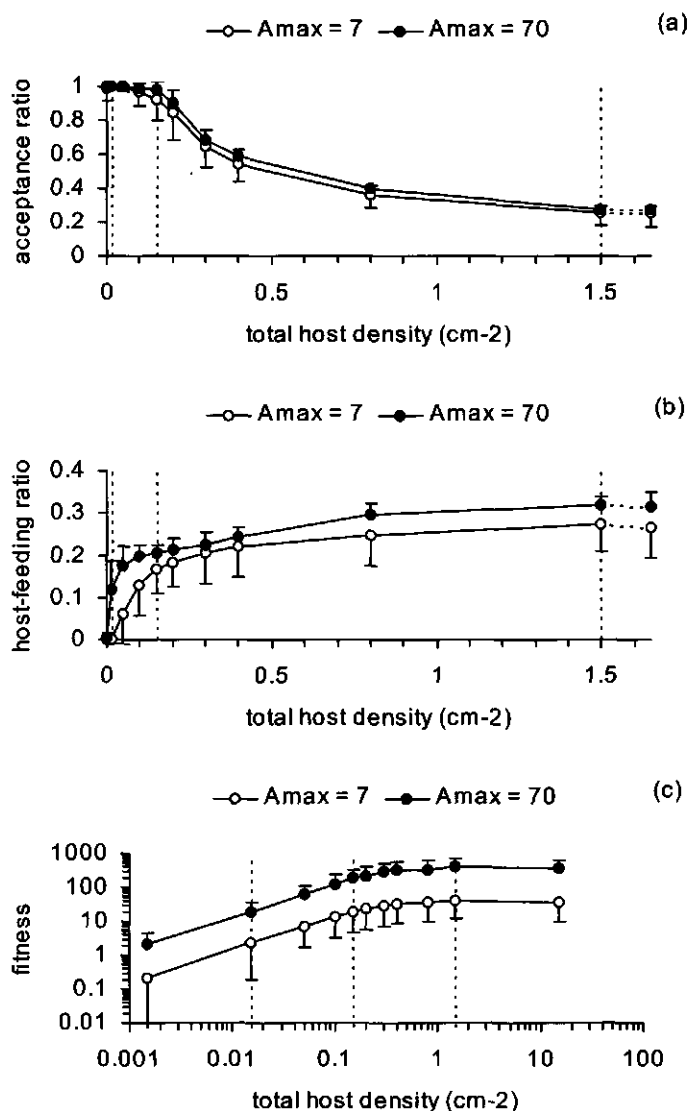


Fig. 2 Simulated mean (\pm sd) (a) fraction of host encounters accepted either for host feeding or oviposition, (b) fraction of accepted hosts fed upon, (c) lifetime reproductive success (lifetime number of deposited eggs surviving until adulthood) of parasitoids that make optimal host-handling decisions, plotted against total host density dens (cm^{-2}) when life expectancy is short (open symbols, $a_{\max} = 7$ days) and long (closed symbols, $a_{\max} = 70$ days). Detached data points on the right hand side of (a) and (b) are results from simulations with a total host density of 15 cm^{-2} . Vertical dotted lines indicate densities used as scenario.

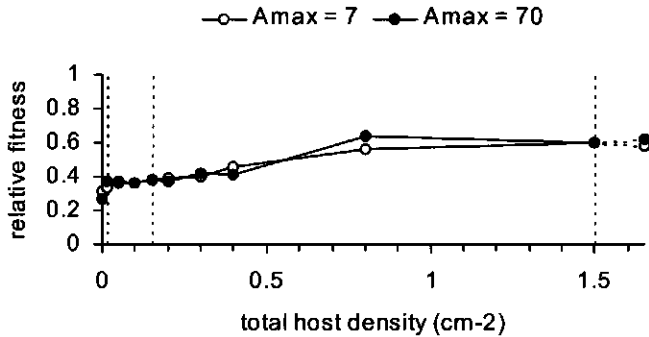


Fig. 3 Simulated mean lifetime reproductive success (lifetime number of deposited eggs surviving until adulthood) of parasitoids that decide randomly whether to reject, host feed (provided that $t \leq t_{max} - t_h(1)$) or oviposit (provided that $x \geq 1$) relative to simulated mean lifetime reproductive success of parasitoids that make optimal host-handling decisions, plotted against total host density $dens$ (cm^{-2}) when life expectancy is short (open symbols, $a_{max} = 7$ days) and long (closed symbols, $a_{max} = 70$ days). Detached data points on the right hand side are results from simulations with a total host density of $15\ cm^{-2}$. Vertical dotted lines indicate densities used as scenario.

However, when life expectancy is long, 12 % (0.120 ± 0.068) of the accepted hosts should be fed upon at field host density. The mean optimal host-feeding ratio initially increases steeply with host density but levels off to 27 % (short life expectancy) and 32 % (long life expectancy) of the accepted hosts. Thus, both host density and parasitoid's life expectancy have a positive effect on the optimal host-feeding ratio, especially at low host densities.

Host density and life expectancy also have a profound impact on lifetime reproductive success (Fig. 2c). Lifetime reproductive success clearly levels off to about 40 ± 30 (short life expectancy) and 380 ± 300 (long life expectancy) when host density exceeds $0.5\ cm^{-2}$. In the field scenario (SF), lifetime reproductive success of parasitoids that make optimal host-handling decisions averages 2.4 ± 2.2 (Fig. 2c). This is close to a stable population, in which parasitoids would replace themselves by having a mean lifetime reproductive success of one.

Fig. 3 shows the lifetime reproductive success of parasitoids that decide randomly whether to reject, host feed (provided that $t \leq t_{max} - t_h(1)$) or oviposit (provided that $x \geq 1$) relative to the lifetime reproductive success of parasitoids that make optimal host-handling decisions (Fig. 2c). Parasitoids that make random decisions gain on average only 35 % ($0.0015\ hosts\ cm^{-2}$) to 60 % ($1.5\ hosts\ cm^{-2}$) of the lifetime reproductive success that parasitoids gain when making optimal decisions, independent of life expectancy. This suggests that there is a strong selection pressure on making optimal decisions and that the selection pressure is highest at low host densities.

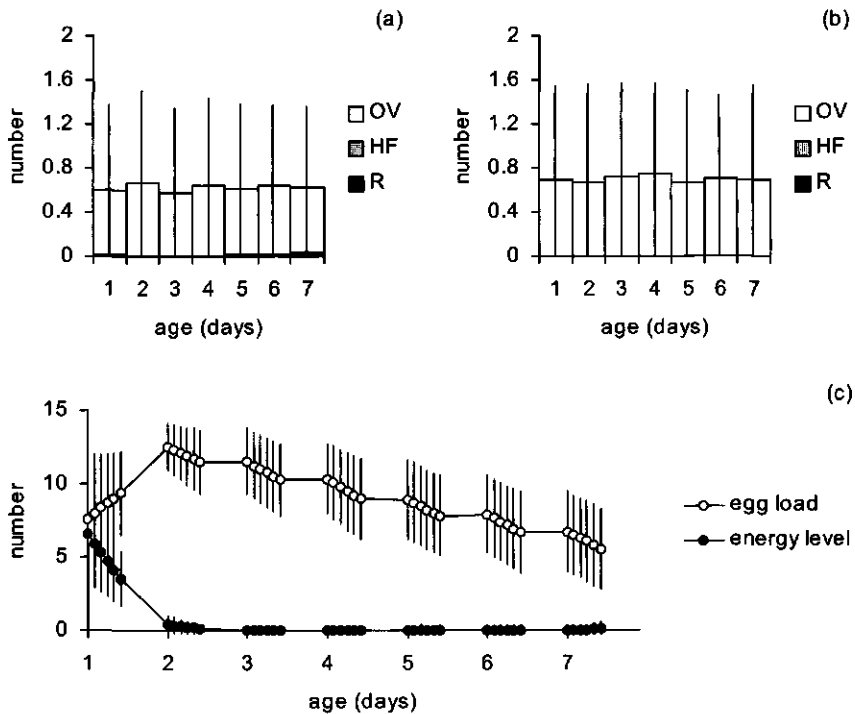


Fig. 4 Output from Monte Carlo simulation for scenario SF where parasitoids have a short life expectancy ($a_{max} = 7$ days) and forage at field host density ($dens = 0.015$ hosts cm^{-2}). Shown are stacked means (\pm sd) for number of (a) bad quality hosts ($W(1)=0.25$) and (b) good quality hosts ($W(2)=0.9$) rejected (black bars), fed upon (grey bars) and used for oviposition (white bars) per day; and (c) mean (\pm sd) egg load (white symbols) and energy level (black symbols) at within-day intervals of 20 time steps (2 hours).

To gain more insight in the patterns observed in Fig. 2, we plotted the stacked means for the number of hosts rejected, fed upon and used for oviposition per day and per host type, and the state dynamics, for the six scenarios (Fig. 4 to 9). Again, it is clear that host feeding is virtually absent when parasitoids are simulated under presumably natural conditions (Fig. 4). Every host encountered should be used for oviposition regardless of host quality or parasitoid's age (Fig. 4a,b). Once all the energy reserves from the immature stage are converted into eggs, the egg load only gradually decreases due to rare events of oviposition (Fig. 4c). The host encounter rate is much slower than the egg maturation rate and parasitoids are clearly host limited. Nevertheless, they encounter and parasitise on average about 0.6 bad quality hosts per day (Fig. 4a) and about 0.7 good quality hosts per day (Fig. 4b) (in total about 1.3 hosts per day).

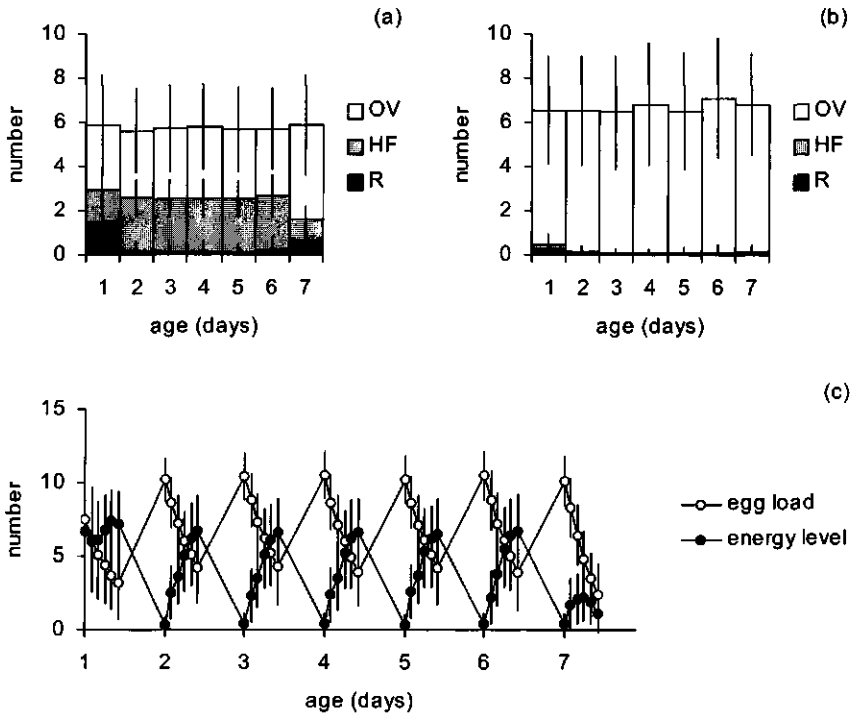


Fig. 5 Output from Monte Carlo simulation for scenario SI where parasitoids have a short life expectancy ($a_{\max} = 7$ days) and forage at intermediate host density ($\text{dens} = 0.15$ hosts cm^{-2}). Shown are stacked means (\pm sd) for number of (a) bad quality hosts ($W(1)=0.25$) and (b) good quality hosts ($W(2)=0.9$) rejected (black bars), fed upon (grey bars) and used for oviposition (white bars) per day; and (c) mean (\pm sd) egg load (white symbols) and energy level (black symbols) at within-day intervals of 20 time steps (2 hours).

At intermediate host density of 0.15 cm^{-2} (Fig. 5), parasitoids encounter on average about 5.7 bad quality hosts per day (Fig. 5a) and about 6.7 good quality hosts per day (Fig. 5b) (in total about 12 hosts per day). All good quality hosts should be used for oviposition (Fig. 5b). Almost half of the bad quality hosts should be fed upon (Fig. 5a). The host-handling behaviour is more or less independent of the parasitoid's age (Fig. 5a) because the probability of instant mortality p_m is constant. Only very young parasitoids host feed less because they can use reserves from the immature stage for oogenesis. Parasitoids approaching the maximum reproductive period (recall that $F(x, y, t_{\max} + i, a_{\max}) = 0$) invest less in future reproduction, i.e. decrease the fraction of accepted hosts used for host feeding. Host feeding occurs mostly at the beginning of each active period when most egg equivalents have matured into eggs overnight (Fig. 5c). At the end of each active period, host feeding decreases as the energy level approaches its capacity y_{\max} .

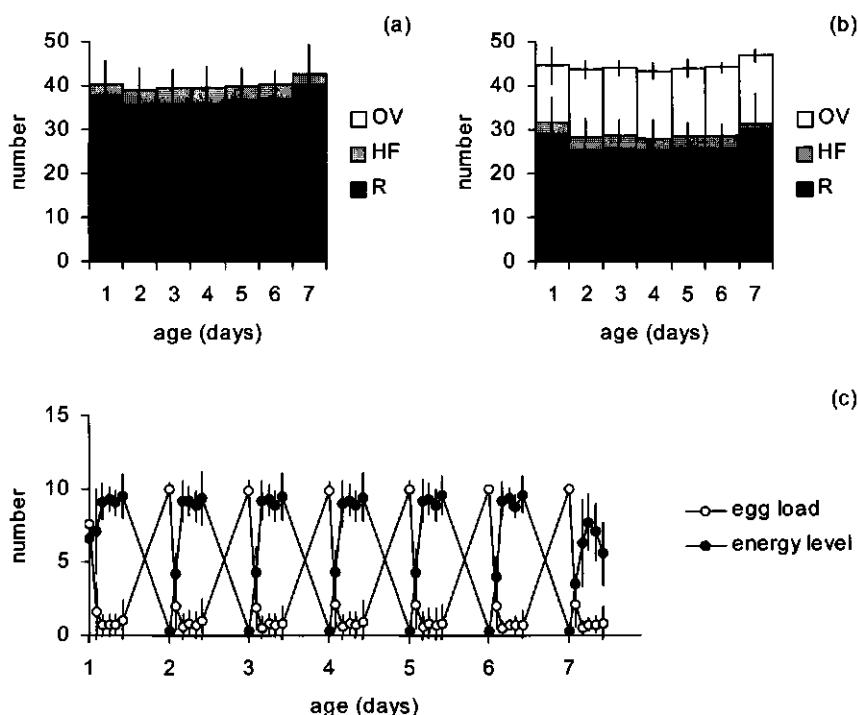


Fig. 6 Output from Monte Carlo simulation for scenario SP where parasitoids have a short life expectancy ($a_{max} = 7$ days) and forage at pest host density ($dens = 1.5$ hosts cm^{-2}). Shown are stacked means (\pm sd) for number of (a) bad quality hosts ($W(1)=0.25$) and (b) good quality hosts ($W(2)=0.9$) rejected (black bars), fed upon (grey bars) and used for oviposition (white bars) per day; and (c) mean (\pm sd) egg load (white symbols) and energy level (black symbols) at within-day intervals of 20 time steps (2 hours).

(Fig. 5c). The oviposition rate is higher than at field host density but host-feeding behaviour prevents temporary egg limitation.

When parasitoids forage at a pest density of 1.5 hosts cm^{-2} (Fig. 6), they encounter on average about 40 bad quality hosts per day (Fig. 6a) and about 44 good quality hosts per day (Fig. 6b) (in total about 84 hosts per day). Recall that at this density, the probability of host encounter is 1 per time step. Parasitoids can be selective and should save all their eggs, i.e. about 15 per day, for oviposition in good quality hosts (Fig. 6a,b). Per day, parasitoids feed on average upon 3.2 bad quality hosts and 2.6 good quality hosts. Although this is enough so that the energy level does not limit egg maturation rate (Equation 8a) and is at its maximum at the end of the active period, egg maturation rate cannot account for the rate of oviposition (Fig. 6c). During the night when no oviposition takes place, the egg load increases but as soon as foraging starts again,

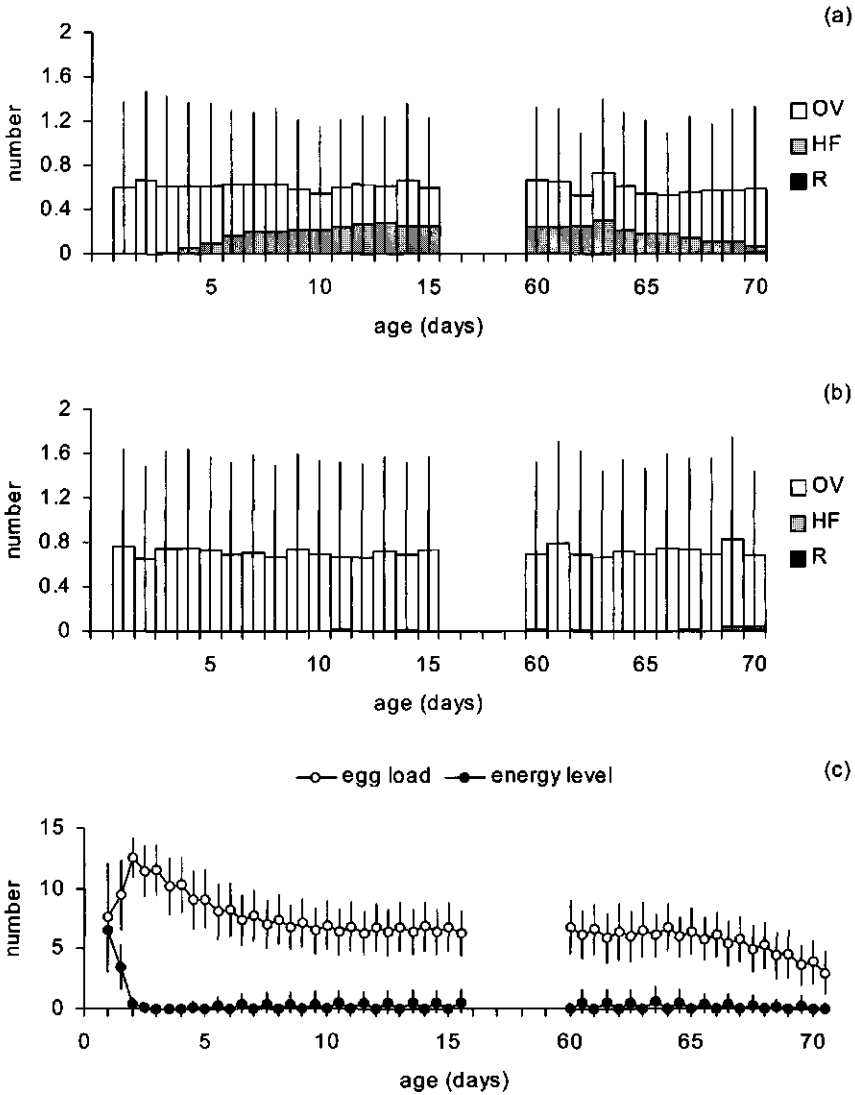


Fig. 7 Output from Monte Carlo simulation for scenario LF where parasitoids have a long life expectancy ($a_{max} = 70$ days) and forage at field host density ($dens = 0.015$ hosts cm^{-2}). Shown are stacked means (\pm sd) for number of (a) bad quality hosts ($W(1)=0.25$) and (b) good quality hosts ($W(2)=0.9$) rejected (black bars), fed upon (grey bars) and used for oviposition (white bars) per day; and (c) mean (\pm sd) egg load (white symbols) and energy level (black symbols) at the beginning and the end of each active period. Data are more or less unchanging between 15 and 60 days and therefore not shown.

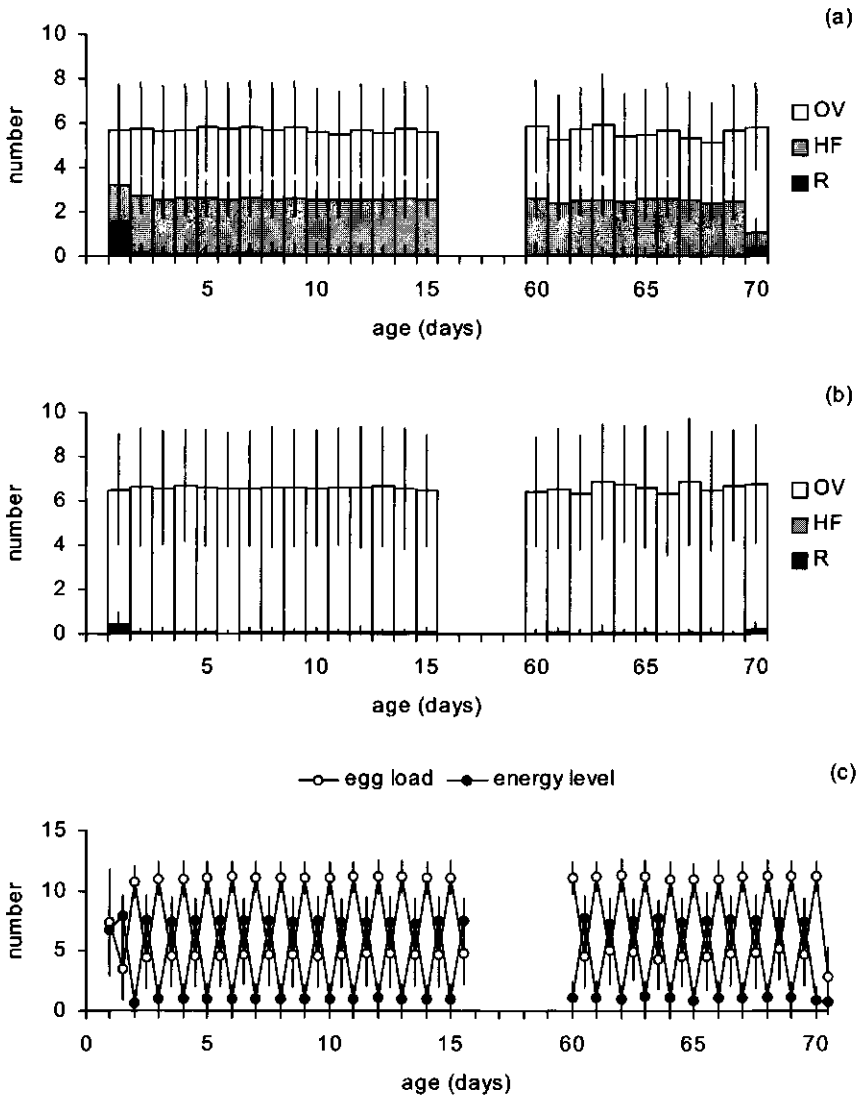


Fig. 8 Output from Monte Carlo simulation for scenario LI where parasitoids have a long life expectancy ($a_{max} = 70$ days) and forage at intermediate host density ($dens = 0.15$ hosts cm^{-2}). Shown are stacked means (\pm sd) for number of (a) bad quality hosts ($W(1)=0.25$) and (b) good quality hosts ($W(2)=0.9$) rejected (black bars), fed upon (grey bars) and used for oviposition (white bars) per day; and (c) mean (\pm sd) egg load (white symbols) and energy level (black symbols) at the beginning and the end of each active period. Data are more or less unchanging between 15 and 60 days and therefore not shown.

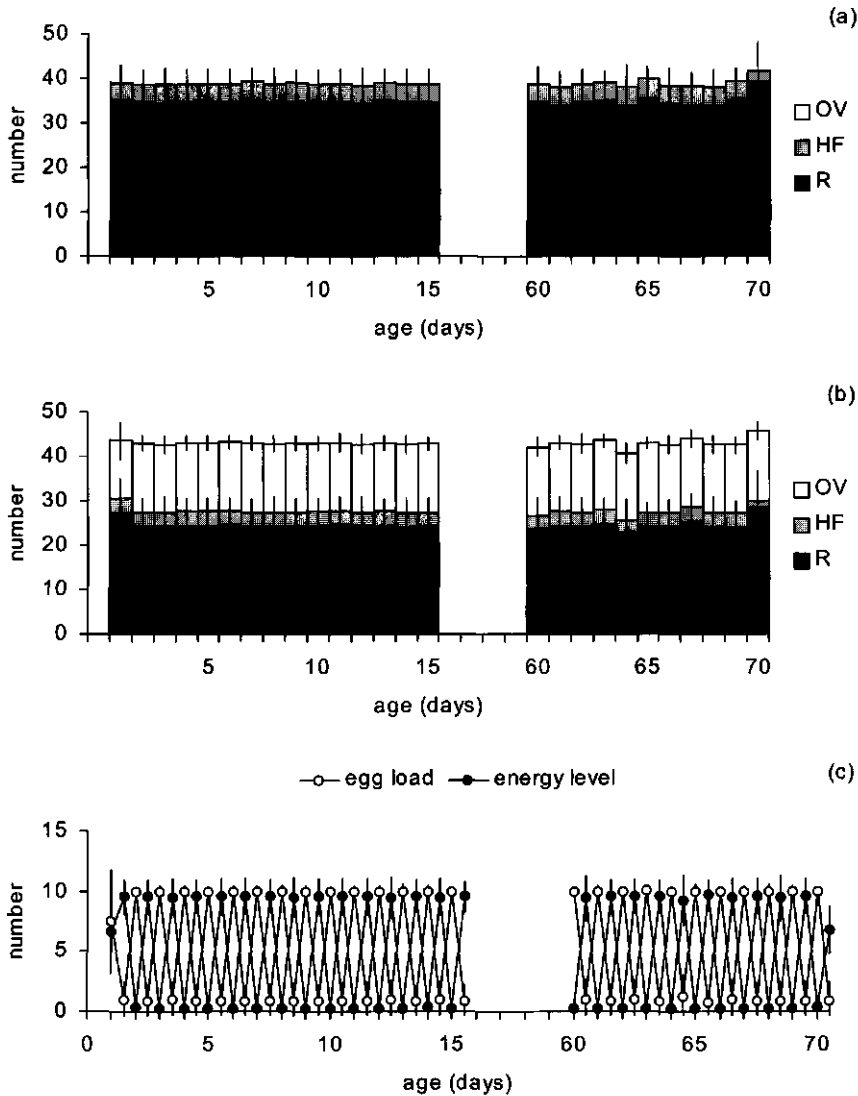


Fig. 9 Output from Monte Carlo simulation for scenario LP where parasitoids have a long life expectancy ($a_{max} = 70$ days) and forage at pest host density ($dens = 1.5$ hosts cm^{-2}). Shown are stacked means (\pm sd) for number of (a) bad quality hosts ($W(1)=0.25$) and (b) good quality hosts ($W(2)=0.9$) rejected (black bars), fed upon (grey bars) and used for oviposition (white bars) per day; and (c) mean (\pm sd) egg load (white symbols) and energy level (black symbols) at the beginning and the end of each active period. Data are more or less unchanging between 15 and 60 days and therefore not shown.

parasitoids quickly become temporarily egg limited. Thus, parasitoids are not able to handle more than about 21 hosts per day because eggs are depleted and the energy level reaches the maximum capacity. This explains the low acceptance ratio of about 27 % (Fig. 2a). The egg maturation rate also limits lifetime reproductive success at higher host densities (Fig. 2c).

When parasitoids have a long life expectancy (Fig. 7 to 9), parasitoids do host feed at low host density, i.e. on average almost half of the bad quality hosts encountered (Fig. 7a). In contrast to parasitoids that have a short life expectancy (Fig. 4c), parasitoids that have a long life expectancy have to host feed to prevent egg limitation even at field host density (Fig. 7c). Preventing egg limitation through host feeding clearly offsets the associated loss of reproductive opportunities. Again, the behaviour is independent of parasitoid's age, except when energy from the immature stage can still be used for oogenesis or when the maximum reproductive period is approached. At higher host densities, life expectancy does not have a clear effect on host-handling behaviour (Fig. 5 vs. 8 and 6 vs. 9). Parasitoids become limited by the rate of egg maturation from host-feeding gains.

Elasticity Analyses

Elasticity analyses were performed for the six scenarios to reveal the impact of each parameter on the model predictions. Elasticity e is defined as the change in a response variable y from y_{old} to y_{new} relative to the change in a parameter p from $p_{default}$ to p_{new} :

$$e = \frac{(y_{new} - y_{old})/y_{old}}{(p_{new} - p_{default})/p_{default}}. \quad (12)$$

Hence, an elasticity of -2 means that the change in the response variable was twice as large as, and in the opposite direction of the change in the parameter. As response variables we used the mean optimal fraction of host encounters that should be accepted ("acceptance ratio"), the mean optimal fraction of accepted hosts that should be fed upon ("host-feeding ratio"), and the mean predicted lifetime reproductive success ("fitness"), predicted from Monte Carlo simulations with $p_{max} = 1000$ parasitoids (Fig. 2). Default parameter values were halved one by one ($p_{new} = 1/2 * p_{default}$). The default value of three discrete parameters were multiplied by a divergent factor, i.e. $3/7$ (a_{max} , scenarios S), $7/15$ (x_{max}), and $1/3$ ($t_h(1)$), to keep the parameter value discrete. Three parameters that depend on host type, i.e. $dm(h)$, $g(h)$ and $W(h)$, were changed per host type and simultaneously for both host types. When the number of host types (h_{max}) was reduced from 2 to 1, parameters values that depended on host type were averaged, i.e. $dm=0.04135$ cm, $g=4$ egg equivalents and $W=0.575$; rc was set to 1.

Table 4 shows the change in the predicted mean acceptance ratio (Table 4a, Fig. 2a), host-feeding ratio (Table 4b, Fig. 2b) and lifetime reproductive output (Table 4c, Fig. 2c), relative to the change in a parameter for the six scenarios. At field host density, the

Table 4a Change in predicted mean optimal fraction of host encounters that should be accepted for either host feeding or oviposition ("acceptance ratio") under default parameter values relative to change in parameter value (elasticity), for six scenarios. Default parameter values were generally halved (see text). Acceptance ratios under default parameter values are given for reference. High elasticities are outlined. a_{max} : parasitoid's age horizon (days); act : fraction of searching time walking actively; $dens$: density of total host population (cm^{-2}); $dm(h)$: diameter of host type h (cm); $g(h)$: gain from feeding on host type h (egg equivalents); h_{max} : number of host types; p_{int} : interruption probability (per time step); $rc(1)$: relative contribution of host type 1 to total host population; sw : parasitoid's searching width (cm); $t_h(1)$: handling time for host feeding (time steps); t_{max} : number of time steps in active period ($t_{max}+i=240$); $temp$: temperature at which oogenesis occurs ($^{\circ}\text{C}$); $W(h)$: direct fitness increment from oviposition in host type h ; ws : parasitoid's walking speed (cm per time step); x_{max} : maximum egg load; y_{max} : maximum energy level.

| Life expectancy | | -----Short ($a_{max} = 7$ days)----- | | | -----Long ($a_{max} = 70$ days)----- | | |
|---|---------|---------------------------------------|--------------|--------|---------------------------------------|--------------|--------|
| Host | density | Field | Intermediate | Pest | Field | Intermediate | Pest |
| (cm^{-2}) | | (0.015) | (0.15) | (1.5) | (0.015) | (0.15) | (1.5) |
| Acceptance ratio under default parameter values | | | | | | | |
| | | 0.996 | 0.922 | 0.256 | 1.000 | 0.978 | 0.276 |
| Elasticity | | | | | | | |
| a_{max} | | 0.001 | 0.058 | 0.117 | 0.000 | 0.025 | 0.014 |
| act | | -0.005 | -0.143 | -0.979 | 0.000 | -0.041 | -1.009 |
| $dens$ | | -0.003 | -0.136 | -0.950 | 0.000 | -0.041 | -1.010 |
| $dm(1)$ | | -0.004 | -0.009 | -0.149 | 0.000 | -0.010 | -0.141 |
| $dm(2)$ | | -0.003 | -0.042 | -0.112 | 0.000 | -0.015 | -0.097 |
| $dm(1,2)$ | | -0.004 | -0.069 | -0.286 | 0.000 | -0.023 | -0.273 |
| $g(1)$ | | -0.007 | -0.045 | -0.117 | 0.000 | -0.018 | -0.128 |
| $g(2)$ | | -0.006 | -0.001 | -0.046 | 0.000 | 0.002 | -0.041 |
| $g(1,2)$ | | -0.007 | -0.043 | -0.231 | 0.000 | -0.017 | -0.275 |
| h_{max} | | -0.007 | -0.113 | -0.373 | 0.000 | -0.029 | -0.391 |
| p_{int} | | -0.005 | 0.004 | 0.261 | 0.000 | 0.006 | 0.234 |
| $rc(1)$ | | -0.004 | -0.034 | -0.255 | 0.000 | -0.003 | -0.201 |
| sw | | -0.005 | -0.100 | -0.404 | 0.000 | -0.031 | -0.439 |
| $t_h(1)$ | | -0.006* | -0.127 | -4.311 | 0.000* | -0.034 | -3.918 |
| t_{max} | | 0.003 | -0.050 | -0.733 | 0.000 | -0.024 | -0.851 |
| $temp$ | | 0.003 | 0.705 | 1.077 | 0.000 | 0.865 | 1.333 |
| $W(1)$ | | -0.005 | 0.072 | 0.026 | 0.000 | 0.038 | -0.006 |
| $W(2)$ | | -0.008 | -0.076 | -0.023 | 0.001 | -0.025 | -0.012 |
| $W(1,2)$ | | -0.006 | 0.009 | 0.018 | 0.000 | 0.003 | -0.007 |
| ws | | -0.002 | -0.141 | -0.971 | 0.000 | -0.041 | -1.007 |
| x_{max} | | -0.006 | 0.190 | 0.644 | 0.000 | 0.216 | 0.705 |
| y_{max} | | -0.007 | 0.077 | 0.238 | 0.000 | 0.190 | 0.307 |

* All hosts were accepted after parameter value was changed.

Table 4b Change in predicted mean fraction of accepted hosts that should be fed upon ("host-feeding ratio") under default parameter values relative to change in parameter value (elasticity), for six scenarios. Default parameter values were generally halved (see text). Host-feeding ratios under default parameter values are given for reference. High elasticities are outlined.

| Life expectancy | Short ($a_{max} = 7$ days) | | | Long ($a_{max} = 70$ days) | | |
|---|-----------------------------|--------------|--------|-----------------------------|--------------|--------|
| Host density | Field | Intermediate | Pest | Field | Intermediate | Pest |
| (cm^{-2}) | (0.015) | (0.15) | (1.5) | (0.015) | (0.15) | (1.5) |
| Host-feeding ratio under default parameter values | | | | | | |
| | 0.001 | 0.169 | 0.274 | 0.120 | 0.205 | 0.318 |
| Elasticity | | | | | | |
| a_{max} | 1.750* | 0.557 | 0.428 | 0.717 | 0.044 | 0.033 |
| act | 1.597 | 0.873 | 0.206 | 0.753 | 0.126 | 0.179 |
| $dens$ | -3.795 | 0.870 | 0.206 | 0.713 | 0.149 | 0.179 |
| $dm(1)$ | -7.222 | 0.067 | -0.027 | 0.077 | 0.018 | -0.006 |
| $dm(2)$ | -11.96 | 0.161 | 0.098 | 0.163 | 0.046 | 0.072 |
| $dm(1,2)$ | -1.547 | 0.270 | 0.080 | 0.200 | 0.055 | 0.051 |
| $g(1)$ | -4.600 | -0.750 | -0.188 | -1.070 | -1.176 | -0.161 |
| $g(2)$ | -10.32 | -0.023 | -0.099 | -0.100 | 0.008 | -0.060 |
| $g(1,2)$ | -11.02 | -0.809 | -0.338 | -1.149 | -1.210 | -0.328 |
| h_{max} | -6.500 | -0.065 | -0.375 | 0.186 | 0.017 | -0.409 |
| p_{int} | -8.289 | 0.007 | 0.431 | -0.086 | 0.014 | 0.340 |
| $rc(1)$ | -9.698 | -0.183 | -0.171 | -0.477 | -0.057 | -0.090 |
| sw | 0.079 | 0.325 | 0.068 | 0.427 | 0.049 | 0.059 |
| $t_h(1)$ | -12.76 | -0.700 | -3.003 | -0.074 | -0.192 | -2.433 |
| t_{max} | 1.614 | 0.697 | 0.378 | 0.770 | 0.136 | 0.440 |
| $temp$ | -14.13 | 0.573 | 0.891 | -0.047 | 0.092 | 0.632 |
| $W(1)$ | -5.765 | 0.043 | -0.021 | -0.169 | 0.015 | 0.006 |
| $W(2)$ | -7.948 | -0.014 | -0.005 | 0.239 | 0.029 | 0.009 |
| $W(1,2)$ | -8.616 | -0.011 | -0.007 | 0.023 | 0.014 | 0.007 |
| ws | 2.000* | 0.830 | 0.201 | 0.727 | 0.126 | 0.174 |
| x_{max} | -96.92 | -0.821 | 0.342 | -1.194 | -0.152 | 0.548 |
| y_{max} | -53.40 | -0.447 | -0.211 | -0.505 | -0.560 | -0.447 |

* Not one host was fed upon after parameter value was changed.

prediction that all hosts should be accepted for host feeding or oviposition is very robust (Table 4a). At higher host densities, fewer hosts are accepted when the maximum rate of oogenesis through temperature $temp$ is decreased, as parasitoids become egg limited sooner. At pest density, where the probability to encounter a host equals 1 per time step, the acceptance ratio increases with decreasing host encounter probability (act , $dens$, ws ; Equation 11b; Fig. 2a) because fewer hosts have to be rejected as a result of egg limitation and energy saturation. Decreasing the active period t_{max} (while $t_{max}+i$ remains

Table 4c Change in predicted mean lifetime reproductive success ("fitness") under default parameter values relative to change in parameter value (elasticity), for six scenarios. Default parameter values were generally halved (see text). Fitness values under default parameter values are given for reference. High elasticities are outlined.

| Life expectancy | | -----Short ($a_{max} = 7$ days)----- | | | -----Long ($a_{max} = 70$ days)----- | | |
|--|---------|---------------------------------------|-------|---------|---------------------------------------|--------|--|
| Host density | Field | Intermediate | Pest | Field | Intermediate | Pest | |
| (cm^{-2}) | (0.015) | (0.15) | (1.5) | (0.015) | (0.15) | (1.5) | |
| Fitness under default parameter values | | | | | | | |
| | 2.46 | 19.3 | 42.4 | 20.3 | 184 | 397 | |
| Elasticity | | | | | | | |
| a_{max} | 0.952 | 0.888 | 0.980 | 0.945 | 1.003 | 1.033 | |
| act | 1.025 | 0.913 | 0.101 | 0.959 | 0.896 | 0.107 | |
| $dens$ | 1.023 | 0.923 | 0.104 | 0.950 | 0.922 | 0.154 | |
| $dm(1)$ | 0.065 | 0.163 | 0.050 | 0.103 | 0.066 | 0.116 | |
| $dm(2)$ | 0.405 | 0.265 | 0.069 | 0.343 | 0.384 | 0.074 | |
| $dm(1,2)$ | 0.530 | 0.374 | 0.150 | 0.358 | 0.430 | 0.044 | |
| $g(1)$ | 0.093 | 0.088 | 0.095 | 0.038 | 0.232 | 0.018 | |
| $g(2)$ | 0.080 | -0.030 | 0.150 | -0.079 | 0.091 | 0.014 | |
| $g(1,2)$ | 0.081 | 0.079 | 0.092 | 0.116 | 0.126 | 0.098 | |
| h_{max} | 0.101 | 0.274 | 0.775 | 0.216 | 0.267 | 0.736 | |
| p_{int} | 0.096 | -0.100 | 0.084 | -0.127 | -0.089 | 0.018 | |
| $rc(1)$ | -0.516 | -0.563 | 0.105 | -0.559 | -0.566 | -0.017 | |
| sw | 0.491 | 0.361 | 0.133 | 0.584 | 0.496 | 0.070 | |
| $t_h(1)$ | 0.042 | -0.092 | 0.072 | -0.053 | -0.098 | -0.034 | |
| t_{max} | 0.985 | 0.846 | 0.460 | 0.975 | 0.968 | 0.458 | |
| $temp$ | 0.150 | 0.428 | 1.085 | -0.044 | 0.489 | 1.217 | |
| $W(1)$ | 0.320 | 0.076 | 0.055 | 0.126 | 0.151 | 0.067 | |
| $W(2)$ | 0.874 | 0.863 | 1.061 | 0.855 | 0.940 | 1.005 | |
| $W(1,2)$ | 1.000 | 0.979 | 1.021 | 1.039 | 1.023 | 0.996 | |
| ws | 1.054 | 0.884 | 0.178 | 0.961 | 0.958 | 0.167 | |
| x_{max} | 0.189 | 0.149 | 0.571 | 0.057 | 0.176 | 0.446 | |
| y_{max} | 0.023 | 0.166 | 0.429 | 0.011 | 0.142 | 0.559 | |

constant) has a similar effect for the same reason. When it is ignored that host feeding takes longer than rejection ($t_h(d)=(1,1,1)$ time step), the acceptance ratio increases to virtually 1 because there is no time advantage of rejection over host feeding. In other words, the decrease in acceptance ratio with host density (Fig. 2a) only occurs when host feeding takes longer than rejection. Finally, the acceptance ratio is decreased with a decrease in the egg load capacity x_{max} , again because parasitoids become egg limited sooner.

Of the accepted hosts, less than 1 % was fed upon when life expectancy is short and host density low (scenario SF) (Fig. 2b). This predicted does not seem robust because most elasticities exceed 1. However, even although the host-feeding ratio has an elasticity of -97 for egg load capacity x_{max} (Table 4b), it still does not exceed merely 3 % of the accepted hosts when the egg load capacity is halved. We are confident from literature and egg load measurements at ideal conditions that this parameter is well estimated. This holds for most parameter values (see section Parameter values). Elasticities for a_{max} and ws reached a maximum value (not one host was fed upon after parameter value was changed). The importance of a_{max} was already shown in Fig. 2b but the elasticity of ws is always in the same order of the elasticity for act (1.6) and $dens$ (-3.8) (Equation 11b). Thus, only a_{max} or t_{max} could potentially explain evolution of host-feeding behaviour at the average field host density. However, even when t_{max} is increased from 100 to 200 time steps (20 hours of foraging per day), the optimal host-feeding ratio is still only 2 % of the accepted hosts (data not shown). This leaves a_{max} as the most important parameter that can explain evolution of host-feeding behaviour at the average field host density. Unfortunately, this parameter was also most difficult to estimate. Furthermore, the host-feeding gain from bad quality hosts has a considerable effect on the host-feeding ratio when the host encounter rate is high enough to risk egg limitation but low enough to prevent egg depletion and energy saturation within a day. At pest host density, parasitoids become egg limited sooner when the maximum rate of oogenesis through temperature $temp$ is decreased. As a result, both the acceptance ratio (Table 4a) and the host-feeding ratio (Table 4b) decrease. When it is ignored that host feeding takes longer than rejection and oviposition ($t_h(d) = (1,1,1)$), there is no time advantage of rejection over host feeding. Therefore, virtually all hosts are fed upon instead of being rejected, which has a strong positive effect on the host-feeding ratio.

The impact of each parameter on the predicted mean fitness (Table 4c) is rather different from their impact on the predicted behaviour (Tables 4a,b). For instance, the parameter $W(h)$ did not considerably affect the predicted behaviour but has a relatively large impact on mean fitness. Vice versa, handling time of host feeding $t_h(1)$ can have a very large impact on behaviour, without affecting mean fitness. Elasticities in mean fitness rarely exceed 1, which means that the change in mean fitness rarely exceeds the parameter change. Independent of scenario, mean fitness is limited by the parasitoid's age horizon a_{max} and the fitness gain from oviposition $W(h)$, especially in the good quality host, $W(2)$. They change mean fitness in the same order of magnitude as the parameter change. At field and intermediate host densities, mean fitness is mainly affected by parameters that determine the host encounter probability (act , $dens$, ws , and to a lesser extent $rc(h)$, sw and $dm(2)$) and by time t_{max} available to find the limited hosts. On the other hand, at pest host density, the most important parameters are temperature $temp$, which determines the rate of egg maturation (Equation 8), and the number of host types h_{max} . The latter is important because hosts that differ in survival probabilities for parasitoid eggs but do not differ in host-feeding gain allow parasitoids to lay eggs in good quality hosts and feed upon bad quality hosts. At high host density, the state capacities x_{max} and y_{max} are also more important than at lower host densities. Thus,

parameters that are expected to drive natural selection are the parasitoid's life expectancy and the survival probability of deposited eggs, independent of host density, the host encounter probability and length of active period when host density is low and the egg maturation rate and number of host types when host density is high.

Discussion

We developed a dynamic state variable model to find a functional explanation for host-handling behaviour in the whitefly parasitoid *E. formosa*. At presumably natural conditions, i.e. low host density (Chapter 4) and short life expectancy (Heimpel *et al.* 1997b, 1998), our model simulation predicts that host feeding is maladaptive. Parasitoids gain maximum lifetime fitness when they accept and oviposit in every host encountered, regardless of host quality and parasitoid's age. Nutrients from the immature stage are sufficient to prevent egg limitation. We also showed that parasitoids that make optimal host-handling decisions gain a considerably higher lifetime fitness than parasitoids that make random host-handling decisions, especially at low host density. This suggests that there would be a strong selection pressure against host-feeding behaviour at field host density. Still, *E. formosa* is known as a host-feeding species (Jervis & Kidd 1986).

Evolution of host-feeding behaviour could be explained when host density was underestimated in the field, because the optimal fraction host feeding initially increases steeply with host density (Fig. 2b). At higher host densities, nutrients from the parasitoid's immature stage are not sufficient anymore to prevent egg limitation and host feeding becomes adaptive. It seems more likely, however, that the overall estimate of natural host density (Chapter 4) is an overestimation of the host population available to the parasitoid, because no distinction was made between dead and alive whitefly nymphs, nor between host and non-host whitefly species, nor between unparasitised and already-parasitised nymphs. In addition, average lifetime reproductive success should be unity for stable field populations. In our field scenario it was 2.4 whereas it increased with host density.

Possibly, ignoring the variation in host density is an important oversimplification. Up to now we only used the average host density described from the field, whereas whiteflies aggregated at several spatial levels (Chapter 4), as in most natural insect populations (Taylor 1961). Houston *et al.* (1992) showed that variation in host encounter rate leads to selection for increased host feeding but assumed that the function of host feeding was to provide energy for a parasitoid with an unlimited egg load. The optimal egg load at emergence increases with stochasticity in oviposition opportunities (Ellers *et al.* 2000; Rosenheim 1996; Sevenster *et al.* 1998). In a stochastic habitat where some patches of relatively high host density are present, parasitoids run a higher risk of becoming egg limited and should therefore produce more eggs than they expect to be able to lay. We already showed that host feeding becomes selectively favourable when it can prevent or reduce egg limitation. Thus, variation in host density might explain evolution of host-feeding behaviour under field conditions.

Walking activity act and walking speed ws had similar effects on model predictions as host density (Equation 11b; Table 4). Walking activity has a maximum value of 1, at which the optimal host-feeding ratio is still less than 1 % of the accepted hosts (data not shown). Moreover, walking activity could be well estimated from literature (van Roermund & van Lenteren 1995a; Sütterlin & van Lenteren 1997, 1999). Walking speed, on the other hand, was extrapolated from measurements per second to a value per time step of 6 minutes. Walking speed was more difficult to parameterise because it varies with leaf structure, temperature and egg load (van Lenteren *et al.* 1976a, 1995; Woets & van Lenteren 1976; Hulspar-Jordaan & van Lenteren 1978; Li *et al.* 1987; Sütterlin & van Lenteren 1993, 1997; van Roermund & van Lenteren 1995b). In contrast to host density, however, walking speed has little effect on host encounter probability at field density (Fig. 1). Thus, incorporating variation in walking activity or walking speed seems less important than incorporating variation in host density.

Host-feeding behaviour also became adaptive when life expectancy (through a_{max}) was considerably higher (Fig. 2b; Table 4b) than our estimate based on field observations of predation on *Aphytis* parasitoids (Heimpel *et al.* 1997b, 1998). Parasitoids were predicted to encounter on average merely 1.3 hosts per day in the field. At this density, parasitoids would become egg limited on average after about 10 days $((x_{init} + y_{init})/1.3)$ if no hosts would be fed upon (extrapolating Fig. 4c). Optimal host-handling strategies at high host density or long life expectancy show that becoming egg limited is more costly than the loss of opportunities to oviposit through destructive host feeding. More field data are needed to support the hypothesis that natural life expectancy is indeed overestimated by data on parasitoid longevity in the laboratory. Unfortunately, alternative age determination methods like the pteridine bioassay (e.g. Mail *et al.* 1983) have not proved successful in hymenopteran parasitoids (J. Ellers, pers. comm.; M. Visser, pers. comm.; see thesis' Appendix).

In the field, on average 12 % of the whitefly nymphs became eventually parasitised (Chapter 4). *E. formosa* is able to distinguish between parasitised and unparasitised hosts (van Lenteren *et al.* 1976b). Both intra- and interspecific competition may enhance the tendency to host feed (Collier & Hunter 2001; Collier *et al.* 2002; Yano 1987), because the fitness increment from oviposition in a parasitised host is lower than from oviposition in an unparasitised host (e.g. Collier & Hunter 2001). On the other hand, superparasitism can be an evolutionary stable strategy (van Alphen & Visser 1990; Mangel 1992; Visser *et al.* 1992). Moreover, *E. formosa* can kill eggs previously laid within the host by jabbing them with their ovipositor before laying an egg themselves (Netting & Hunter 2000). To study whether competition could explain evolution of host-feeding behaviour at field host density, the decisions to ovicide+parasitise and superparasitise should be incorporated into the strategy set of the model in addition to the decisions to reject, host feed and parasitise, and parasitised host types should be included. Furthermore, qualitative changes may occur in host hemolymph after attack by an insect parasitoid (Cox 1970). Thus, the host-feeding gain from parasitised host may differ from the host-feeding gain from unparasitised hosts.

We assumed in our model that parasitoids use the host-feeding gain to mature eggs (Chapter 3) and that parasitoids obtain nutrients for maintenance and activity from non-host or host-derived food sources such as nectar and honeydew. Chan & Godfray (1993) and Collier (1995b) showed that optimal host-feeding behaviour changes when the host-feeding gain is used for maintenance. Nevertheless, our assumption seems reasonable (Jervis *et al.* 1996), especially for homopterid parasitoids, which can drink honeydew from their host's anus. The energy state of a parasitoid, however, can be replenished not only by feeding on non-host, host-derived and host food sources, but also by resorption of eggs (Bell & Bohm 1975; van Vianen & van Lenteren 1986b). The ability of oosorption allows parasitoids to respond more flexible to their environment, for instance to survive meagre periods when hosts and honeydew are limiting. This flexibility in nutrient allocation may change the value of host feeding and thus optimal host-handling strategies (Rivero & Casas 1999a). However, incorporating egg resorption may not explain evolution of host-feeding behaviour at low host density. A dynamic state variable model with egg resorption predicted lower critical egg loads at and below which parasitoids should host feed than a model without egg resorption (Collier 1995b). When parasitoids have access to sugar-rich foods, egg resorption is unlikely to initiate egg limitation (Rosenheim *et al.* (2000).

The model assumes that each handling behaviour takes at least 6 min, whereas parasitoids can reject a host within 5 to 35 seconds after contact with their antennae (van Roermund & van Lenteren 1995b). Parasitoids can gain searching time when rejection times would be 30 s rather than 6 min. More searching time can result in an increase in host encounter rate, risk of egg limitation and thus host-feeding ratio. However, at field conditions, none (0.0 %) of the encountered hosts should be rejected (Fig. 2a). Incorporating antennal rejection by decreasing the length of a time step from 6 min ($t_{max} = 100$) to e.g. 30 s ($t_{max} = 1200$) would only increase the size of the optimal-decision matrix and computing time.

Some previous models on optimal host-feeding behaviour predict that the tendency to host feed should decrease with host availability, sometimes with an initial increase (Jervis & Kidd 1986; Chan & Godfray 1993; Collier 1995b). Others predict an increase (Collier *et al.* 1994; Heimpel *et al.* 1994; McGregor 1997) or no effect (Heimpel *et al.* 1998). We found that the optimal fraction host feeding initially increases steeply with host density and then levels off. Chan & Godfray (1993) showed that a domed relationship between host encounter rate and the host-feeding ratio only occurs when the host-feeding gain is (also) used for maintenance. We assumed that *E. formosa* uses the host-feeding gain only for oogenesis because parasitoids can also feed non-destructively on honeydew, which is produced by the host and an excellent food source for *E. formosa* (van Lenteren *et al.* 1987; Chapter 2). The reason for the initial increase in host-feeding ratio with host density is the autogenous biology of our parasitoid. Autogenous parasitoids do not need to host feed before being able to lay some eggs (Jervis & Kidd 1986). Instead, nutrients from the parasitoid's immature stage can be used for the initial egg production. In our model, parasitoids start with $0 < x_{init} < x_{max}$ eggs and $y_{init} = \text{chop}\{x_{max} - x_{init}, y_{max}\}$ egg equivalents that are matured into eggs during the first days.

This is conform *E. formosa*'s biology (van Vianen & van Lenteren 1986b; van Lenteren *et al.* 1987; Chapter 3). As a consequence, parasitoids do not run the risk of becoming egg limited as long as host encounter rate is slow and life expectancy short, which could be the natural situation. The optimal host-feeding ratio only increases when the risk of egg limitation increases, i.e. when host encounter rate or life expectancy increases. The host-feeding ratio levels off at high host densities when parasitoids become egg depleted and energy satiated.

Elasticities can be used to predict new means for other parameters values ($y_{new} = y_{old} * (1 + e * (p_{new} - p_{default}) / p_{default})$; Equation 12). This use of elasticities results in reasonable estimates for mean fitness, but in poorer estimates for fractions, which range between 0 and 1. Moreover, elasticities varied between scenarios, thus parameter effects interact. Because not only sensitivity but also elasticity depends on parameter change, caution should be exercised making generalisations to other systems. However, elasticity analyses have proved a useful tool in revealing the relative importance of the parameters under different scenarios. Parameters that are expected to drive natural selection are the parasitoid's life expectancy and the survival probability of deposited eggs, independent of host density, the host encounter probability and length of active period when host density is low, and the egg maturation rate and number of host types when host density is high.

After having calculated optimal host-handling decisions, empirical data are needed to validate model predictions. At high host density when hosts are unlimited, parasitoids are predicted to lay on average about 15 eggs per day in good quality hosts only and to feed upon on average 6 to 7 hosts per day (30 % of the accepted hosts). The rest of the encountered hosts is rejected because of egg limitation. This corresponds reasonably well to empirical studies on *E. formosa* not used for parameterisation, although the predicted daily number of host feedings is somewhat high. For example, Arakawa (1982) found that *E. formosa* generally lays about 20 eggs per day, and feeds upon about 4 hosts per day. Fransen & van Montfort (1987) also found an upper limit of 15 to 20 parasitised hosts and 1 to 4 host feedings within 24 h, depending on the host stage offered. Sütterlin & van Lenteren (1999) found that 12 to 25 % of the accepted hosts was used for host feeding on leaves with 20 hosts, depending on host plant cultivar. Van Alphen *et al.* (1976) found that 17 % of the accepted hosts were used for host feeding. In Chapter 2, we found that parasitoids with access to sugar and honeydew laid on average 5.8 eggs before attempting to host feed (a host-feeding ratio of 15 %). Lower temperatures or longer host feeding times could account for lower host-feeding ratios (Table 4b). Validation experiments with low host densities are needed to test our prediction that parasitoids do not host feed at the low average field host density (see Chapter 6).

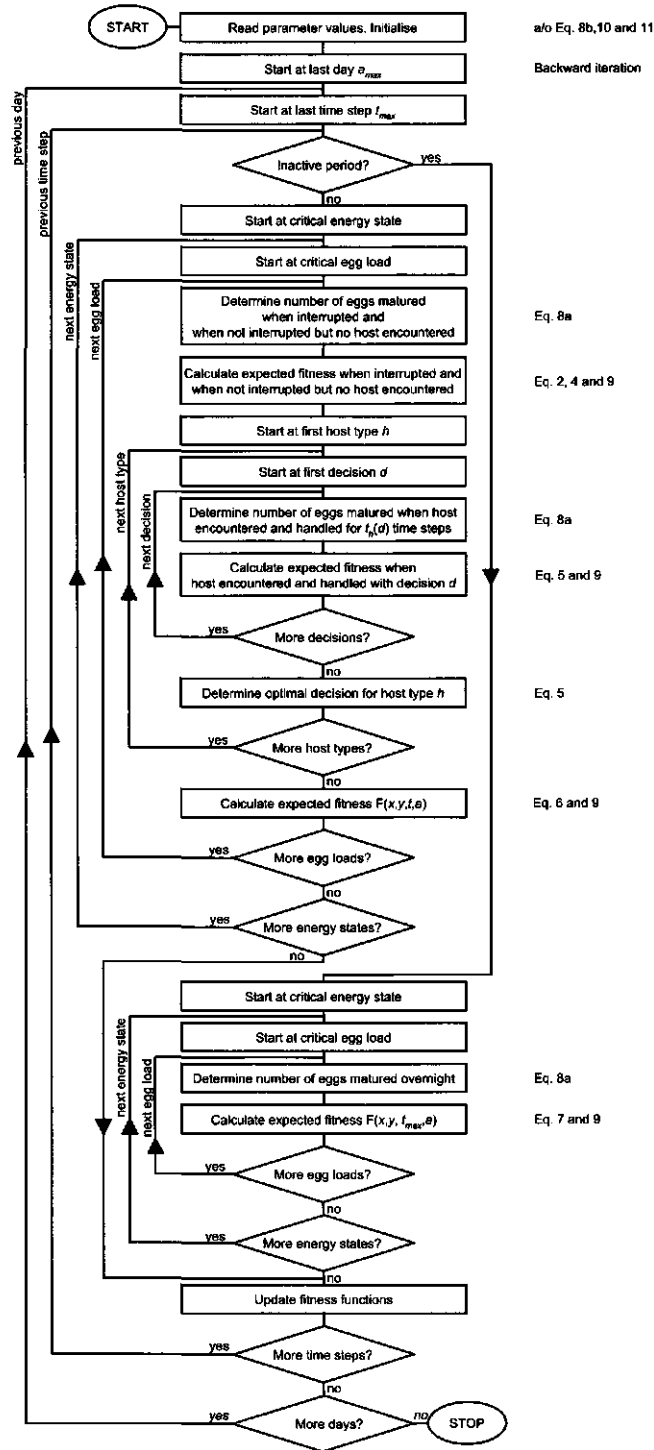
In conclusion, the model predicts that when the function of host feeding is to gain nutrients for egg maturation, the host-feeding gain does not offset the destruction of opportunities to oviposit under presumably field conditions. In the field, egg limitation is prevented without host feeding by a combination of *E. formosa*'s autogenous biology, the low host encounter rate and low parasitoid's life expectancy. Explaining evolution of

host-feeding behaviour under natural conditions may require incorporation of variation in host density, incorporation of parasitised host types, or field data showing that life expectancy in the field is not as short as we assumed. Incorporating variation in walking speed, egg resorption, or antennal rejection are not expected to reveal an explanation. Furthermore, we have illustrated that a strong selection pressure can be expected on optimal decision making over random decision making, especially at low host densities. Elasticity analyses revealed that the relationship between host density and acceptance ratio is most sensitive to the relative handling time of host feeding and the temperature at which oogenesis takes place. The relationship between host density and host-feeding ratio is most sensitive to the relative handling time of host feeding and the host-feeding gain from the bad quality host. Parameters that have a large impact on lifetime reproductive success and thus fitness are the parasitoid's life expectancy and the survival probability of deposited eggs (independent of host density), the host encounter probability and length of the active period (when host density is low) and the egg maturation rate and number of host types (when host density is high). Finally, *E. formosa* seems to be adapted to low host densities, since egg maturation rate limits the number of hosts that can be handled at high host densities. This is likely to be a characteristic of synovigenic parasitoids in general.

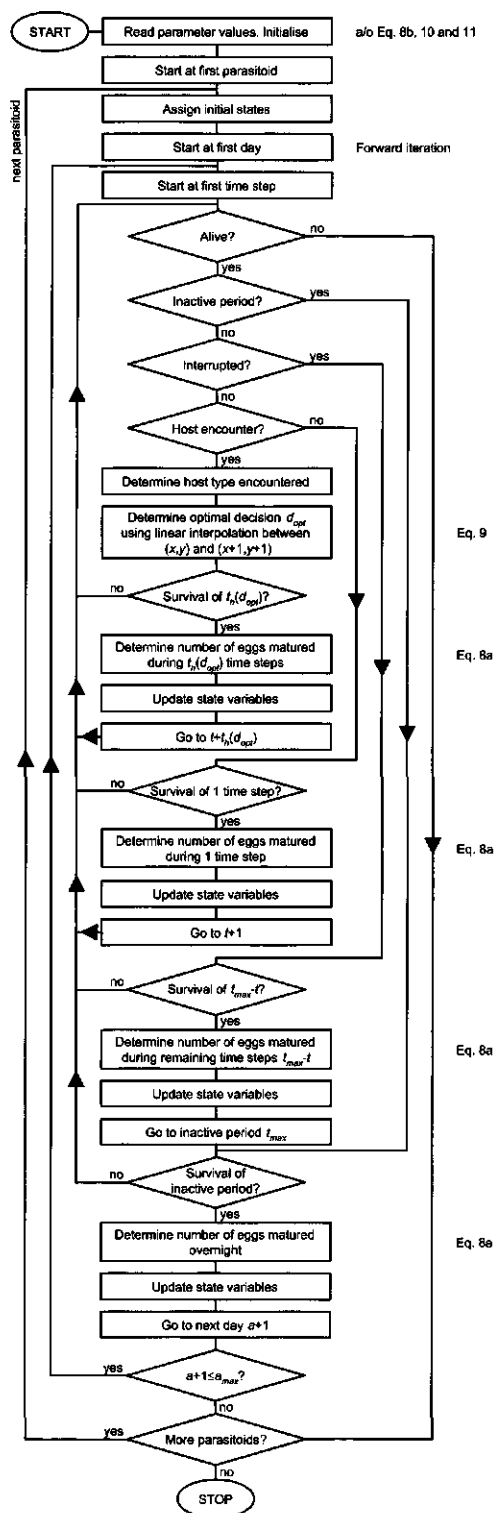
Acknowledgements

We would like to thank Man-Suen Chan for sending relevant chapters of her thesis. The first author expresses his thanks to Mark Kramer and Sjoukje Osinga for programming advice.

Appendix 1 Flow chart of backward iteration.



Appendix 2 Flow chart of Monte Carlo simulation.



6

Effect of host distribution and parasitoid experience on foraging decisions of *Encarsia formosa*

J.M.S. Burger, Y. Huang, L. Hemerik, J.C. van Lenteren & L.E.M. Vet

Abstract

We tested (1) the prediction from a dynamic state variable (DSV) model that the whitefly parasitoid *Encarsia formosa* (Hymenoptera, Aphelinidae) should never host feed at the low average host density found in the field (0.015 hosts per cm²), (2) the hypothesis that host aggregation enhances host feeding behaviour and (3) the effect of host distribution and parasitoid experience on patch-leaving behaviour. Each parasitoid was observed 10 hours per day for 6 consecutive days in a 3-D set-up with 21 tomato leaflets containing either always 1 host per leaflet (uniform distribution), or on average 1 host per leaflet with variation described from field data (aggregated distribution). Fourth instars of greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera, Aleyrodidae) were used as host. On average, parasitoids encountered about 12 hosts per day in both treatments and host-fed about once a day or upon about 11 % of the accepted hosts. A proportional hazards model showed that after about 4 days since the start of the observation, the tendency to host feed when host distribution was aggregated exceeded the tendency to host feed when host distribution was uniform, independent of time since the latest host acceptance. Another proportional hazards model showed that host encounter *decreased* the leaving tendency on a leaflet with 1 host when time since the latest host encounter was *short*, but *increased* the leaving tendency when time since the latest host encounter was *long*, independent of host distribution. Time since start of observation had a positive effect on leaving tendency. We conclude that (1) the formula by Skellam (1958) used in the DSV model and simulation models by van Roermund *et al.* (1996, 1997a,b,c) underestimates host encounter rate from host density, (2) variation

in host density has a positive effect in the course of time on the tendency to host feed and (3) *E. formosa* uses time since the latest host encounter to decide whether to stay or leave after a host encounter, which may be an efficient strategy in the natural environment where infested leaves contain most likely a single host but sometimes many hosts.

Introduction

Organisms evolve by natural selection to optimally allocate their limited time and resources among competing demands. Examples of competing demands include trade-offs between reproduction and survival and between current and future reproduction (Stearns 1992; Roff 1992). In many species of insect parasitoids, both trade-offs are reflected in the decision between host feeding and oviposition (Jervis & Kidd 1986; Heimpel & Collier 1996). Host feeding is the consumption of the host by the adult female parasitoid and provides nutrients that can be used to mature eggs and to increase longevity. However, host feeding kills the host and hence destroys an opportunity to oviposit. Although under some conditions non-destructive feeding on honeydew can be an alternative to destructive host feeding (Chapter 2), host feeding can have an evolutionary advantage over feeding on honeydew, especially at high host densities when egg maturation becomes the limiting factor for oviposition (Chapter 3).

We study the decision between host feeding and oviposition in the whitefly parasitoid *Encarsia formosa* (Hymenoptera, Aphelinidae) both theoretically and empirically. *E. formosa* is successfully applied in most countries with an important greenhouse industry as a biological control agent against whitefly pests (van Lenteren *et al.* 1996; van Lenteren 2000). An individual-based population dynamical simulation model showed that host density, host distribution and the parasitoid's searching efficiency are the most important factors that determine the success of this parasitoid as biological control agent in greenhouses (van Roermund *et al.* 1997c; van Lenteren & van Roermund 1999). This model does not provide, however, a functional explanation of *E. formosa*'s behaviour. Because host feeding results in whitefly mortality without direct parasitoid offspring, host-handling decisions can have important implications for population dynamics and the final outcome of biological control. It is therefore crucial to understand these decisions from an evolutionary point of view.

In Chapter 5, a dynamic state variable (DSV) model was developed to predict the optimal fraction of encountered hosts that should be accepted for oviposition or host feeding and the optimal fraction of accepted hosts that should be fed upon to achieve maximum reproductive output. Fieldwork in *E. formosa*'s presumable area of origin (Central America) has revealed a quantitative description of natural whitefly densities and distributions (Chapter 4). Under these conditions the parasitoid's behaviour most likely has evolved. Model simulation at the average field host density of 1 host per leaflet (with two-sided leaf area of 67 cm²) revealed that maximum reproductive output is achieved when parasitoids use each encountered host for oviposition, regardless of

host quality or parasitoid age (Chapter 5). The first aim of this paper was thus to test this model prediction that the whitefly parasitoid *E. formosa* does not host feed at the average field host density of 1 host per leaflet.

In addition, analysis of the whitefly distribution in the field showed spatial dependence (aggregation) at several spatial scales (Chapter 4). Parasitoids may experience above-average host encounter rates and may run a higher risk of egg limitation in host aggregates. Simulation of foraging behaviour of *E. formosa* on a plant indeed showed that the overall mean number of encountered hosts, ovipositions and host feedings was always higher when host distribution was clustered than when it was discrete uniform (van Roermund *et al.* 1997b). We therefore tested the hypothesis that the aggregation of whitefly nymphs in the field in combination with arrestment behaviour in *E. formosa* (van Roermund & van Lenteren 1995a,b; van Roermund *et al.* 1994) enhances host feeding. To test this hypothesis, we compared host-handling decisions between a set-up with a uniform host distribution of *always* 1 host per leaflet and a set-up with an aggregated host distribution of *on average* 1 host per leaflet including the natural variation.

A third aspect of this paper concerned the effect of host distribution and parasitoid experience on patch leaving decisions by *E. formosa*. Classical patch-leaving models like Charnov's (1976) marginal value theorem assumed that the forager is omniscient. Limited experience was incorporated in simple rules of thumb, i.e. leave after a fixed time, after a fixed number of encounters or after a fixed giving up time (see e.g. Godfray 1994). Iwasa *et al.* (1981) showed that the best rule depends on the distribution of prey (or hosts). Waage (1979) developed a more sophisticated patch-leaving rule where oviposition has an incremental effect on giving up time. Using statistical analysis of behavioural rules (e.g. Haccou & Hemerik 1985), Driessen *et al.* (1995) proposed a count-down mechanism where oviposition has a decremental effect on giving up time. Host distribution is an important factor determining which mechanism is adaptive, an incremental mechanism being adaptive when hosts are aggregated and a decremental mechanism when hosts are uniformly distributed (see also Vos *et al.* 1998; Driessen & Bernstein 1999).

Although whitefly nymphs aggregated at several spatial scales in the field, still a leaflet with only 1 host was the second most likely type of leaflet after empty leaflets and the probability to encounter a leaflet with more than 1 host decreased with the number of hosts on the leaflet (Chapter 4). In such an environment, a single host encounter only is not enough to decide whether to stay or leave. Therefore, we quantified the leaving tendency of *E. formosa* under such conditions. Furthermore, changing behaviour with experience (learning) can be an adaptive mechanism when foraging cues are highly variable in a predictable way (Vet & Dicke 1992; Papaj & Lewis 1993; Vet *et al.* 1995). In the field, the number of hosts on a leaflet was highly variable and the spatial dependence provides parasitoids with some degree of predictability on presence of hosts (Chapter 4). Greenhouse data showed that the whitefly distribution gradually changed from clustered towards regular in the course of

time (Eggenkamp *et al.* 1982). We therefore tested whether parasitoids learn to leave sooner when host distribution is uniform than when host distribution is aggregated.

Thus, the aim of this empirical study was threefold. First, we tested the prediction from a DSV model as described in Chapter 5 that the whitefly parasitoid *E. formosa* does not host feed at the average host density found in the field. Second, we tested the hypothesis that host aggregation enhances host feeding behaviour. Third, we quantified the effects of host distribution and parasitoid experience on patch-leaving behaviour.

Materials & Methods

Living material

Tomato plants (Solanaceae, *Solanum lycopersicum* L. cv. Moneymaker) were reared by Unifarm, Wageningen, the Netherlands, at 21°C, 70% R.H. and L:D=16:8 hours. Nymphs of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) were obtained from the whitefly culture on tomato at the Laboratory of Entomology, Wageningen, the Netherlands (21°C, 70% R.H. and L:D=16:8 hours). Pupae of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) parasitoids were obtained from Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands.

Experimental set-up

We decided to observe parasitoids continuously because (1) host feeding is difficult to determine after the parasitoid has left the host, (2) the DSV model predicted merely one or two host encounters per day and (3) preliminary experiments showed that a relatively large, 3-D set-up was necessary to evoke natural leaf hopping behaviour, which complicated the use of camera recording. The experimental arena also had to be small enough to keep track of the parasitoids that are less than 1 mm in size. To balance these opposing demands on the size of the experimental arena, we designed the following set-up (Fig. 1).

The experimental arena was confined by two rectangular, colourless plastic containers (Multistar, Starplast) encompassing an area of 27×18×22 cm (l×w×h). In each container three holes (Ø 1.8 cm) were melted and covered by fine gauze for ventilation. Six wooden blocks (3.2×3.2×1.8 cm) were attached to the bottom of the arena in a 3×2 grid using double-sided adhesive tape, each pierced to hold a vertical wooden stick (Ø 5 mm) mimicking a plant's branch. In total 21 tomato leaflets were alternately attached to the sticks in three horizontal layers (a 3×2 grid has seven inter-stick spaces). Distance between sticks was about 9 cm, between layers about 6 cm. Each leaflet was put in a 1.5 ml micro tube that was filled with water and closed by parafilm. Each micro tube was suspended in two rings of iron wire attached to one of the sticks. In this way, leaflets could be replaced and parasitoids were able to visit more than 21 leaflets present in the

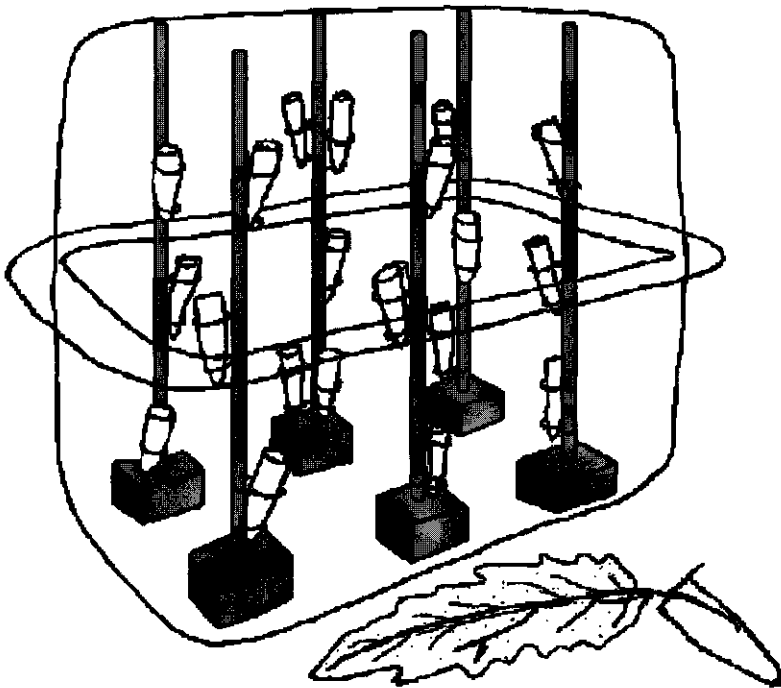


Fig. 1 Experimental set-up ($l \times w \times h = 27 \times 18 \times 22$ cm). Each micro tube was filled with water, closed by parafilm and holding one tomato leaflet (see foreground).

set-up (see section "Observations"). Droplets of sucrose solution were provided on a piece of parafilm on the bottom of the arena to mimic presence of nectar.

Treatments

The effect of host distribution was studied using two treatments. In the treatment with a uniform host distribution (U), each leaflet contained 1 host on the lower side, which was about the average number of nymphs found in the field and in the treatment with an aggregated host distribution. In the DSV model (Chapter 5) we used a host density of 0.015 cm^{-2} , or 1 host per 67 cm^2 leaf area, which is about the two-sided area of a tomato leaflet. In the treatment with an aggregated host distribution (A), the number of nymphs on the lower side of a leaflet was drawn from a Poisson distribution with mean and variance equal to λ . Field sampling of plants within spots along transects (Chapter 4) revealed that $\log(\lambda)$ could be described by $-1.422 + \text{tsct}_i + \text{spot}_{j(i)} + \text{plant}_{k(ij)}$, where tsct_i is the effect of the i th randomly selected transect, $\text{spot}_{j(i)}$ is the effect of the j th spot

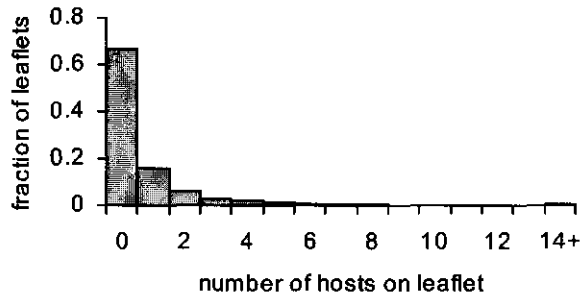


Fig. 2 Frequency distribution of number of hosts per leaflet used in the treatment with aggregated host distribution, based on field data on number of whitefly nymphs on the lower side of leaflets collected from plants within spots within transects. The number of hosts per leaflet was drawn 2500 times from a $\text{Poisson}(\lambda)$ distribution with $\log(\lambda) = -1.422 + \text{tsct}_i + \text{spot}_{j(i)} + \text{plant}_{k(ij)}$, where tsct_i is the effect of the i th randomly selected transect and assumed independent $N(0, 0.522)$, $\text{spot}_{j(i)}$ the effect of the j th spot randomly selected along the i th transect and assumed independent $N(0, 0.197)$, and $\text{plant}_{k(ij)}$ the effect of the k th plant randomly selected within the j th spot along the i th transect and assumed independent $N(0, 2.485)$. Parameter estimates result from generalised linear mixed modelling (Chapter 4).

randomly selected along the i th transect, and $\text{plant}_{k(ij)}$ is the effect of the k th plant randomly selected within the j th spot along the i th transect (Chapter 4). Random effects tsct_i , $\text{spot}_{j(i)}$ and $\text{plant}_{k(ij)}$ were assumed normally distributed with mean 0 and variance σ^2 , where $\sigma_{\text{tsct}}^2 = 0.522$, $\sigma_{\text{spot}}^2 = 0.197$ and $\sigma_{\text{plant}}^2 = 2.485$ (Chapter 4). For 2500 leaflets, the number of nymphs on the lower side was drawn from a Poisson distribution each with another λ , by replacing the random effects tsct_i , $\text{spot}_{j(i)}$ and $\text{plant}_{k(ij)}$. The resulting frequency distribution is highly skewed to the right (Fig. 2). The average over these 2500 leaflets was about 1.0 (± 3.0 sd) host per leaflet. Nymphs were carefully transferred from their original feeding site onto the leaflets. Previous experiments revealed that parasitoids still accept transferred nymphs for both oviposition and host feeding, and that *E. formosa* does not exploit herbivore-induced plant volatiles to locate hosts from a distance (Noldus & van Lenteren 1990; Romeis & Zebitz 1997; Sütterlin & van Lenteren 2000). Only fourth nymphal stages were used because younger stages are easily damaged during transfer. The fourth stage is preferred for oviposition and is also fed upon in a no-choice situation (Nell *et al.* 1976).

Observations

Parasitoids were kept and observed in a climate room at 30 °C, about 60 % RH and L:D=12:12 h. Preliminary observations revealed that parasitoids were basically inactive during the day of emergence. On day 0, parasitoids were allowed to emerge during one

to two hours in a Petri dish containing droplets of sucrose solution (10 % w/w) on a piece of parafilm and kept herein until the next day. On day 1, one parasitoid per arena was introduced on a leaflet between 8 and 9 o'clock (a.m.). Two parasitoids, one at the uniform host distribution (U) and one at the aggregated host distribution (A), were observed simultaneously by two observers taking turns. The following behaviours were recorded continuously in time (min): landing on leaflet, changing leaflet side, antennal rejection, insertion of ovipositor, host feeding, honeydew feeding, leaving host, leaving leaflet, sucrose feeding, leaving sucrose source. Whitefly nymphs in which the parasitoid had inserted its ovipositor were dissected under a light microscope to check the number of ovipositions (usually 1 or 0). If dissection failed or the host was lost, which happened only 7 times out of hundreds of dissections, an oviposition was scored when ovipositorial insertion lasted 3 min or longer, and a rejection when this time was shorter (see also van Lenteren *et al.* 1976b). Once a leaflet was visited, it was replaced when the parasitoid landed on another leaflet, actively fed from the sucrose solution or was off the leaf during 15 min. In treatment U, this implied that the whitefly nymph was replaced if it was encountered. In treatment A, this implied that all nymphs were removed and replaced by a number drawn from a Poisson distribution with a new λ (see section "Treatments"). Parasitoids were removed 10 hours after introduction into the arena and kept in a Petri dish with sucrose solution until the next day. The next day, all nymphs were replaced in the latest configuration by newly-transferred nymphs from the rearing. To incorporate patch depletion, parasitoids were introduced on the same leaflet from which they were taken off, with the number of nymphs on that leaflet decreased by the number encountered on that leaflet the previous day. Each parasitoid was observed individually from day 1 until it died, escaped or reached the end of day 6.

Sucrose solution was supplied between observations because the DSV model assumed that parasitoids have unlimited access to non-host food sources to obtain nutrients for maintenance and activity. On a diet of glucose solution, mean life span of *E. formosa* is 22 days at 20 °C, but 5 to 6 times lower at 30 °C (van Roermund & van Lenteren 1992b). Mean longevity in the experiment therefore approximates mean longevity of about 3 days in the DSV model.

Statistical analysis

Data were analysed using a proportional hazards model (Cox 1972; Kalbfleish & Prentice 1980; Haccou & Hemerik 1985). It models the effect of p time-independent predictor variables X_i ($i=1, \dots, p$) on the hazard rate $h(t, X_1, \dots, X_p)$, which is the tendency (a probability per unit time) that the event of interest occurs at time t , given that it has not occurred yet:

$$h(t, X_1, \dots, X_p) = h_0(t) * \exp\left(\sum_{i=1}^p \beta_i * X_i\right), \quad (1)$$

Table 1 Renewal points, censors and tested predictor variables of proportional hazards models on tendency to host-feed and tendency to leave a leaflet with 1 host.

| Event (= failure) | Renewal points | Censors | Predictor variables tested |
|--|--------------------|---|--|
| Host feeding ^(a) | Landing on leaflet | (Un-)intentionally leaving leaflet ^(c) | Treatment (U/A) = (0/1) ^(d) |
| | Leaving host | Rejection | Time since start of observation (days) ^(e) |
| | | Oviposition | Time since the latest host acceptance (min) |
| | | Parasitoid out of sight | Number of hosts accepted since start of day |
| Intentionally leaving a leaflet with 1 host ^(b,c) | Landing on leaflet | Unintentionally leaving leaflet ^(c) | Treatment (U/A) = (0/1) ^(d) |
| | Leaving host | Host encounter | Time since start of observation (days) ^(e) |
| | | Parasitoid out of sight | Time since the latest encounter with any host (min) |
| | | | Host encountered since landing on current leaflet (no/yes) = (0/1) |

^(a) Times only used when time since the latest host acceptance was non-missing.

^(b) Times only used when time since the latest host encounter was non-missing. Leaving tendency was only considered on leaflets with 1 host to test the effect of previous experience rather than host density.

^(c) Intentionally: without interference by the observer; unintentionally: disturbed or removed after 10 hours of observation.

^(d) Host distribution either uniform (U) or aggregated (A).

^(e) Time was measured in minutes and divided by 1440 min per day.

where $h_0(t)$ is the baseline hazard, the time-dependent but unspecified hazard rate when all predictor variables are 0, and β_i is the parameter that shows the direction and relative strength of the effect of the i th predictor variable. Because the model was originally developed to analyse survival data where death is the event of interest, the event is also called a failure. In our data, an event occurs when the parasitoid feeds upon a host or intentionally leaves a leaflet with 1 host, i.e. without interference by the observer (Table 1). We analysed the leaving tendency only on leaflets with 1 host to test the effect of experience rather than host density. If the cumulative leaving tendency increases more or less linearly with time, the baseline hazard $h_0(t)$ is approximately constant and can be estimated by dividing the number of events by the sum of censored times and times until the event. The median time until the event can then be estimated by dividing $\ln(2)$ by the baseline hazard (van Roermund *et al.* 1994). The baseline hazard is reset after so-called renewal points, which we defined here as the moment of landing on a leaflet and the moment of leaving a host (Table 1).

The time from a renewal point until host feeding was censored when the parasitoid left the leaflet, rejected or parasitised a host or the observer lost sight of the parasitoid (Table 1). Predictor variables of interest were treatment, time since start of observation (measured in min but translated into days), time since the latest host acceptance (min), and the number of hosts accepted since start of day (Table 1). Only data were used when the time since the latest host acceptance was non-missing.

The time from a renewal point until the parasitoid intentionally left the leaflet was censored when the parasitoid left unintentionally, e.g. after 10 hours of observation, when it encountered a host, or when the observer lost sight of the parasitoid (Table 1). Predictor variables of interest were treatment, time since start of the observation (measured in min but translated into days), time since the latest host encounter (min), and whether or not the host was encountered since landing on the leaflet (Table 1). Only data were used when the time since the latest host encounter was non-missing.

Partial deviance tests were used to decide which combination of main effects and one-level interactions was the most parsimonious (Neter *et al.* 1996). Interactions were only considered when accessory main effects were also included. When predictor variables X_1 to X_p are selected in the most parsimonious model, 95 % confidence limits for given values of X_1, \dots, X_p , denoted by X_{h1}, \dots, X_{hp} , are given by:

$$h(t, X_{h1}, \dots, X_{hp}) = h_0(t) * \exp \left(\left(\sum_{i=1}^p \beta_i * X_{hi} \right) \pm \sqrt{t \left(1 - \frac{\alpha}{2}; n - p \right) * \begin{bmatrix} X_{h1} & \dots & X_{hp} \end{bmatrix} * \begin{bmatrix} \sigma^2(b_1) & \sigma(b_1, b_2) & \dots & \sigma(b_1, b_p) \\ \sigma(b_2, b_1) & \sigma^2(b_2) & \dots & \sigma(b_2, b_p) \\ \dots & \dots & \dots & \dots \\ \sigma(b_p, b_1) & \sigma(b_p, b_2) & \dots & \sigma^2(b_p) \end{bmatrix} * \begin{bmatrix} X_{h1} \\ \dots \\ X_{hp} \end{bmatrix}} \right), \quad (2)$$

where $\sigma^2(b_i)$ is the variance of the estimated regression coefficient b_i , and $\sigma(b_i, b_j)$ the covariance of the estimated regression coefficients b_i and b_j ; $t(1-\alpha/2; n-p)$ was approximated by 2.

Results

The first two aims of this paper were to test a model prediction that the whitefly parasitoid *E. formosa* does not host feed at a low uniform host distribution of 1 host per leaflet and the hypothesis that host feeding is enhanced by an aggregated host distribution. Fig. 3 shows the behaviour of the parasitoids for both host distributions. Bars show the stacked mean (\pm se) daily numbers (Fig. 3a and b) and estimated rates (per day) (Fig. 3c) of encountered hosts that were rejected, fed upon and parasitised. There was no clear effect of age on parasitoid behaviour, supporting the DSV-model assumption of a constant probability of instant mortality (Chapter 5). When host distribution was uniform, overall 75 % of the encountered hosts was accepted, 1.00 ± 0.20 hosts were fed upon per day (12 % of accepted hosts) and 7.45 ± 1.02 eggs were laid per day (Fig. 3a). Thus, in contrast to the model prediction, parasitoids did feed upon a considerable percentage of accepted hosts, even when variation in host density was ignored. When host distribution was aggregated, overall 89 % of the encountered hosts was accepted, 1.12 ± 0.22 hosts were fed upon per day (11 % of accepted hosts) and 9.27 ± 4.41 eggs were laid per day (Fig. 3b). Contrary to the 1.3 host encounters per day predicted by the DSV model, host encounter rate during the experiment averaged 11.3 ± 1.23 (se) per day when host distribution was uniform ($n=5$ parasitoids) (Fig. 3c). Variation in host density only increased variation in host encounter rate but not the mean rate (11.7 ± 5.26 per day, $n=5$ parasitoids) (Fig. 3c). As explained in the materials and methods section, we replaced leaflets to allow parasitoids to visit more than the 21 leaflets simultaneously present in the set-up. Parasitoids indeed visited more than 21 leaflets during the 10 hours of observation per day, i.e. on average 25.8 ± 3.65 (uniform distribution) and 27.4 ± 10.1 (aggregated distribution), weighed by the number of observation days per parasitoid. Overall, parasitoids were out of sight during 5.7 % of the 497.8 foraging hours (median 33 min, $n=44$ incidents).

Although the overall host-feeding rate averaged around 1 per day for both treatments, intra- and inter-patch experience of individual parasitoids was not taken into account. The most parsimonious proportional hazards model on the tendency to host feed was $h(t, X_1, X_2, X_3) = h_0(t) * \exp(-2.050 * X_1 - 0.398 * X_2 - 0.013 * X_3 + 0.470 * X_1 * X_2 + 0.004 * X_2 * X_3)$, where X_1 is treatment (U/A) = (0/1), X_2 is time since start of observation (days) and X_3 is time since the latest host acceptance (min). Incorporation of the number of hosts accepted since start of day thus did not significantly improve the model. Fig. 4 shows $h(t, X_1, X_2, X_3)$, the tendency to host feed (per min), for both treatments in relation to time since start of observation (days) and time since the latest host acceptance. The baseline hazard $h_0(t)$ was estimated by dividing the number of events by the sum of censored times and times until the event, i.e. 39/17706 per min. Dividing $\ln(2)$ by the

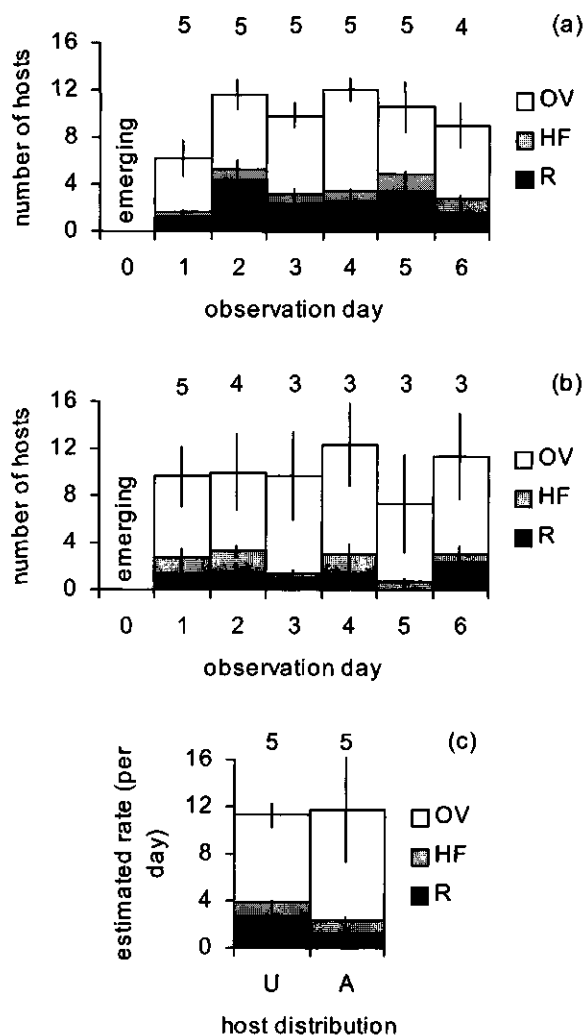


Fig. 3 Stacked mean (\pm se) number of encountered hosts rejected (black), fed upon (grey) and used for oviposition (white) per day by *Encarsia formosa* parasitoids when host distribution was (a) uniform and (b) aggregated. The number of parasitoids that survived the day is shown above the stacked bars. (c) Stacked mean (\pm se) estimated rate (per day) of encountered hosts rejected (black), fed upon (grey) and used for oviposition (white) when host distribution was uniform (U, $n=5$ parasitoids) and aggregated (A, $n=5$ parasitoids), weighed by the number of observation days per parasitoid (Sokal & Rohlf 1995).

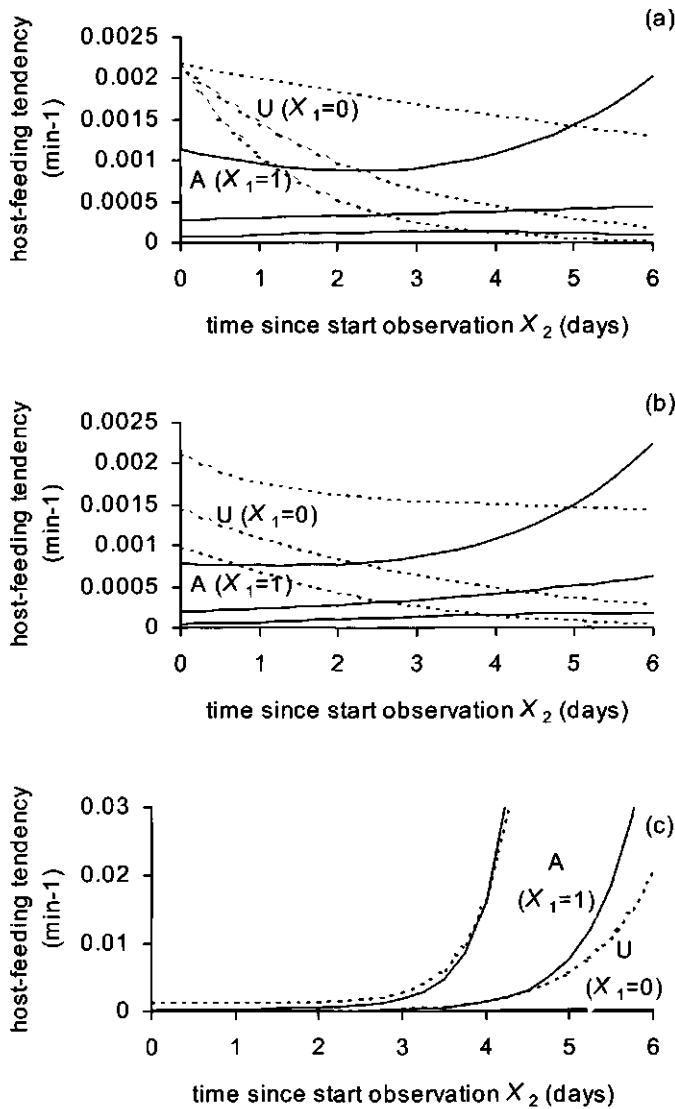


Fig. 4 Host-feeding tendency (per min) including 95 % confidence limits in relation to time since start of observation (days) when host distribution was uniform (U, dotted lines) and aggregated (A, solid lines), and when time since the latest host acceptance was (a) 1 min (minimum), (b) 32 min (median) and (c) 420 min (maximum). Most parsimonious proportional hazards model: $h(t; X_1, X_2, X_3) = 39/17706 \cdot \exp(-2.050 \cdot X_1 - 0.398 \cdot X_2 - 0.013 \cdot X_3 + 0.470 \cdot X_1 \cdot X_2 + 0.004 \cdot X_2 \cdot X_3)$, where X_1 is treatment (U/A) = (0/1), X_2 is time since start of observation (days) and X_3 is time since the latest host acceptance (min). The number of hosts accepted since start of day was included in the analysis but not significant. Times were only used when time since the latest host acceptance was non-missing.

baseline hazard results in a median time until host feeding of 315 min (5.25 h). The tendency to host feed always increased in time when host distribution was aggregated ($\beta_2 + \beta_4 = -0.398 + 0.470 = 0.072$ per day). On the other hand, it decreased in time when host distribution was uniform ($\beta_2 = -0.398$ per day), unless time since the latest host acceptance exceeded 100 min (β_2/β_5). After about 4 days since the start of the observation (β_1/β_4), the tendency to host feed when host distribution was aggregated exceeded the tendency to host feed when host distribution was uniform, independent of time since the latest host acceptance (Fig. 4). Thus, host aggregation did enhance host feeding in the course of time.

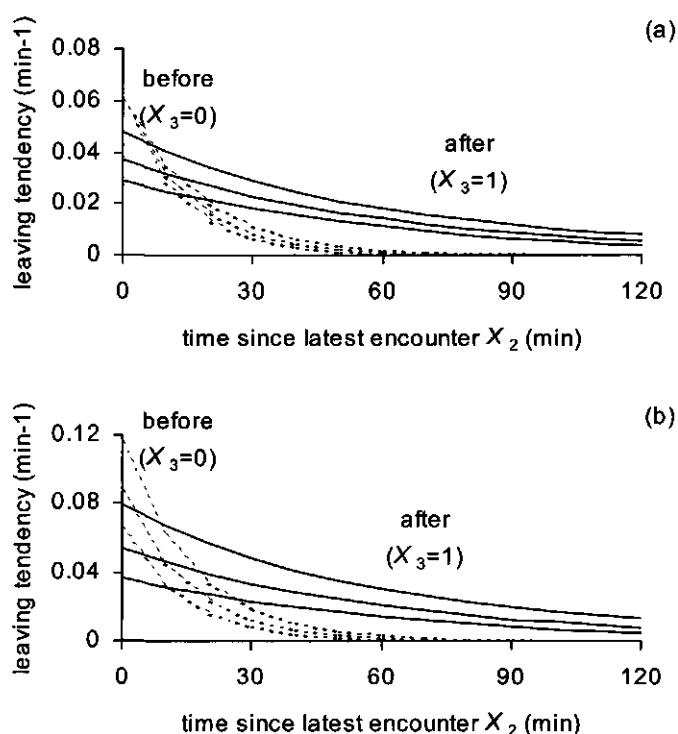


Fig. 5 Leaving tendency (per min) including 95 % confidence limits on leaflets with 1 host in relation to time since the latest host encounter (min) when the host was not encountered since landing on the leaflet ("before", dotted line) and when it was ("after", solid line), and when time since start of observation was (a) 0 days and (b) 6 days. Most parsimonious proportional hazards model: $h(t, X_1, X_2, X_3) = 714/11964 * \exp(0.062 * X_1 - 0.065 * X_2 - 0.475 * X_3 + 0.049 * X_2 * X_3)$, where X_1 is time since start of observation (days), X_2 is time since the latest host encounter (min) and X_3 is host encounter since landing on current leaflet (no/yes) = (0/1). Treatment was included in the analysis but not significant. Times were only used when time since the latest host encounter was non-missing.

The third aim of this paper was to quantify the effect of host distribution and experience on patch-leaving behaviour. The most parsimonious proportional hazards model on the tendency to leave a leaflet with 1 host was $h(t, X_1, X_2, X_3) = h_0(t) * \exp(0.062 * X_1 - 0.065 * X_2 - 0.475 * X_3 + 0.049 * X_2 * X_3)$, where X_1 is time since start of observation (days), X_2 is time since the latest host encounter (min) and X_3 is host encounter since landing on the leaflet (no/yes) = (0/1). Incorporation of treatment thus did not significantly improve the model. Fig. 5 shows $h(t, X_1, X_2, X_3)$, the tendency to leave a leaflet with 1 host (per min), before and after the host was encountered in relation to time since the latest host encounter (min) and time since start of observation (days). The baseline hazard $h_0(t)$ was estimated again by dividing the number of events by the sum of censored times and times until the event, i.e. 714/11964 per min. Dividing $\ln(2)$ by the baseline hazard results in a median residence time on a leaflet with 1 host of 12 min. The tendency to leave decreased with time since the latest host encounter ($\beta_2 = -0.065$ per min) but increased over time ($\beta_1 = 0.062$ per day). Parasitoids that did not encounter the host had a *higher* tendency to leave than parasitoids that already encountered the host when the time since the latest host encounter was *less* than 10 min (X_2 where hazard rates for $X_3 = 0$ and $X_3 = 1$ intersect, i.e. β_3/β_4). When the time since the latest host encounter was *more* than 10 min, parasitoids that did not encounter the host had a *lower* tendency to leave than parasitoids that already encountered the host. Alternatively, suppose a parasitoid lands on a leaflet with 1 host and the time since the latest host encounter is e.g. 20 min (the median) ($X_2 = 20$ and $X_3 = 0$). When it encounters the host, its leaving tendency *increases* from $h(t, X_1, 20, 0)$ to $h(t, X_1, 0, 1)$ (Fig. 5). In general, this holds when time since the latest host encounter is longer than 7 min (X_2 where $h(t, X_1, X_2, 0) = h(t, X_1, 0, 1)$, i.e. β_3/β_2). When time since the latest host encounter is shorter than 7 min, the leaving tendency *decreases* after host encounter. The latter is more likely when the leaflet contains more than 1 host. In other words, a host encounter arrests the parasitoid only when the latest host encounter occurred within 7 minutes.

Discussion

Contrary to the predictions of the DSV model (Chapter 5), *E. formosa* does host feed at the average host density described from field data (Chapter 4), namely on average about once a day or upon 11 % of the accepted hosts. Also when the DSV model was adjusted to experimental conditions with only good quality hosts and a temperature of 30 °C (increase in maximum egg maturation rate from 0.71 to 0.89 per hour), the predicted overall mean fraction host feeding remained less than 1 % of the accepted hosts (J.M.S. Burger, unpublished data). The most likely explanation for the discrepancy is that the adjusted DSV model predicted an average host encounter rate of 1.4 per day, whereas in the experiment average host encounter rate was eight times higher at 11.3 per day. The DSV model reasonably assumed that parasitoids emerge with enough resources from their immature stage to lay on average 10 to 15 eggs without host feeding (van Vianen & van Lenteren 1986b; Heimpel *et al.* 1998). At a mean host encounter rate of

1.4 per day, parasitoids have to survive about 10 days to become egg limited (Chapter 5). In the field, such a long life span is considered unlikely (Heimpel *et al.* 1997b, 1998). At a mean host encounter rate of 11.3 per day, however, parasitoids are far more likely to become egg limited even when life expectancy is as short as 2 days. The higher host encounter rate in the experiment could therefore explain the need to host feed.

When mean host encounter rate is increased in the adjusted DSV model from 1.4 to about 11 per day, the predicted mean fraction host feeding is about 14 % (mean longevity of 3 days) to 20 % (mean longevity of 30 days) of the accepted hosts (J.M.S. Burger, unpublished data). The first percentage is a much better prediction of the actual behaviour. In the DSV model, the host encounter rate r_e was calculated by multiplying the sum of the width of the parasitoid's searching path sw and mean host diameter dm , with the parasitoid's walking speed ws , walking activity act and host density $dens$: $r_e = (sw+dm)*ws*act*dens$ (van Roermund *et al.* 1996 after Skellam 1958). To increase mean host encounter rate in the adjusted DSV model from 1.4 to 11 per day, the product of the parameter estimates had to be increased by a factor eight to nine, which is highly unrealistic. A more likely deficit of the DSV model is the translation of host density into host encounter probability. Since *E. formosa* searches at random on a leaf (van Lenteren *et al.* 1976a; van Roermund & van Lenteren 1995a), it seems legitimate to derive the encounter probability p_e per t time steps from the Poisson distribution, using the encounter rate: $p_e = 1 - \exp(-r_e*t)$ (van Roermund *et al.* 1996). Thus, the calculation of the encounter rate derived by Skellam (1958) is probably unrealistic.

Skellam adjusted a formula by W.B. Yapp (cited in Skellam 1958), who used a formula from classical kinetic theory of colliding gas molecules to estimate population densities of a mobile organism from encounters between a moving observer and the mobile organism. Skellam (1958) relaxed Yapp's assumption that organisms move rectilinearly between encounters. Van Roermund *et al.* (1996) added the walking activity of the "observer" (a foraging parasitoid), a contour of the "organism under study" (a host) and discarded the average velocity of the hosts (sessile whitefly nymphs). They used the resulting formula for host encounter rate also in models of foraging behaviour on higher spatial scales (van Roermund *et al.* 1997a,b) and in a population-dynamical simulation model (van Roermund *et al.* 1997c). In our DSV model (Chapter 5), we used the formula by van Roermund *et al.* (1996) to translate whitefly density into a rate of host encounter by the whitefly parasitoid *E. formosa*. The only laboratory experiment carried out to provide empirical support to the theoretical formula by Skellam was a set-up where particles (or hosts) were represented by pins moving randomly in six directions on triangular graph paper and the observer (or a foraging parasitoid) by a hexagonal frame moving across the paper and encountering pins when they entered the frame (Skellam 1958). Mols (1993) adjusted Skellam's formula by including recrossing as a result of turning by the forager and concluded that Skellam's formula highly overestimates the encounter rate. The difference in host encounter rate between the DSV model and our experiment suggest an underestimation. Apparently, encounters between gas molecules cannot simply be applied to encounters between organisms. Bio-assays are needed to quantify the relationships between the parameters

sw, *dm*, *ws*, *act* and *dens* on the one hand and the host encounter rate on the other. The relationships may have regression coefficients other than 1, or may be non-linear. This may explain the difference in host encounter rate between the DSV model and our experiment.

Another difference between the DSV model and our experiment is that the model assumes that parasitoids forage on an infinitely large, continuous leaf surface, whereas in reality and in the experiment, leaves are discrete units in space with an infested lower and an uninfested upper leaf side. Leaf edges may positively affect the host encounter rate on infested lower leaf sides. On the other hand, leaf edges may increase residence times on upper sides or uninfested leaves. Despite leaf edges, hairs and veins, *E. formosa*'s walking pattern seems to be random on leaves (van Lenteren *et al.* 1976a; Li *et al.* 1987; van Roermund & van Lenteren 1995a; Sütterlin & van Lenteren 1997). Furthermore, the model assumes that each handling behaviour takes at least 6 min, whereas parasitoids can reject a host within 5 to 35 seconds after contact with their antennae (van Roermund & van Lenteren 1995b). However, a shorter rejection time in the model would not increase available searching time and thus host encounter rate, because none (0.0 %) of the encountered hosts should be rejected. The observed overall mean rejection rate of about 2 hosts per day can be explained by damage to a host during transfer from its original feeding site or breaking of the parasitoid's egg during dissection before it was observed.

We think that to improve the DSV model, adjusting the equation for host encounter rate could be more important than incorporating variation in host density. Nevertheless, the proportional hazards model on the tendency to host feed showed a positive interaction between treatment and time since start observation ($\beta_4 = 0.470$). This suggests that in the course of time, variation in host density can indeed have a positive effect on the tendency to host feed. It supports the hypothesis that host aggregation in combination with arrestment behaviour of the parasitoid results in above-average host encounter rates and in an increased risk of egg depletion.

The fact that the overall mean number of encountered hosts did not differ between host distributions could be explained by the fact that visited leaflets were replaced when the parasitoid landed on another leaflet. This was done to allow parasitoids to visit more leaflets than simultaneously present in the set-up. The number of leaflets was limited for practical reasons explained in the materials and methods section. The unavoidable drawback is that parasitoids cannot return to favourable patches. The limited possibility to return negatively affects only parasitoids foraging in the aggregated distribution treatment. This should be taken into account when incorporating variation in host density into the DSV model.

Oviposition should have a decremental effect on giving up time when host distribution is uniform (Driessen *et al.* 1995) and an incremental effect when host distribution is aggregated (Waage 1979). Although whitefly nymphs aggregated at several spatial scales in the field, still a leaflet with only 1 host was the second most likely type of leaflet after empty leaflets (Chapter 4). In such an environment, a host encounter only is not enough to decide whether to stay or leave. Our analysis suggests

that arrestment behaviour is conditional upon the time since the latest host encounter. The leaving tendency on a leaflet with 1 host decreased after host encounter when time since the latest host encounter was short, but increased after host encounter when time since the latest host encounter was long, independent of treatment. Van Roermund *et al.* (1994) found that the leaving tendency of *E. formosa* decreased significantly after one or more ovipositions in unparasitised hosts. In contrast to our experiment, however, they only observed intra-patch behaviour on a single leaflet and therefore could not consider inter-patch experience. The behavioural response that we found possibly allows the parasitoid to deal efficiently not only with highly infested leaflets but also with leaflets containing 1 host only, using the time since the latest host encounter to decide whether to stay or leave. This is supported by the similar host encounter rates in both treatments (Fig. 3c).

We hypothesised that *E. formosa* changes its behaviour with experience because the number of hosts in the field varied in a predictable way as a result of spatial dependence (Chapter 4). We found that the tendency to leave a leaflet with 1 host was significantly affected by whether or not the host was encountered, the time since the latest host encounter and the time since the start of the observation. This confirms that *E. formosa* indeed uses experience to decide whether to stay or leave, provided that senescence has a negligible effect on behaviour during the first week of life. Contrary to our hypothesis, however, parasitoids in the uniform distribution treatment did not leave sooner after host encounter than parasitoids in the aggregated distribution treatment. This supports the previous suggestion that the time since the latest host encounter allows parasitoids in both treatments to deal efficiently with leaves that contain only 1 host.

In conclusion, at host densities found in the field, *E. formosa* does host feed on average about once a day and lays on average about 8 eggs per day. The discrepancy between model prediction and observed behaviour could be explained by an underestimation of the host encounter rate from host density using the formula by Skellam (1958), but we also showed that variation in host density increased the tendency to host feed in the course of time. *E. formosa* uses time since the latest host encounter to decide whether to stay or leave after a host encounter. This may be an efficient strategy in the natural environment where infested leaves contain most likely a single host but sometimes many hosts.

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7

General discussion

Introduction

The main aim of evolutionary biology is to explain the adaptation of form, function and behaviour of organisms to their environment. In this thesis, I studied host-handling behaviour of the whitefly parasitoid *Encarsia formosa* from such an evolutionary point of view. In this chapter, I will briefly summarise the most important results, expand the discussion, draw the main conclusion and give suggestions for future research.

Summary of results

In **Chapter 2**, I studied what the benefits are to the parasitoid of one destructive host feeding over non-destructive feeding on honeydew. In contrast to my expectation, parasitoids that were allowed to feed upon one host did not have a significantly higher egg load 20 hours or 48 hours after host feeding than parasitoids prevented from host feeding. Host feeding did not increase the estimated number of eggs matured within these periods, neither did the time spent host feeding positively affect any of these response variables. On the other hand, presence of honeydew did have a positive effect on egg load 20 hours and 48 hours after host feeding compared with parasitoids deprived of honeydew. Parasitoids with access to honeydew were estimated to mature more eggs within these periods than honeydew-deprived parasitoids. Host feeding did have a positive effect on life expectancy, but this effect was nullified when honeydew was supplied after the host-feeding attempt. These results suggested that feeding on honeydew could be an advantageous alternative to host feeding, especially in homopterid parasitoids, which can drink honeydew directly from the host's anus. I hypothesised that host feeding is unavoidable to be able to produce anhydrotic eggs,

which may have an advantage over hydronic eggs in the parasitoid's natural environment.

In **Chapter 3**, I extended the observation period from 1 to 5 days and measured the effect of host feeding on the number of ovipositions, longevity and egg volume, and the effect of alternative food sources on egg load dynamics. Even in the presence of honeydew, parasitoids allowed to host feed laid significantly more eggs per hour of foraging per host-feeding attempt (1.25 ± 0.38) than parasitoids prevented from host feeding (0.84 ± 0.43). Although parasitoids allowed to host feed laid more eggs during the experiment than parasitoids prevented from host feeding (27.57 ± 7.59 vs. 19.44 ± 5.68), they did not differ in survival probability (median longevity 31 vs. 29 days), nor in the change in estimated egg volume over time ($-3E3 \pm 6E3$ vs. $-1E3 \pm 8E3 \mu m^3$ per day). Parasitoids with only access to water quickly started resorbing eggs and all died within 6 days. The average egg load of parasitoids with access to food initially increased to about 10 (sucrose), 12 (honeydew) and 14 (sucrose and honeydew) eggs at day 2. Between day 2 and 10, net oosorption was about 0.42 eggs per day. These experiments showed that destructive host feeding can have an advantage over non-destructive feeding on honeydew, especially for egg-limited parasitoids. At low host densities, however, feeding on non-host food sources could supply enough nutrients to prevent egg limitation. Time- or host-limited parasitoids may benefit from host feeding by resorbing the extra eggs to increase life-span and thus searching time, or by using the extra eggs to exploit rare host aggregates.

To understand evolution of foraging behaviour in *E. formosa*, I quantified natural densities and distributions of whitefly in *E. formosa*'s presumed area of origin, the Neotropics (**Chapter 4**). At most sites, an average of 0.5 to 2 whitefly nymphs per leaflet was found. Of the randomly collected leaflets, 71 % was empty, whereas some carried up to several dozens of nymphs. Usually over 95 % of the whitefly nymphs was on the lower side, and 20 to 90 % was in a developing stage. At the canopy (21 to 37 m above ground), on average only 0.06 nymphs per leaflet were found, compared with 1.3 at the forest floor (0 to 3 m) in the same area. Generalised linear mixed modelling revealed that the number of hosts on the lower side of a leaflet of an average plant within an average spot of an average transect could be described by a Poisson distribution with mean and variance equal to 0.241, in a ratio of 11:12:13:14 = 0.14:0.23:0.26:0.37. The Poisson mean was largely affected by the plant ($\sigma_{plant}^2 = 2.485$) and less by the spot ($\sigma_{spot}^2 = 0.197$) or transect ($\sigma_{transect}^2 = 0.522$). Variation between plants could hardly be explained by variation in leaf area ($\sigma_{plant}^2 = 3.605$ when only plant was incorporated as random effect and $\sigma_{plant}^2 = 2.698$ when leaf area was added). Based on the shape of the opening in vacated puparia, the probability that a whitefly became eventually parasitised was 0.12 on an average leaflet. Parasitism was more patchily distributed among spots ($\sigma_{spot}^2 = 1.006$) but more evenly distributed among plants within spots ($\sigma_{plant}^2 = 0.952$) than whitefly nymphs. Semivariance analysis was used to quantify the degree and scale of spatial dependence of whitefly nymphs. In 1 of 3 short transects, the numbers of whiteflies on leaves were spatially dependent. This was not a result of patchiness in host plants because it was in 1 of the 2 transects where leaves were collected from a

continuous understorey of a single host plant genus (*Piper* sp., Piperaceae). In 4 of 7 three-dimensional plots at least one level of spatial dependence could be detected, 3 of 4 within a single host plant species. In one plot, patches of about 16 cm were nested within patches of about 37 cm. In the other three, similar patch sizes of 41, 20 and 15 cm were found in seclusion. Results provided ecological information that was used in the subsequent chapters to understand the evolution of foraging behaviour by *E. formosa*. Because mean whitefly densities were low in the field, whitefly parasitoids were expected to be under strong selection pressure to handle a host optimally, i.e. in a way that maximises their lifetime reproductive success.

In **Chapter 5**, a dynamic state variable (DSV) model was developed to predict optimal host-handling strategies under different scenarios of natural and artificial environments. The most important prediction is that host feeding is maladaptive under presumable field conditions of low mean host density (0.015 cm^{-2} or 1 host per 67 cm^2 leaf area) and short life expectancy (mean longevity about 3 days). Egg limitation is prevented without host feeding because parasitoids can mature some eggs from the immature stage, host encounter rate is slow and parasitoid's life expectancy is short. Both host density and parasitoid's life expectancy had a large positive effect on the optimal host-feeding ratio. Parasitoids that make random decisions gain on average only 35 % to 60 % of the lifetime reproductive success of parasitoids that make optimal decisions, depending on host density. Elasticity analyses revealed that the relationship between host density and acceptance ratio is most sensitive to the relative handling time of host feeding and the temperature at which oogenesis takes place. The relationship between host density and host-feeding ratio is most sensitive to the relative handling time of host feeding and the host-feeding gain from the bad quality host. Parameters that have a large impact on lifetime reproductive success and thus fitness are the parasitoid's life expectancy and the survival probability of deposited eggs (independent of host density), the host encounter probability and length of the active period (when host density is low) and the egg maturation rate and number of host types (when host density is high). Explaining evolution of host-feeding behaviour under natural conditions may require incorporation of variation in host density, incorporation of parasitised host types, or field data showing that life expectancy in the field is not as short as I assumed. Incorporating variation in walking speed, egg resorption, or antennal rejection are not likely to be able to explain the evolution of host-feeding behaviour under natural conditions.

The DSV model predicted that parasitoids encounter and parasitise on average 1.3 hosts per day at 1 host per 67 cm^2 leaf area. In contrast, the experiment in **Chapter 6** showed that on average, parasitoids encountered about 12 hosts per day and host fed about once a day or about 11 % of the accepted hosts for both host distributions. A proportional hazards model showed that after about 4 days since the start of the observation, the tendency to host feed when host distribution was aggregated exceeded the tendency to host feed when host distribution was uniform, independent of time since the latest host acceptance. Another proportional hazards model showed that host encounter decreased the leaving tendency on a leaflet with 1 host when time since the

latest host encounter was relatively short, but increased the leaving tendency when time since the latest host encounter was relatively long, independent of host distribution. Time since start of observation had a positive effect on leaving tendency. These results suggested that the formula by Skellam (1958) used in the DSV model and simulation models by van Roermund *et al.* (1996, 1997a,b,c) underestimates host encounter rate from host density. Although variation in host density had a positive effect in the course of time on the tendency to host feed, adjusting Skellam's formula in the DSV model seems more important than incorporating variation in host density. Moreover, *E. formosa* uses time since the latest host encounter to decide whether to stay or leave after a host encounter. This allowed it to forage as efficiently in an environment with a uniform host distribution as in the natural environment with an aggregated host distribution.

The DSV model showed that evolution of host-feeding behaviour could be explained under the low mean host density found in the field when parasitoid's life expectancy in the field is not as short as assumed. Predators, bad weather or food deprivation are expected to considerably reduce a parasitoid's life expectancy in the field compared with that in the relatively safe laboratory. However, field data that support this hypothesis are rare. Therefore, three age determination methods were tested in the **Appendix** to estimate the age of a field-caught specimen. Unfortunately, none proved a useful technique. *E. formosa* did have detectable fluorescent compounds and there was a significant effect of age on fluorescence, but the scatter was too large to be of predictive value, even when temperature and hind tibia length were used as covariates to reduce the error variance. Moreover, a positive rather than a negative effect of age was expected physiologically. There was no effect of age on degree of wing fray, probably because wing movement, predatory attack or abrasion by the habitat did not occur under laboratory conditions. The method of relative residual longevity failed because the number of female whitefly parasitoids in the field sample was too small, no offspring could be obtained as a control and there was a large interspecific variation in survival among commercially produced whitefly parasitoids even under controlled conditions. In conclusion, no suitable age determination method was found for *E. formosa* or whitefly parasitoids in general. As a result, parameter values for life expectancy in the field remain largely hypothetical in models on the trade-off between current and future reproduction.

On adaptation

The aim of my project was to understand host-handling behaviour in the whitefly parasitoid *Encarsia formosa* from an evolutionary perspective. Under presumable field conditions, parasitoids did not behave in a way predicted by the optimal-foraging model. The model predicted that host feeding is maladaptive at the low mean host density found in the field, whereas parasitoids fed upon about 11 % of the accepted hosts under similar conditions in the experiment. A number of reasons can be the underlying cause. Explaining form, function or behaviour from an adaptive viewpoint, the so-called

adaptationist programme, has been largely criticised (e.g. Lewontin 1978; Gould & Lewontin 1979). However, the target of criticism should not be the adaptationist programme as such but an improperly conducted one (Mayr 1983; Williams 1985). I argue that host-handling behaviour is likely to have evolved by natural selection as an adaptation to the parasitoid's environment for at least two reasons. Generally, there is a direct link in insect parasitoids between behavioural decisions and the parasitoid's lifetime reproductive output. Their behaviour is therefore under direct natural selection. Second, host-feeding behaviour in *E. formosa* kills the host and therefore destroys an opportunity to oviposit. This creates a strong selection pressure against host-feeding behaviour if it was not offset by a selective advantage. Indeed, parasitoids making random host-handling decisions were predicted to gain a considerably lower lifetime reproductive success than parasitoids making optimal host-handling decisions. This effect was strongest at the low mean host density found in the field.

Other potential explanations for the discrepancy between model prediction and experimental observation are that the population may be stuck at a local optimum or environmental changes may have shifted the optimum (Mangel & Clark 1988). The latter is important, because the experiments have been carried out with commercially produced parasitoids, rather than field-caught parasitoids. Although rearing conditions are a trade secret (Koppert Biological Systems, pers. comm.), it is likely that commercially produced parasitoids experience and adapt to host densities much higher than those found in the field. Price (1997) refers to the evolution of industrial melanism in the peppered moth *Biston betularia* (Kettlewell 1959) and the very rapid evolution of insecticide resistance (Georghiou 1972) to illustrate the large evolutionary potential in arthropods. The obvious solution is to use specimens reared from whitefly nymphs collected in natural vegetation. Unfortunately, attempts to rear parasitoids from whitefly nymphs collected in Costa Rica failed, probably because of high humidity. Moreover, *E. formosa* is relatively rare in the Caribbean and Latin America compared with other hymenopterous parasitoids of *Bemisia* spp. (Schuster *et al.* 1998).

There are more explanations that can apply if the organism fails to behave according to predictions. *E. formosa* is an asexual species. Sex has been referred to as "the masterpiece of nature" (Bell 1982), but also as "a kind of crisis in evolutionary biology" (Williams 1975), "one of the greatest unsolved problems in evolutionary biology" (Godfray 1994) and even "an enigma within a mystery" (Hurst & Peck 1996). A population of asexually reproducing females can have twice the growth rate of an anisogamous sexual population without paternal care. Numerous hypotheses have been put forward to explain the evolution and maintenance of sex (Maynard Smith 1978a; Bell 1982; Michod & Levin 1988). One class of hypotheses is that the main characteristic of sex, genetic recombination, creates genetic variance and this may accelerate adaptive evolution. Because the asexual *E. formosa* produces genetically identical offspring, it may have been unable to adapt to its environment during the course of evolution and hence not behave according to optimal-foraging theory. However, lack of adaptability would also imply that *E. formosa* is an evolutionary dead end. In *E. formosa*, the thelytokous parthenogenesis is *Wolbachia*-induced (Stouthamer

1997). The infection by this symbiont is likely to be an ancient association, since antibiotic treated females produce males that are not able to inseminate conspecific females (Zchori-Fein *et al.* 1992). Apparently the selection pressure for mating behaviour has been absent for many generations. Furthermore, a negative effect of the *Wolbachia* infection on offspring production in *E. formosa* could not be detected (Stouthamer *et al.* 1994). These results suggest that *E. formosa* does not have a short evolutionary lifetime. Possibly, *E. formosa* is able to maintain the genetic variance it had before it was infested by *Wolbachia* or is phenotypically plastic. Some other asexual lineages, i.e. the bdelloid rotifers, darwinulid ostracods and brine shrimp, seem to have persisted for tens of millions of years without sex (e.g. Judson & Normak 1996; Butlin 2002).

Still, the most likely explanation for the discrepancy between predicted and observed behaviour is an inadequate model (Mangel & Clark 1988). As Maynard Smith (1978b) pointed out, the aim of an optimisation model is not to test the hypothesis of adaptation but to test the hypotheses of the model. Parasitoids do not fail to behave according to predictions, but the model fails to predict the behaviour of the parasitoids. Simulations under different scenarios and elasticity analyses suggested that evolution of host-feeding behaviour under presumable field conditions may require incorporation of variation in host density, incorporation of parasitised host types or field data showing that life expectancy is not as short as assumed in the model. Incorporating variation in walking speed, egg resorption, or antennal rejection was unlikely to reveal an explanation. The validation experiment additionally revealed that the model underestimated host encounter rate from host density.

Main conclusion

In addition to the rather qualitative studies reviewed in Heimpel & Collier (1996), this study focussed on a well-known species to quantify several aspects of the evolution of host-handling behaviour. The main conclusion is that host-handling decisions in the whitefly parasitoid *Encarsia formosa* may have evolved as an adaptation to a spatially and possibly temporally heterogeneous environment. The parasitoid's host-handling behaviour allows the parasitoid to deal with variation in both host density and host distribution. I showed that destructive host feeding has an evolutionary advantage over non-destructive feeding on honeydew. Parasitoids can use the host-feeding gain to increase their oviposition rate. This can be advantageous when parasitoids encounter patches with high host densities. Although the average host density was low in the field, I found several indices for host aggregation. Previous studies showed that *E. formosa* does not use herbivore-induced plant volatiles to detect its host from a distance, probably because its host range is too wide or hosts are too polyphagous. As a result, parasitoids are likely to experience low host densities when leaving a depleted patch of high host density. Leaving high host density patches before depletion might also be an adaptive strategy to spread the risk of hyperparasitism (Rosenheim 1998). During such meagre periods, it pays to have invested in anhydropic eggs. Parasitoids with hydropic eggs do

not have to destructively host feed and can quickly lay many eggs, but parasitoids with anhydrotic eggs have an egg resorption capability, which allows them to reallocate their nutrients into maintenance when hosts are rare. Results from the final experiment suggested that parasitoids used a patch-leaving mechanism based on time since latest host encounter. This allows them to be flexible and to forage efficiently at different host distributions.

Future perspectives

To truly understand the evolution of host-handling behaviour, the main gaps to be filled lie in understanding the physiological, biochemical and genetic mechanisms; the effect of spatial and temporal heterogeneity in host availability on the risk of egg limitation; and the parasitoid's life expectancy in the field. Adult female parasitoids can obtain nutrients from the immature stage, from feeding on host hemolymph, honeydew and nectar and from egg resorption. Parasitoids need nutrients for maintenance, activity, egg maturation and survival; maybe some nutrients invested in eggs can also increase egg viability. Understanding this complex of supply and demand requires in the first place more qualitative and quantitative biochemical analyses of compounds present in the different food sources. This may be complicated by the fact that the composition of food sources depends on environmental conditions (e.g. Cox 1970; Crafts-Brandner 2002). Radioactive labelling of nutrients can reveal how different nutrients are allocated to different organs and how they are used over time (e.g. Boggs 1997; Rivero & Casas 1999a,b; Rivero *et al.* 2001). Furthermore, field data are needed to quantify the availability and possibility for parasitoids to exploit different food sources (e.g. Queiroz & Oliveira 2001) and to quantify the temporal changes in host availability. Models incorporating the complex of nutrient supply and demand and the heterogeneity in host availability should be developed to define a parameter space wherein host feeding is an adaptive trait.

Stouthamer (1997) pointed out that involvement of cytoplasmic factors in causing parthenogenesis disqualifies such parthenogenetic organisms to a large extent for experimental studies on the evolution of sex. However, it remains to be clarified what is the role of genetic variability and adaptive phenotypic plasticity in the evolution of host-feeding behaviour in *E. formosa* and other host-feeding parasitoids. Thus, future studies should try to integrate genetics, physiology, biochemistry and behavioural ecology.

Appendix

Life expectancy and the value of future reproduction in the field

Introduction

The trade-off between current and future reproduction is one of the most studied trade-offs in life-history theory (Stearns 1992). Resources allocated to current reproduction cannot be used for future reproduction. Natural selection favours the allocation of resources that maximises fitness. The optimal allocation depends among others upon environmental conditions. In a rich or safe environment, it is more rewarding to invest in future reproduction than in a poor or hazardous environment. Here we aim at quantifying the probability for an insect parasitoid to survive in its natural environment.

The trade-off between current and future reproduction is reflected in adult, female insect parasitoids when deciding whether to parasitise or feed upon a host (Jervis & Kidd 1986; Heimpel & Rosenheim 1995; Heimpel & Collier 1996). Parasitism is the deposition of an egg that can result in new offspring (current reproduction), whereas host feeding is the destructive consumption of the host that can result in new parasitoid eggs (future reproduction), which can also be reallocated to survival through resorption.

The parasitoid *Encarsia formosa* (Hymenoptera, Aphelinidae) is successfully applied worldwide to control whitefly pests in greenhouses (van Lenteren *et al.* 1996; van Lenteren 2000). Simulation models by van Roermund *et al.* (1996, 1997a,b) explain from a mechanistic point of view how *E. formosa* parasitoids realise the observed level of parasitism. In Chapter 5, we modelled from an evolutionary point of view why *E. formosa* parasitoids decide to host feed or oviposit. This decision can affect population dynamics because host feeding results in whitefly mortality without direct parasitoid offspring. The model showed that the optimal decision depends upon the life expectancy of the parasitoid. Given the average host density found in the field, the optimal fraction

of accepted hosts that should be fed upon averaged only 0.006 when survival was 10 % after 7 days but was much higher, i.e. 0.243, when survival was 10 % after 70 days.

In laboratory studies, van Lenteren *et al.* (1987) found an average longevity for *E. formosa* of 37 days on honey and 52 days when hosts were present. Vet & van Lenteren (1981) even found an average longevity of 99 days at 15.6 °C. No data are available under field conditions, but predators, bad weather or food deprivation are expected to considerably reduce life expectancy of an adult parasitoid (Rosenheim 1998). Heimpel *et al.* (1998) estimated that overall only 1 % of *Aphytis* parasitoids (Hymenoptera, Aphelinidae) survived 8 days of foraging in the field, based on observations of predation in an orchard (Heimpel *et al.* 1997b). Parasitoids are able to change their behaviour in response to rain or wind (Fink & Völkl 1995) or to changes in barometric pressure associated with thunderstorms (Roitberg *et al.* 1993). Bad weather might therefore not directly reduce survival, but indirectly by reducing the available searching time. Food deprivation has a large effect on parasitoid longevity in the laboratory (van Lenteren *et al.* 1987).

Here we tested three age determination methods in order to quantify life expectancy in the field of *E. formosa* and related whitefly parasitoids and to test the hypothesis that parasitoid longevity in the laboratory is an overestimate of natural life expectancy.

Materials & Methods

Age determination method

General

Hayes & Wall (1999) reviewed several age-grading techniques that have been used to determine the age of an adult insect in the field. They discern three types based on changes in the reproductive system, somatic changes and cuticular deterioration. Ovarian dissection, wing fray and pteridine analysis are the three most commonly used techniques. The pteridine bioassay is a practical technique when field specimens have to be stored for several weeks before analysis (Lehane & Mail 1985). Pteridines are end products of nitrogen metabolism (Ziegler & Harmsen 1969) and can be measured using a fluorescence spectrophotometer (Mail *et al.* 1983). In several Dipteran species, pteridine levels significantly increase with age (e.g. Mail *et al.* 1983; Lehane *et al.* 1986). For accurate age grading, Hayes & Wall (1999) suggested to use two or more complementary techniques. Wing fray is damage to the trailing edge of the wing. The degree of wing fray is a relative measure of physiological age and can easily be applied to field-caught specimens. Because neither pteridine nor wing fray appeared useful (see results), we tested relative residual longevity as a third method (see below).

Pteridine bioassay

To determine the relationship between pteridine level and parasitoid age, female *Encarsia formosa* parasitoids were obtained from Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands. Parasitoids were allowed to emerge during 24 hours in plastic dishes (Ø 6 cm, height 2 cm) with fine gauze in the lid for ventilation and irradiation. Honey was provided throughout the experiment. Dishes were kept in climate cabinets (Gallenkamp) at 15, 20 or 25 °C. The light regime was set at L:D=12:12 hours. Each cabinet contained 5 natural daylight fluorescent lamps (Philips TL 8W/965) and 1 UV-A fluorescent lamp (Philips TL 8W/05). UV-A light (320–400 nm) was added because it was lacking in the natural daylight fluorescent lamps but is present in natural daylight and may affect accumulation of pteridine pigments. The inner side of each cabinet was covered with aluminium foil to increase irradiation. A spectral power distribution was measured using a scanning spectroradiometer (Macam SR 9910) with a side-view cosine sensor, which revealed that light intensity gradually increased with wavelength from about 0.16 W/m² per nm at 340 nm (UV-A) to about 0.50 W/m² per nm at 650 nm (red), with peaks of about 2 to 3 W/m² per nm at 405 nm (violet), 436 nm (blue-violet) and 547 nm (green).

After the parasitoids had aged a certain time, dishes containing parasitoids were wrapped in aluminium foil and stored at -80 °C for some minutes to quickly kill the parasitoids. Each parasitoid was decapitated. Hind tibia length was measured at 400 power as measure of parasitoid size. The head was ground in a 1.5 ml micro tube using a melted tip of a Pasteur pipette. After adding 250 µl of 0.05 M tris/HCl buffer at pH 8, the micro tube was briefly swirled on a vortex and centrifuged for 4 min at rotation speed of 10,000 rpm (Ø 11 cm). Relative fluorescence was read from a fluorescence spectrophotometer (Perkin-Elmer 2000) with excitation wavelength set at 367 nm and emission wavelength set at 454 nm.

Relative fluorescence was regressed to age, hind tibia length and temperature using general linear modelling. Partial F-tests were used to decide which combination of main effects and one-level interactions was the most parsimonious (Neter *et al.* 1996). Interactions were only considered when accessory main effects were also included.

Wing fray

The parasitoids used in the pteridine bioassay were also used to study the relation between ageing and the degree of wing fray. Before the hind tibia length was measured, wings were removed and inspected under a light microscope at 400 power for degree of fray.

Relative residual longevity

To quantify relative residual longevity, specimens (of unknown age) were caught in the field (see section "Fieldwork") and held under optimal conditions until they died.

The time between capture and death was defined as the residual longevity. This residual longevity was compared with the time between emergence and death of a control group kept under the same conditions. Ideally, the control group was the offspring of the field-caught specimen. We tried to obtain offspring by allowing field-caught specimens to oviposit in a whitefly nymph on an infested leaf. Plants were obtained from local market gardeners and stall holders and infested with adult whiteflies from local populations. Although the whitefly rearing proved successful, wasps did not oviposit when offered an infested leaf. To obtain an alternative control group, *Encarsia formosa* females and *Eretmocerus mundus* males and females (Hymenoptera, Aphelinidae) were ordered from Koppert Biological Systems. They were kept in the Netherlands under similar conditions as the field-caught specimens, that is, individually in 1.5 ml micro tubes with a drop of honey in a climate chamber at a light regime of L:D=13:11 hours and a temperature regime of L:D=35:23 °C. Survival was checked twice a day. The difference between

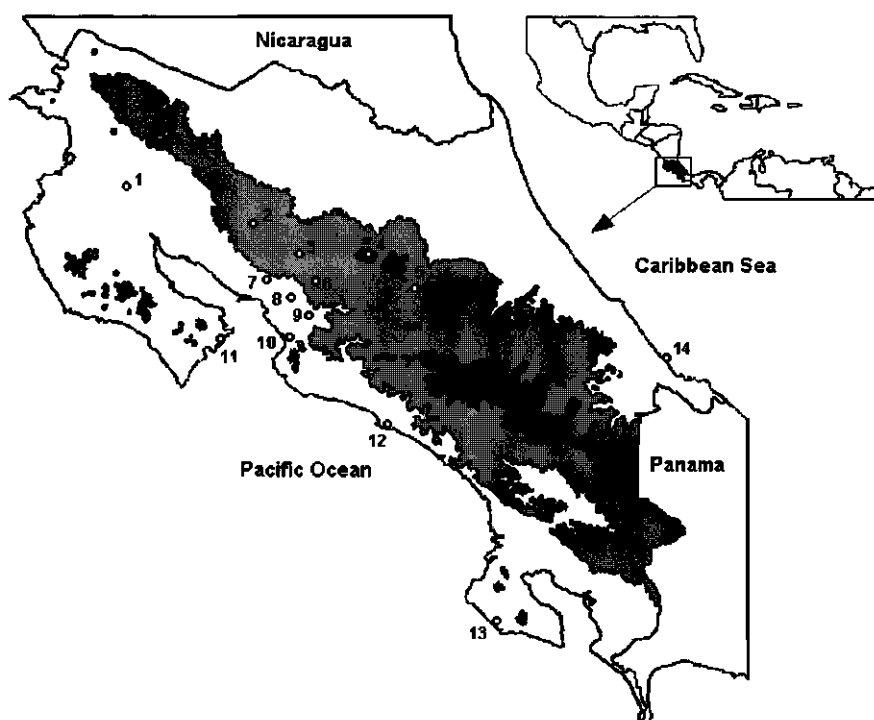


Fig. 1 Map of Costa Rica (8-11° N, 82-86° W) with 500 m and 2000 m contour lines, showing sampling sites: 1 Lomas Barbudal Biological Reserve; 2 Santa Elena Forest Reserve; 3 Los Alpes Refuge; 4 Volcán Poás National Park; 5 Braulio Carrillo National Park; 6 Enpalme; 7 Miramar; 8 Finca Alisa, La Angostura; 9 Finca CUP, Orotina; 10 Carara Biological Reserve; 11 Curú National Wildlife Refuge; 12 Manuel Antonio National Park; 13 Corcovado National Park; 14 Cahuita National Park.

residual longevity of field-caught specimens and longevity of the control group is a relative measure of life expectancy in the field. If the difference is small, field-caught specimens were probably young and field conditions reduce life expectancy compared with laboratory conditions. If the difference is large, field-caught specimens were probably old and field conditions do not reduce life expectancy compared with laboratory conditions.

Fieldwork

E. formosa is assumed to originate from the Neotropics and fieldwork was carried out in Costa Rica (see Chapter 4; Polaszek *et al.* 1992; Babcock *et al.* 2001; van Lenteren *et al.* 1996; van Roermund & van Lenteren 1995a; Vet *et al.* 1980; Speyer 1927; Schuster *et al.* 1998). Minute, adult, wasp-like specimens were searched for in the areas indicated in Fig. 1. Table 1 shows Holdridge life zones according to Janzen (1983), sampling dates and the number of specimens caught per sampling site. *Finca* Alisa at La Angostura consisted of shrubs on pasture and forested river banks. Specimens were searched by visual inspection of leaves, caught alive using an aspirator and used for the method of relative residual longevity. Wasps were kept individually in 1.5 ml micro tubes with a

Table 1 Holdridge Life Zones (Janzen 1983), sampling dates and number of specimens per Costa Rican sampling site.

| Site ^(a) | Holdridge Life Zone ^(b) | Sampling dates (2001) | Number of specimens |
|-------------------------------|------------------------------------|-------------------------------|---------------------|
| 1 Lomas Barbudal | Tropical dry forest | 5 April, 12 June | 36 |
| 2 Santa Elena | Tropical lower montane wet forest | 18, 19 April | 7 |
| 3 Los Alpes | Tropical premontane wet forest | 7 May | 20 |
| 4 Volcán Poás | Tropical lower montane rain forest | 19 May | 14 |
| 5 Braulio Carrillo | Tropical premontane rain forest | 15 May | 3 |
| 6 Enpalme | Tropical premontane wet forest | 2 May | 7 |
| 7 Miramar | Tropical moist forest | 11 May | 7 |
| 8 La Angostura ^(c) | Tropical moist forest | 26 March - 25 May | 83 |
| 9 Orotina | Tropical moist forest | 23 May | 6 |
| 10 Carara | Tropical moist forest | 31 March - 2 April; 5, 6 June | 18 |
| 11 Curú | Tropical moist forest | 30 March | 23 |
| 12 Manual Antonio | Tropical wet forest | 14 June | 11 |
| 13 Corcovado | Tropical wet forest | 18, 19 June | 12 |
| 14 Cahuita | Tropical moist forest | 23, 24 April | 7 |

^(a) Numbers of sampling sites correspond to those in Fig. 1 and Table 2.

^(b) Latitudinal region: tropical; altitudinal gradient: sea level, premontane, lower montane, montane, subalpine; precipitation gradient: dry, moist, wet, rain.

^(c) Residence area.

drop of honey and checked twice a day for survival. During the first 51 days, tubes were kept under ambient conditions in the shade. Daily minimum temperatures were around 23 °C, daily maximum temperatures between 31 °C and 36 °C. To test the effect of maximum temperature on survival, tubes were transferred to a hole in the ground on day 52 (17 May), where daily maximum temperatures varied between 25 °C and 27 °C. Wasps were stored in alcohol after death for identification.

Results

Pteridine bioassay

Fig. 2 shows relative fluorescence in relation to parasitoid age, temperature and hind tibia length. At higher temperatures the number of data points decreased with age because survival decreases with temperature. Relative fluorescence decreased significantly with age about 1.1 units per day. It also decreased significantly with about 4.1 units per °C and increased significantly with about 1.7 units per μm hind tibia. All three effects were independent. The most parsimonious model, however, explained only 11.7 % of the variance (adjusted coefficient of determination $R_a^2 = 9.9\%$) and still 10.4 % ($R_a^2 = 8.8\%$) when age was excluded (second most parsimonious model; partial F-test, 1 df: $P = 0.018$). Thus, although ambient temperature and hind tibia length could be used as covariates to reduce error variance, the relationship between age and fluorescence was too weak to be of practical use. Moreover, a positive rather than a negative effect of age was expected physiologically.

Wing fray

No effect of age on degree of wing fray was observed (data not shown). Even 30-day-old parasitoids did not show any degree of wing fray compared with newly emerged parasitoids. This showed that wing fray, if present in field-caught specimens, cannot be calibrated by a control population under simple laboratory conditions.

Relative residual longevity

Table 2 shows identity, origin and biology of the specimens caught in the field. From a total of 254, 182 were identified as Hymenoptera, belonging to at least 14 families. At least 18 specimens were whitefly parasitoids according to Gerling (1990) or Hanson & Gauld (1995), one of which belonged to the genus *Encarsia*. Despite the low absolute number of whitefly parasitoids, they were collected from all altitudinal regions that were sampled (Table 2; Fig. 1). At least 11 of the *Euderomphale* sp. were males. The low number of female whitefly parasitoids in the total sample explains why no offspring could be obtained as a control group.

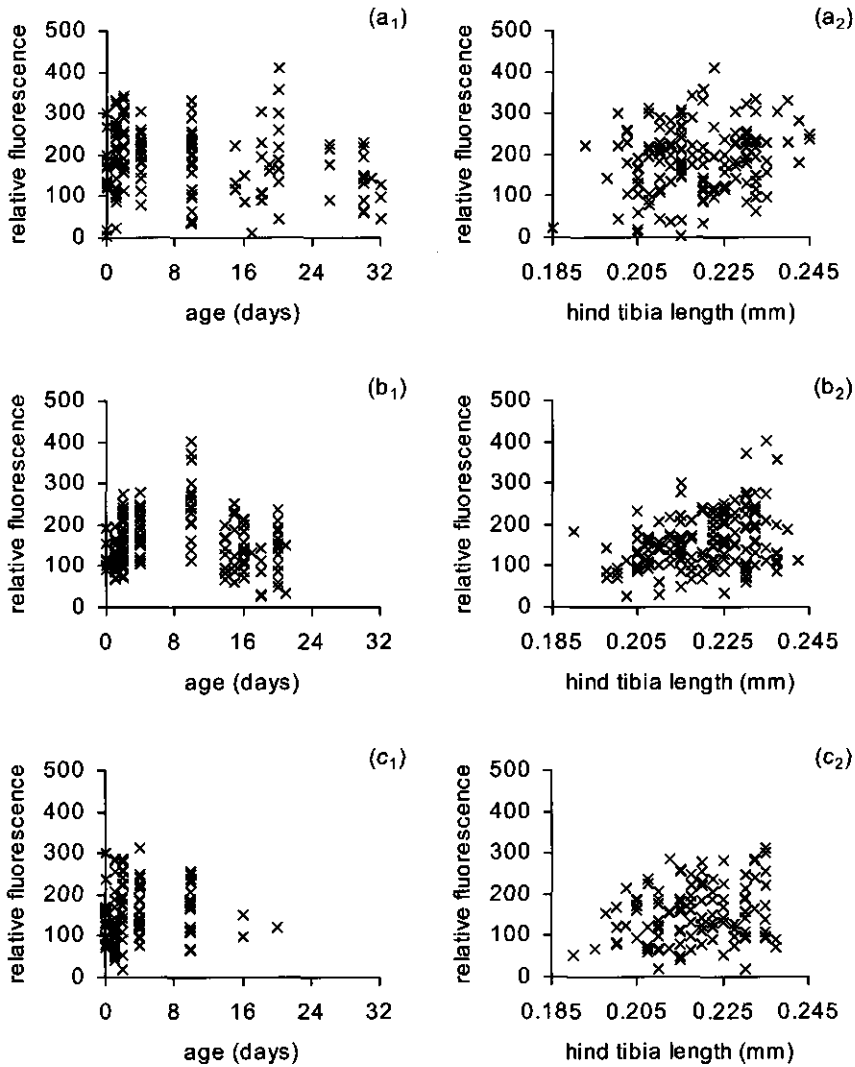


Fig. 2 Relative fluorescence of adult female *Encarsia formosa* heads plotted against parasitoid age (days after emergence) (left panels) and parasitoid size measured as hind tibia length (mm) (right panels). Parasitoids were kept on honey at (a) 15 °C, (b) 20 °C and (c) 25 °C. Most parsimonious general linear model: $Y = -108.332 + 1669.922 \cdot X_1 - 4.1426 \cdot X_2 - 1.0997 \cdot X_3$, where Y is relative fluorescence, X_1 is hind tibia length (mm), X_2 is temperature (°C) and X_3 is age (days).

Appendix

Table 2 Identity, origin and biology (whitefly parasitoid according to Gerling 1990 or Hanson & Gauld 1995) of 254 "minute, adult, wasp-like" specimens caught alive by visual inspection of leaves in Costa Rican sampling sites.

| Order | Species | Number of specimens | Site nos. ^(a) | Whitefly parasitoid |
|-----------------------------|-------------------------|---------------------|--------------------------|---------------------|
| Family | | | | |
| Hymenoptera | | | | |
| Aphelinidae | <i>Encarsia</i> sp. | 1 | 3 | + |
| | <i>Eretmocerus</i> sp. | 1 | 8 | + |
| Braconidae | Unidentified | 2 | 8, 12 | - |
| Ceraphronidae | Unidentified | 32 | 1,3,5,8,10,12,13,14 | - |
| Chalcididae | Unidentified | 1 | 11 | - |
| Cynipidae | Unidentified | 1 | 2 | - |
| Diapriidae | Unidentified | 12 | 4,8,10,11,13 | - |
| Encyrtidae | Unidentified | 4 | 6,8,10,11 | - |
| Eulophidae | <i>Euderomphale</i> sp. | 13 | 8 | + |
| | <i>Horismenus</i> sp. | 1 | 8 | - |
| | <i>Neopomphe</i> sp. | 1 | 3 | + |
| | Unidentified | 8 | 3,8,10,13 | - |
| Eurytomidae | Unidentified | 5 | 1,8 | - |
| Mymaridae | Unidentified | 3 | 1,2,14 | - |
| Platygastridae | <i>Amitus</i> sp. | 2 | 3,4 | + |
| | <i>Synopeas</i> sp. | 1 | 8 | - |
| | Unidentified | 38 | 1,2,3,4,6,8,12,14 | - |
| Pteromalidae | Unidentified | 2 | 8,11 | - |
| Scelionidae | Unidentified | 51 | 1,3,4,6,8,9,10,11,12,13 | - |
| Trichogrammatidae | Unidentified | 1 | 14 | - |
| Unidentified ^(b) | | 2 | 8,10 | - |
| Diptera | | | | |
| Unidentified | | 30 | 1,5,6,7,8,9,10,12,13,14 | - |
| Unidentified ^(b) | | 42 | | - |

^(a) Numbers of sampling sites correspond to those in Fig. 1 and Table 1.

^(b) Damaged or lost.

In Fig. 3a, the survival curve (residual longevity) of the first 110 Hymenoptera that were caught in the field and kept under ambient conditions in the shade is compared with the survival curve of 41 commercially produced *En. formosa* females that emerged in the laboratory and kept under similar conditions. The survival curve of the laboratory-emerged specimens was concave, indicating that the probability of mortality was higher in older than in younger specimens and that parasitoids probably died of senescence. The survival curve of the field-caught specimens, on the other hand, was convex, indicating that survival was exponentially distributed and that the probability of mortality was more or less constant over time. The large difference in median survival time between field-caught specimens (1.7 days) and laboratory-emerged *En. formosa* (11.7 days) suggests that field-caught specimens were already old and that field

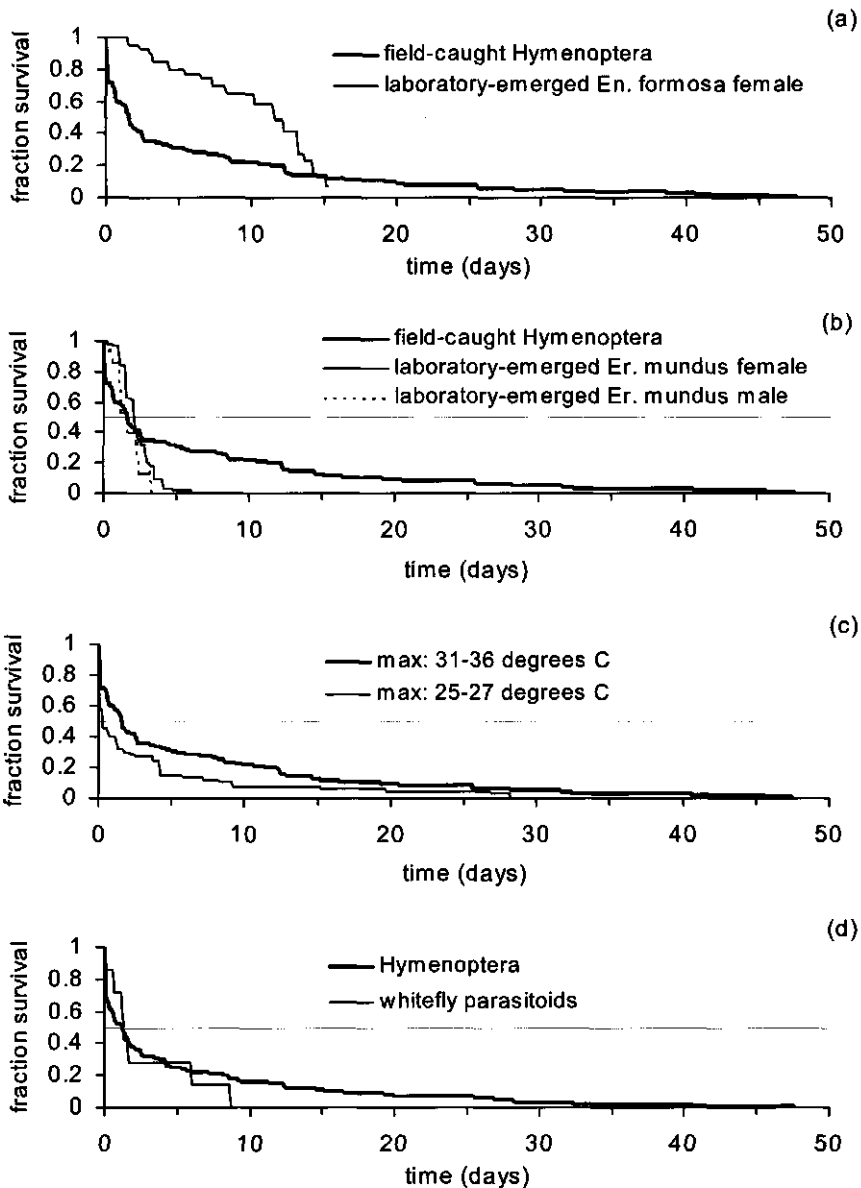


Fig. 3 (a-c) Survival curve of adult Hymenoptera caught in the field and kept on honey in the shade where minimum temperature was about 23 °C and maximum temperatures ranged between 31 and 36 °C (bold solid line; $n=110$) compared with survival curves of (a) adult *Encarsia formosa* females emerged in the laboratory and kept on honey at L:D=35:23 °C (thin solid line; $n=41$), (b) adult *Eretmocerus mundus* females (thin solid line; $n=57$) and males (thin dotted line; $n=15$) emerged in the laboratory and kept on honey at L:D=35:23 °C, and (c) adult Hymenoptera caught in the field and kept on honey in a hole in the ground where maximum temperatures ranged between 25 and 27 °C (thin solid line; $n=67$). (d) Survival curves of all adult Hymenoptera caught in the field (bold solid line; $n=177$) and of female and unsexed whitefly parasitoids only (thin solid line; $n=7$).

conditions do not reduce life expectancy compared with laboratory conditions. However, the difference in median survival time was only minor between field-caught specimens and laboratory-emerged *Er. mundus* females (2.1 days) and males (1.6 days), another whitefly parasitoid species (Fig. 3b). Opposite to Fig. 3a, this suggests that field-caught specimens were still young and that field conditions do reduce life expectancy compared with laboratory conditions.

The exponential distribution in survival of field-caught Hymenoptera and the short longevity of all three control groups could have been the result of high daily maximum temperatures. Although high temperatures could limit life expectancy in the field, parasitoids might profit from cooler microclimates created by plants when evaporating water from their leaves. In Fig. 3c, the survival curve of the first 110 field-caught Hymenoptera that were kept under ambient conditions in the shade (daily maximum temperatures ranging between 31 and 36 °C) is compared with the survival curve of the second 67 field-caught Hymenoptera that were kept in a hole in the ground (daily maximum temperatures ranging between 25 and 27 °C). The reduction in daily maximum temperature did not have a positive effect on survival, nor did it change the shape of the survival curve (Fig. 3c). This suggests that survival at laboratory conditions was not limited by high temperatures.

There is a large interspecific variation in survival even among whitefly parasitoids under controlled conditions (Fig. 3a,b). The field-caught specimens form an even more diverse group belonging to different families, originating from different areas and varying in their biology (Table 2; Hanson & Gauld 1995). The small subsample of 7 female and unsexed whitefly parasitoids does not strikingly deviate in survival from the total sample of 177 field-caught Hymenoptera for which the residual longevity was measured (Fig. 3d).

Discussion

We hypothesised that parasitoid longevity measured in the laboratory overestimates natural life expectancy, because the laboratory is a relatively safe environment that lacks predators or bad weather present in the field. Unfortunately, we could not test this hypothesis because none of the three age determination methods proved a useful technique to estimate the age of a field-caught specimen.

E. formosa did have detectable fluorescent compounds and there was a significant effect of age on fluorescence, but the scatter was too large to be of predictive value, even when temperature and hind tibia length were used as covariates to reduce the error variance. The effect of age-related fluorescent pteridines may be masked by age-unrelated fluorescent pteridines (Mail & Lehane 1988; Tomic-Carruthers *et al.* 1996). Extraction and analysis may destabilise pteridines (Ziegler & Harmsen 1969). Because this depends on pH, buffers with different pH values could be tested. Possibly, we did not use the optimal rotation speed or time because most authors mention rotation speed in revolutions per min without the accompanying diameter of the centrifuge. Because pteridines have a secondary function in light filtering, light regime and light intensity

may affect the rate of accumulation (Lehane & Mail 1985). The light intensity in the climate cabinets was rather low compared with measurements in direct sunlight at noon in February in the Netherlands (52° N) that showed light intensities up to 6 W/m² per nm (J.M.S. Burger, unpublished data). In tsetse flies, fluorescent pigments accumulate in the head while losing them from the abdomen (Msangi & Lehane 1991). Although unlikely, accumulation of pteridines in other parts of the body while losing them from the head could explain the negative effect of age on fluorescence in *E. formosa* heads.

In the laboratory, we did not find any effect of age on the degree of wing fray in *E. formosa*. The degree of wing fray is a standard method of age-grading male tsetse flies and has been applied reliably in a mosquito and blowfly species (Hayes & Wall 1999; Hayes *et al.* 1998). Although wing fray can be correlated to longevity, it is caused by wing movement, predatory attack or abrasion by the habitat (Allsopp 1985). The laboratory conditions were apparently too safe to inflict any degree of wing fray. Stimulation of activity might result in a positive correlation between age and wing fray, although this requires simulation of natural field conditions for calibration of field-caught specimens.

The method of relative residual longevity failed because the number of female whitefly parasitoids in the field sample was too small and no offspring could be obtained as a control. In addition, the large interspecific variation in survival among commercially produced whitefly parasitoids under controlled conditions showed that only intraspecific comparisons should be made. In the field, it is difficult to catch a representative number of alive, adult specimens of a single whitefly parasitoid species. Furthermore, the exponentially distributed survival of the field-caught Hymenoptera and the short longevity of the control groups indicated that laboratory conditions were not as optimal as planned. Because reducing the daily maximum temperature did not increase median survival time nor the shape of the survival curve, parasitoids may have suffered from other negative factors such as a high humidity. Crystallisation of honey was fast in the climate chambers in the Netherlands, but virtually absent in Costa Rica. Kajita (1979) showed that mean longevity of *E. formosa* is about 19 days at 74 % RH but only 2.2 at 100 % RH. Moreover, the method of relative residual longevity would not work at all if laboratory conditions in micro tubes were worse than the field conditions under which a specimen lived before it was captured.

Hayes & Wall (1999) reviewed several alternative age determination methods. In addition, Ellers (1996) showed that fat reserves decreased linearly with age in *Asobara tabida* (Hymenoptera) using the ether extraction method by David *et al.* (1975). Fat reserves also depended on the presence or absence of food, however, which requires knowledge on availability and use by parasitoids of food sources in the field. Desena *et al.* (1999a,b) showed a relationship between cuticular hydrocarbons and age in *Aedes aegypti* (Diptera) using gas chromatography. Heimpel *et al.* (1997b) estimated a predation rate by directly observing *Aphytis* parasitoids (Hymenoptera) in the field. Applying these methods to whitefly parasitoids or improving the methods tested here still require an increased number of whitefly parasitoids caught in the field, which could be achieved using bait traps. Another alternative would be to introduce newly emerged

parasitoids in field cages, although survival and recapture may be difficult to separate in cages large enough to mimic natural conditions.

In conclusion, no suitable age determination method was found for *E. formosa* or whitefly parasitoids in general. As a result, parameter values for life expectancy in the field remain largely hypothetical in models on the trade-off between current and future reproduction.

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Nederlandse inleiding en samenvatting

De etiquette der evolutie: gastheerbehandeling in *Encarsia formosa*, een parasitoïd van wittevlieg

Proloog: het Laatste Oordeel en evolutie door natuurlijke selectie

“Sst! Stilte alstublieft!” Het geroezemoes ebt weg. Maar niet voor lang. De bezoekers kunnen hun opwinding niet voor zichzelf houden. Al snel neemt het gefluister toe tot onorthodoxe hoogten totdat de suppoost de mensen weer tot respectvol gedrag maant. Respectvol want ze staan in de Sixtijnse Kapel in Rome, kijkend naar een van de meesterwerken van de Renaissance dat Michelangelo op de altaarwand schilderde tussen 1537 en 1541. Het fresco beeldt het Laatste Oordeel af, waar een baardloze en gespierde Christus aan het einde der tijden beslist of mensen zich goed of slecht hebben gedragen. Goede mensen gaan naar de hemel, slechte naar de hel. Onder het midden van het schilderij tonen trompetterende engelen etiquetteboeken over hoe je te gedragen om niet voor eeuwig gedoemd te zijn.

Dit proefschrift gaat over etiquette. Echter niet vanuit Christelijk oogpunt maar vanuit een evolutionair standpunt. Het belangrijkste doel van evolutionaire biologie is het verklaren van de aanpassing van vorm, functie en gedrag van organismen aan hun omgeving. Het centrale concept in de evolutionaire biologie werd gepubliceerd door Charles Darwin (1859) in *De oorsprong der soorten door natuurlijke selectie of het behoud van begunstigde rassen in de strijd om het leven*. Het eerste punt in het betoog is dat levende organismen voortplanten en oneindig in aantal zullen toenemen als hulpbronnen (bijvoorbeeld energie, tijd, voedingsstoffen) onbeperkt zijn. Ten tweede produceren zich voortplantende organismen nakomelingen die op hun ouders lijken omdat ten minste sommige kenmerken overerfbaar zijn. Mechanismen van overerving zijn bijvoorbeeld genen of opvoeding. Ten derde verschillen organismen in kenmerken waarvan sommige de overleving en voortplanting beïnvloeden; met andere woorden organismen verschillen in geschiktheid (“fitness”). Darwin redeneerde dat als organismen moeten concurreren om beperkte hulpbronnen, dan zullen organismen met overerfbare kenmerken die het gunstigst zijn voor overleving en voortplanting de meeste

nakomelingen hebben en de nakomelingen zullen die kenmerken ook hebben door overerving. Door dit mechanisme van natuurlijke selectie verandert (evolueert) de frequentie van kenmerken tussen generaties in een populatie van een soort. Darwins evolutietheorie voorspelt dat organismen vervangen zullen worden door organismen met kenmerken die ze beter in staat stelt te overleven en voort te planten in hun omgeving. Zij kan daarom de aanpassing van organismen aan hun omgeving verklaren (binnen fylogenetische, fysische, genetische, fysiologische en ecologische grenzen).

Het Laatste Oordeel illustreert enkele principes van de evolutionaire gedragsbiologie. De stervelingen zijn voor te stellen als de organismen die verschillen in gedrag. Christus zou men kunnen zien als de natuurlijke selectie die het ene type gedrag boven het andere stelt. De etiquetteboeken symboliseren de omgevingsvoorwaarden die de relatieve geschiktheid van de verschillende gedragstypen bepalen. De hemel stelt de omgeving voor waar de geschiktste organismen overleven en voortplanten. Een belangrijk verschil tussen de Christelijke en de evolutionaire benadering van etiquette is dat het Laatste Oordeel aan het einde der tijden plaatsvindt, terwijl evolutie een continu proces is omdat selectiedrukken veranderen met de dynamiek van de fysische en biologische omgeving.

Leven in tijd en ruimte

We zijn gekomen van de geocentrische kosmologieën van Ptolemaeus en zijn voorgangers, via de heliocentrische kosmologie van Copernicus en Galileo, tot het moderne beeld waarin de aarde een middelgrote planeet is die om een gemiddelde ster wentelt in de buitenste buitenwijken van een gewoon spiraalvormig sterrenstelsel, dat zelf slechts een van de duizend miljard sterrenstelsels is in het waarneembare heelal.

Hawking (1996); mijn vertaling

Het doel van wetenschap is het begrijpen van het heelal in ruimte en tijd. Biologie is de wetenschap van het leven. Exobiologie bestudeert de oorsprong, evolutie en verspreiding van het leven in het heelal. De meeste biologen concentreren zich op de aarde, waar het leven ogenschijnlijk aanwezig is, het gemakkelijkst bestudeerd kan worden en inzicht verschaft in onze eigen (menselijke) oorsprong. Het leven zoals wij het kennen is het product van een lange evolutionaire geschiedenis. De leeftijd van het heelal is zo'n tien tot twintig miljard jaar (de tijd die er voor nodig is om van Wageningen naar Rome te reizen bij een snelheid van 0,1 mm per jaar!); onze zon en de planeten die er omheen wentelen vormden zo'n vijf miljard jaar geleden. Het vroegste directe bewijs van leven op aarde komt van drieëneuhalf miljard jaar oude microbiële fossielen die in Noordwest-Australië zijn gevonden. Deze lijken op cyanobacteriën, organismen zonder kernmembraan die net als planten zonlicht als energiebron gebruiken voor het maken van biomoleculen. Cyanobacteriën kunnen tegenwoordig nog steeds gevonden worden in gebieden variërend van hete bronnen tot hete en ijsskoude woestijnen. De fossielen hebben al cellen en dit suggereert dat het leven al aanzienlijk eerder is ontstaan.

De eerste gedetailleerde en toetsbare hypothese over de oorsprong van het leven werd gepubliceerd door Oparin in 1938. Hij stelde voor dat het leven was ontstaan in een oersoep van organische verbindingen die gesynthetiseerd werden in een reducerende atmosfeer van methaan, ammoniak, water en waterstof. Begin jaren '50 circuleerde Miller deze verbindingen langs een elektrische lading (bliksem nabootsend) en identificeerde verschillende aminozuren na een week. Sindsdien heeft de abiotische synthese van de bouwstenen der leven (aminozuren, suikers, vetzuren en nucleotiden) aanzienlijke belangstelling gekregen. Alternatieve bronnen van organische verbindingen op energiebronnen als elektrische ladingen zijn levering door asteroïden- en komeetinslagen of interplanetaire stofdeeltjes, en synthese uit schokgolven bij een inslag. De belangrijkste bron hangt sterk af van de samenstelling van de vroege atmosfeer, welke nog steeds een punt van geschil is.

Bij bijvoorbeeld hoge concentraties van anorganische polyfosfaten kunnen de organische monomeren samenvoegen tot polymeren zoals polynucleotiden en polypeptiden. Deze kunnen als mal dienen voor hun complement. Zo'n vermogen tot zelfvermenigvuldiging is een van de belangrijkste eigenschappen van leven. Toen replicerende moleculen eenmaal waren ontstaan was evolutie door natuurlijke selectie een onvermijdelijk gevolg, resulterend in een toename van moleculaire complexiteit en organisatie zoals in cellulaire organismen.

De theorie der levensgeschiedenis

Een kleine kip legt elke dag, een struisvogel eens per jaar.

Nederlands spreekwoord

De organismen die we vandaag de dag observeren zijn het resultaat van een lange evolutionaire geschiedenis. Natuurlijke selectie wordt gezien als het belangrijkste mechanisme dat de vorm, functie en gedrag van een organisme heeft gevormd, hoewel genfrequenties of kenmerken ook kunnen veranderen door toeval (niet-selectieve krachten als natuurlijke vijanden, rampen of willekeurige genetische drift in stichterpopulaties (het niet-representatieve bevruchte vrouwtje dat op een onbewoond eiland aanspoelt), of seksuele selectie (bijvoorbeeld de staart van een pauw). De theorie der levensgeschiedenis heeft als doel het verklaren van variatie tussen en binnen soorten in de kenmerken van een levensgeschiedenis, zoals groeisnelheid, leeftijd en grootte bij volwassenheid, aantal en grootte van de nakomelingen, verhouding tussen zonen en dochters, en levensduur. De optimale waarde van een kenmerk der levensgeschiedenis is de waarde die de geschiktheid over het hele leven maximaliseert en zal worden bevoordeeld door natuurlijke selectie, waarbij geschiktheid wordt gedefinieerd als de bijdrage van een individu aan de genenverzameling in de volgende generatie. Hulpbronnen die geïnvesteerd worden in een kenmerk dat de geschiktheid doet toenemen kunnen echter niet geïnvesteerd worden in een ander kenmerk dat ook de geschiktheid doet toenemen. Als hulpbronnen beperkt zijn, resulteert dit in een fysiologisch dilemma. Bij sprinkhanen bijvoorbeeld is er een negatief verband tussen het

aantal eitjes en het gewicht van een eitje. Verder kan het veranderen van een kenmerk der levensgeschiedenis dat de geschiktheid doet toenemen, tegelijkertijd een kenmerk der levensgeschiedenis veranderen dat de geschiktheid doet afnemen. Dit resulteert in een microevolutionair dilemma. Seksuele activiteit bijvoorbeeld reduceert de levensduur van mannelijke fruitvliegen. Dit proefschrift gaat over het microevolutionair dilemma tussen nu en straks voortplanten.

Het gebruik van modellen

Net als een fotomodel staat een wiskundig model voor een geïdealiseerde wereld waarin getracht wordt de werkelijkheid tot de kern te reduceren. Wiskundig modelleren is een techniek die kan helpen bij het verklaren en voorspellen van natuurlijke verschijnselen. Optimalisatiemodellen stellen ons in staat om de optimale vorm, functie en gedrag van een organisme te berekenen onder bepaalde beperkingen en hoe een organisme om moet gaan met dilemma's, gegeven dat natuurlijke selectie tot de overleving van de geschiktste leidt. De vier essentiële onderdelen van een optimalisatiemodel zijn een optimalisatiegrootte ("Wat wordt geoptimaliseerd?"), een set strategieën ("Wat is het scala aan beslissingen?"), een toestandsbestek ("Welke interne factoren beïnvloeden de beslissing?") en een set beperkingen ("Welke factoren belemmeren een perfecte aanpassing?"). Stel je bijvoorbeeld je bankrekening voor. Wat zou je moeten doen om je saldo te verhogen: sparen met een zekere rente of beleggen in aandelen voor een onzekere maar mogelijk hogere opbrengst? Je saldo zou de optimalisatiegrootte zijn. De set strategieën bestaat uit sparen en beleggen. Het toestandsbestek zou kunnen bestaan uit het beginsaldo en je afhankelijkheid van het saldo voor levensonderhoud. Beperkingen die je belemmeren oneindig rijk te worden zijn de rentevoet en je sterfelijkheid.

Hoewel levenslange geschiktheid de ultieme optimalisatiegrootte is, nemen de meeste biologen hun toevlucht tot indirecte grootheden zoals lichaamsgrootte, vruchtbaarheid of levensduur. De klassieke optimalisatietheorie optimaliseert gewoonlijk de gemiddelde snelheid van netto energiewinst. Dynamisch toestandsvariabel modelleren omvat de toestandsdynamiek als een vijfde onderdeel van een optimalisatiemodel. Dynamische toestandsvariabele modellen zijn flexibeler dan modellen die opnamesnelheid maximaliseren, en gebruiken het voortplantingssucces tijdens het gehele leven als optimalisatiegrootte, welke nauw gerelateerd is aan fenotypische geschiktheid. Vergeleken met statische optimalisatiemodellen kunnen meer kwantitatieve en toetsbare voorspellingen gedaan worden. Deze techniek heb ik gebruikt om het microevolutionaire dilemma tussen nu en straks voortplanten te modelleren.

Parasitoïde insecten

Parasitoïde insecten zijn een ideaal modelsysteem gebleken voor de gedrags- en evolutionaire ecologie. Hun biologie verschaft een waardevol inzicht in vele aspecten van natuurlijke selectie en aanpassing. Het volwassen vrouwtje bezit een legboor die ze

gebruikt om een of meer eitjes te leggen op of in bepaalde stadia van een andere geleeedpotige, haar gastheer. De larf van de parasitoïd ontwikkelt zich in het lichaam van de gastheer door deze op te eten, waarbij de gastheer uiteindelijk sterft (in tegenstelling tot bij een parasiet). De gedragsbeslissingen die een volwassen vrouwelijke parasitoïd neemt bij het zoeken naar en behandelen van een gastheer hebben een direct effect op haar voortplantingssucces en staan onder sterke selectie. Godfray (1994) bespreekt uitvoerig het theoretische en proefondervindelijke werk aan de gedragsbeslissingen waarmee parasitoïde insecten geconfronteerd worden tijdens het foerageerproces. (Merk op dat door het hele proefschrift heen onder foerageren het zoeken naar zowel voedsel als gastheren wordt verstaan.) Het foerageerproces wordt onderverdeeld in habitatlocatie, gastheerlocatie en gastheeracceptatie. Gedragsbeslissingen die tijdens het foerageren worden gemaakt zijn de beslissing te zoeken naar voedsel of gastheren, waar te foerageren, hoe lang te blijven, een ontmoete gastheer af te wijzen of te accepteren, van een geaccepteerde gastheer te eten of in deze (nogmaals) een eitje leggen, hoeveel eitjes te leggen, en in welke verhouding dochters en zonen te produceren (c.q. een eitje wel of niet te bevruchten). Het microevolutionaire dilemma tussen nu en straks voortplanten wordt weerspiegeld in de beslissing om een gastheer te parasiteren of ervan te eten.

Gastheerbehandeling

Het onderzoek in dit proefschrift richt zich op de beslissing hoe een gastheer te behandelen nadat hij is ontmoet. Een parasitoïd kan een ontmoete gastheer afwijzen, ervan eten of parasiteren. Deze beslissing kan verdeeld worden in de beslissing om een ontmoete gastheer af te wijzen of te accepteren, en de beslissing om van een geaccepteerde gastheer te eten of deze te parasiteren. Het klassieke optimaal-dieetmodel voorspelt dat predatoren (of parasitoïden) de minst winstgevende prooi (of gastheer) zouden moeten afwijzen wanneer haar winstgevendheid (opbrengst per tijdseenheid) onder een zekere drempel valt, onafhankelijk van de ontmoetingssnelheid met de minst winstgevende prooi. Andere factoren die de beslissing om een ongeparasiteerde gastheer af te wijzen beïnvloeden zijn bijvoorbeeld gastheertype-afhankelijke sterfterisico's voor een eileggende parasitoïd door gastheerverdediging of een aanval van een predator, eilimitatie, gastheerherkenningstijd, en de levensverwachting van de parasitoïd. Afwijzing is dus een belangrijke beslissing in de set van strategieën bij het bestuderen van gastheerbehandeling.

De beslissing om van een geaccepteerde gastheer te eten of om deze te parasiteren weerspiegelt het dilemma tussen nu en straks voortplanten. Gastheervoeden is de consumptie van gastheerblood en lichaamsweefsel door de volwassen vrouwelijke parasitoïd. Jervis & Kidd (1986) vatten de taxonomische verspreiding samen van gastheervoeden bij de vliesvleugeligen (o.a. wespen, bijen, mieren), bespraken de fysiologie van gastheervoeden, en bouwden enkele analytische en simulatiemodellen om het effect van gastheerdichtheid te onderzoeken op de beslissing tussen gastheervoeden en eileg. Zij vonden vermeldingen van gastheervoeden in meer dan 140 vliesvleugelige

parasitoïdensoorten (sluipwespen) in 17 families. Het meest voorkomende type van gastheervoeden is niet-concurrerend (verschillende gastheren worden gebruikt voor gastheervoeden en eileg) en destructief (gastheervoeden doodt de gastheer). Na het uitkomen kunnen sommige soorten geen eitjes leggen zonder gastheervoeden vooraf (anautogeen), in tegenstelling tot autogene soorten die dat wel kunnen met gebruikmaking van reserves uit het larvale stadium. Flanders (1950) onderscheidde proövigene soorten, wier vrouwtjes alle eitjes rijpen voordat ze uitkomen, van synovigene soorten, wier vrouwtjes de meeste eitjes rijpen tijdens hun volwassen leven waarbij ze afhankelijk zijn van voedsel, met name gastheervoeden. Jervis *et al.* (2001) lieten zien dat er een continuüm is in de fractie van het maximum potentiële levenslange aantal eitjes dat rijp is bij uitkomen.

Heimpel & Collier (1996) borduurden voort op het werk van Jervis & Kidd (1986) door het bijwerken van hun bespreking over de fysiologie van gastheervoeden, het beschouwen van de kosten en baten van gastheervoeden en het bespreken van de theoretische voorspellingen en proefondervindelijke bewijzen voor strategieën van gastheervoeden. Rosenheim & Heimpel (1994) bespreken ook verscheidene bronnen van variatie tussen en binnen soorten in gastheervoedgedrag van *Aphytis* sluipwespen. Een gastheervoedmaal is rijk aan eiwitten, vitaminen en mineralen, in tegenstelling tot niet-gastheer-voedsel zoals nectar. De verkregen voedingsstoffen zijn vaak onontbeerlijk voor het rijpen van eitjes. Met behulp van radioactieve labels toonden Rivero & Casas (1999b) aan dat de winst uit gastheervoeden wordt opgeslagen en geleidelijk wordt gebruikt tijdens het leven van de sluipwesp. In sommige soorten heeft gastheervoeden ook een positief effect op levensduur, hoewel niet-gastheer-voedsel hiervoor over het algemeen effectiever is. Eitjes die gerijpt worden van de winst uit gastheervoeden kunnen ook weer omgezet worden door resorptie, wat de levensduur kan verlengen. Hoewel de sluipwesp haar eitjesvoorraad kan vergroten door gastheervoeden, resulteert destructief gastheervoeden in het verlies van een mogelijkheid om een eitje te leggen ("nu voortplanten"). Een andere prijs van gastheervoeden is een toename van behandeltijd, wat de zoektijd verkort en het risico van een predatoraanval kan vergroten. De beslissing tussen eileg en gastheervoeden weerspiegelt dus een dilemma tussen nu en straks voortplanten. Sluipwespen realiseren "nu voortplanten" door eileg maar synovigene soorten (met tenminste enkele onrijpe eitjes bij uitkomen) kunnen hun levenslange voortplantingssucces vergroten door te investeren in "straks voortplanten" door middel van gastheervoeden.

Biologische bestrijding en de parasitoïd *Encarsia formosa*

Sluipwespen worden gedefinieerd door het destructieve eetgedrag van de *larven*. Bij soorten die destructief gastheervoeden, eet ook de *volwassen* vrouwelijke sluipwesp van de gastheer. In deze hoedanigheid wordt zij gedefinieerd als predator. Het destructieve eetgedrag van sluipwespen wordt gebruikt om sluipwespen toe te passen in de biologische bestrijding van plaaginsecten. De larvale sluipwesp *Encarsia formosa* is een beroemd voorbeeld van een succesvolle biologische bestrijder van wittevlies in kasteelt.

Wittevliegen zijn geen vliegen maar behoren met de bladluizen tot de gesnauwde insecten. Er zijn zo'n 1450 soorten wittevlieg benoemd waarvan slechts enkele tientallen als mogelijke plaag kunnen worden beschouwd. Wittevliegen veroorzaken schade aan gewassen door zich te voeden met plantensappen, het produceren van honingdauw (suikerwaterachtige poep) en het overdragen van virussen. In 1926 viel een teler de zwarte nimfen op van de gewoonlijk witte nimfen van kaswittevlieg in een kas in Groot-Brittannië. De sluipwespen die uit de zwarte nimfen kwamen werden geïdentificeerd als *Encarsia formosa* door Gahan, die de soort toentertijd zojuist beschreven had aan de hand van exemplaren die in Idaho (VS) waren verzameld. Binnen een paar jaar werd *E. formosa* commercieel geëxploiteerd. Na de Tweede Wereldoorlog werd de gewasbescherming sterk chemisch georiënteerd in de jaren '50 maar de belangstelling voor natuurlijke vijanden herleefde na de ontwikkeling van resistentie tegen pesticiden.

Hoewel Speyer enige biologie and levensgeschiedenis van *E. formosa* beschreef, werden schema's met hoe en wanneer de natuurlijke vijand te introduceren in eerste instantie met vallen en opstaan ontwikkeld. Om duurzaam plaagbeheer en betrouwbaardere introductieschema's te ontwikkelen werd het onderzoek fundamenteeler georiënteerd op de biologie en levensgeschiedenis van *E. formosa* en wittevlieg.

In de jaren '80 werd begonnen met een lange-termijn project dat zich richtte op het verkrijgen van kwantitatief inzicht in het tritrofische systeem van gewas, wittevlieg en *E. formosa* om zo het lukken of mislukken van de biologische bestrijding te verklaren. De biologische bestrijding van kaswittevlieg met *E. formosa* was erg betrouwbaar op tomaat, paprika en augurk maar niet in aubergine en komkommer. Van Roermund vulde belangrijke hiaten in de reeds uitgebreide kennis over het foerageergedrag van *E. formosa*, vatte de levensgeschiedenisparameters van zowel kaswittevlieg als *E. formosa* samen en incorporeerde de gedetailleerde studies in simulatiemodellen om de belangrijkste oorzaken van het lukken of mislukken van biologische bestrijding van wittevliegplagen in kassen met *E. formosa* aan te wijzen.

Doel

De mechanistische modellen van Van Roermund *et al.* verklaren hoe *E. formosa* sluipwespen het waargenomen niveau van parasitering in kassen realiseren in termen van zoek efficiëntie, gastheerbehandeling en beschikbare eitjes. Zij verklaren echter niet waarom dit gedrag is geëvolueerd in evolutionaire termen van selectiedrukken die op gedrag werken. Het foerageergedrag van *E. formosa* onder natuurlijke omstandigheden is nooit bestudeerd, noch de vraag onder welke omstandigheden dit gedrag adaptief is. Functionele verklaringen voor het gedrag van de sluipwesp kunnen populatiedynamische modellen die zich richten op het begrijpen van interacties tussen sluipwesp en gastheer verbeteren. Zowel gastheervoeden als eileggedrag resulteren in wittevliegsterfte maar alleen eileg resulteert in directe nakomelingen. Daardoor kunnen beslissingen van de sluipwesp hoe een gastheer te behandelen belangrijke implicaties hebben voor populatiedynamiek en de uiteindelijke uitkomst van biologische bestrijding. Dit

proefschrift gaat daarom over de functionele verklaring voor gastheerbehandeling door *E. formosa* in aanvulling op de mechanistische verklaringen van Van Roermund.

Het doel van mijn onderzoek dat wordt weergegeven in dit proefschrift is het bevorderen van de gedragsecologie, in het bijzonder de evolutie van gastheerbehandeling in de witevliegparasitoïd *Encarsia formosa*. Voor het toetsen van evolutionaire hypothesen heeft de focus op een vermaarde soort het voordeel dat realistische beperkingen meegenomen kunnen worden in optimaal-foergeermodellen en dat realistische, gedetailleerde en kwantitatieve voorspellingen gedaan en gevalideerd kunnen worden. Het modelleren van foergeerbeslissingen van *E. formosa* wordt vereenvoudigd door een aantal bijkomende voordelen. *E. formosa* legt slechts één eitje in een gastheer. De sluipwesp maakt daarom geen beslissing over de grootte van het eicluster. Bij de meeste parasitaire insecten worden onbevuchte eitjes mannetjes en bevruchte eitjes vrouwtjes. In veel soorten binnen de familie van *E. formosa* (de Aphelinen) kunnen de twee seksen zich slechts op verschillende gastheertypen ontwikkelen, bijvoorbeeld mannetjes die zich ontwikkelen op of in vrouwtjes van hun eigen of andere soort. *E. formosa* zelf plant zich echter zonder seks voort: maagdelijke vrouwtjes produceren dochters uit onbevuchte eitjes. Deze vorm van ongeslachtelijke voortplanting wordt door *Wolbachia* bacteriën geïnduceerd. *E. formosa* maakt dus ook geen beslissingen over de te produceren verhouding zonen en dochters. Tot slot wordt het parametriseren van een model vergemakkelijkt door de gedetailleerde laboratoriumstudies naar de biologie, levensgeschiedenis en foerageergedrag van de sluipwesp.

Opzet en resultaten

Over het algemeen hebben eerdere studies al laten zien dat voedingsstoffen uit een gastheervoeding gebruikt worden om eitjes te rijpen en dat niet-gastheer-voedsel zoals nectar en honing essentiële voedingsstoffen (voornamelijk aminozuren) niet in voldoende mate bevatten. Het zoeken naar nectar heeft als bijkomend nadeel dat het de tijd om naar gastheren te zoeken vermindert. Honing is in het veld niet eens beschikbaar voor een sluipwesp omdat het door bijen wordt geproduceerd en opgeslagen. Honingdauw aan de andere kant kan rijk aan aminozuren zijn en direct gedronken worden uit de anus van de gastheer. Sluipwespen die honingdauwproducerende gastheren aanvallen, zoals *E. formosa*, verliezen met het zoeken naar honingdauw tijd noch energie voor het zoeken naar gastheren. Natuurlijke selectie zou sterk werken tegen individuen die schaarse mogelijkheden tot voortplanting zouden vernielen als een beschikbare non-destructieve voedingsbron een alternatief zou zijn. In de **Hoofdstukken 2 en 3** zocht ik naar een evolutionair voordeel van destructief gastheervoeden ten opzichte van non-destructief voeden met honingdauw in termen van vruchtbaarheid, levensduur en eikwaliteit. In deze laboratoriumexperimenten mochten sluipwespen in aan- of afwezigheid van honingdauw eitjes leggen totdat ze probeerden te voeden van een gastheer waarbij dit in de ene behandeling werd toegestaan en in de andere werd

voorkomen. Deze experimenten borduren ook voort op eerdere studies aan *E. formosa* waarin geen voordeel van gastheervoeden kon worden aangetoond.

In tegenstelling tot mijn verwachting hadden wespen die mochten gastheervoeden geen grotere eitjesvoorraad 20 uur of 48 uur na de poging dan wespen die dat niet mochten (**Hoofdstuk 2**). Gastheervoeden noch de duur van gastheervoeden had een positief effect op het geschatte aantal eitjes wat gedurende deze perioden werd gerijpt. Aan de andere kant had de aanwezigheid van honingdauw wel een positief effect op de eitjesvoorraad en het geschatte aantal eitjes dat was gerijpt. Gastheervoeden had wel een positief effect op de levensverwachting maar dit effect werd tenietgedaan als honingdauw aanwezig was na de poging tot gastheervoeden. Deze resultaten suggereerden dat eten van honingdauw een voordelig alternatief op gastheervoeden zou kunnen zijn, vooral in parasitoïden van honingdauwproducerende insecten. Ik maakte daarom de hypothese dat gastheervoeden onvermijdelijk is om anhydropische eitjes te produceren. Dit zijn eitjes die in tegenstelling tot hydropische eitjes niet meer zwellen na leg. Of dit type voordeliger is dan hydropische eitjes hangt af van omgevingsfactoren (**Hoofdstuk 4**).

Uit de vervollexperimenten waarin wespen over meerdere dagen werden geobserveerd (**Hoofdstuk 3**) bleek dat zelfs in de aanwezigheid van honingdauw, wespen die mochten gastheervoeden meer eitjes per uur foerageren per poging tot gastheervoeden legden dan wespen die niet mochten gastheervoeden. Hoewel wespen die mochten gastheervoeden meer eitjes legden tijdens het experiment verschilden ze niet in levensduur of geschatte verandering in eivolume over tijd van wespen die niet mochten gastheervoeden. Wespen met alleen toegang tot water begonnen snel eitjes te resorberen en gingen allemaal binnen 6 dagen dood. De gemiddelde eitjesvoorraad van wespen met toegang tot voedsel nam in eerste instantie toe tot ongeveer 10 (suikerwater), 12 (honingdauw) en 14 (suikerwater en honingdauw) eitjes op dag 2. Tussen dag 2 en 10 was de netto resorptie ongeveer 0,42 per dag. Deze experimenten toonden aan dat destructief gastheervoeden een voordeel kan hebben ten opzichte van non-destructief voeden met honingdauw, vooral voor door eitjes beperkte sluipwespen. Bij lage gastheerdichtheden zou voeden met niet-gastheer-voedsel voldoende voedingsstoffen kunnen opleveren om eibeperking te voorkomen. Sluipwespen die door tijd of gastheren zijn beperkte zouden kunnen profiteren van gastheervoeden door het resorberen van de extra eitjes om levensduur en dus zoektijd te verlengen, of door de extra eitjes te gebruiken voor het exploiteren van zeldzame gastheerclusters.

Het vertrekpunt voor een functionele verklaring van het gedrag van *E. formosa* zijn de natuurlijke omstandigheden waaronder de sluipwesp geëvolueerd is voordat de soort commercieel geproduceerd werd. Er waren geen gegevens over deze omstandigheden. In **Hoofdstuk 4** werd een kwantitatieve beschrijving gemaakt van natuurlijke wittevliegdichtheden en -verdelingen uit veldwerk in het vermoede oorspronkelijke verspreidingsgebied van *E. formosa*. Wittevliegdichtheden en -verdelingen kunnen belangrijke factoren zijn die het dilemma tussen nu en straks voortplanten beïnvloeden. Van wittevliegdichtheden werd verwacht dat ze aanmerkelijk lager in het veld zijn dan in kunstmatige gewassystemen omdat wittevliegen in het veld te maken hebben met niet-

waardplanten, slecht weer, ziekteverwekkers, predatoren en parasitoïden. Hoewel het onzeker is waar *E. formosa* vandaan komt, besloot ik het veldwerk in Costa Rica te doen omdat (1) morfologie en gegevens over nucleotidevolgorde *E. formosa* in de *luteola* groep plaatsen, welke uit het Amerikaanse continent voortkomt, (2) de intrinsieke groeisnelheid bij 30 °C piekt en loop- en vliegactiviteit nauwelijks worden waargenomen onder de 20 °C, wat een (sub)tropische oorsprong suggereert, en (3) *E. formosa* daar relatief algemeen was blijkens een veldstudie in Florida, het Caribisch gebied en Latijns Amerika. Tijdens twee veldseizoenen in 1999 en 2001 werden bladeren verzameld op verschillende ruimtelijke niveaus in verschillende gebieden en bekeken op wittevlieg nimfen. De ruimtelijke niveaus van bemonsteren werden geïncorporeerd als willekeurige effecten in statistische modellen. Deze modellen maken de simulatie mogelijk van het aantal gastheren op een blaadje dat een willekeurig zoekende sluipwesp als *E. formosa* tegenkomt. Geostatistiek werd gebruikt om de mate en schaal van ruimtelijke afhankelijkheid van gastheren te kwantificeren langs transecten en binnen planten.

Het veldwerk leverde de volgende resultaten op. Op de meeste plekken werd een gemiddelde van 0,5 tot 2 wittevlieg nimfen per blaadje gevonden. Van de willekeurig verzamelde blaadjes was 71 % leeg terwijl sommige enkele tientallen nimfen bevatten. Meestal zat meer dan 95 % van de nimfen op de onderzijde, en was 20 tot 90 % in een stadium van ontwikkeling. In de kroonlaag (21 tot 37 m boven de grond) werd een gemiddelde van slechts 0,06 nimfen per blaadje gevonden, vergeleken met 1,3 op de bosbodem (0 tot 3 m) in hetzelfde gebied. De statistische modellen lieten zien dat het aantal gastheren op de onderkant van een blaadje op een gemiddelde plant binnen een gemiddeld plekje langs een gemiddeld transect beschreven zou kunnen worden door een Poissonverdeling met gemiddelde en variantie gelijk aan 0,241, in een verhouding van 1^{ste}:2^{de}:3^e:4^e stadium = 0,14:0,23:0,26:0,37. Het gemiddeld aantal gastheren werd enorm beïnvloed door de plant en minder door de plek of transect. De variatie tussen planten kon nauwelijks verklaard worden door variatie in bladgrootte. Op basis van de vorm van de opening in lege pophuidjes was de kans dat een wittevlies uiteindelijk geparasiteerd werd 12 % op een gemiddeld blaadje. Parasitering was meer geclusterd tussen plekken maar gelijkmatiger verdeeld over planten binnen plekken dan wittevlieg nimfen. In 1 van de 3 korte transecten waren de aantallen wittevlieg op blaadjes ruimtelijk afhankelijk. Dit was niet het resultaat van geclusterde waardplanten omdat het in 1 van de 2 transecten was waar bladeren uit een continue ondergroei van een enkel waardplantengeslacht waren verzameld. In 4 van de 7 driedimensionale vakken kon tenminste één niveau van ruimtelijke afhankelijkheid worden vastgesteld; 3 van de 4 binnen een enkele waardplant. In een vak waren clusters van ongeveer 16 cm genesteld binnen clusters van ongeveer 37 cm. In de andere drie vakken werden vergelijkbare clustergrootten van 41, 20 en 15 cm afzonderlijk gevonden. De resultaten van dit veldwerk toonden aan dat wittevlieg dichtheden gemiddeld laag zijn maar dat wittevlieg op verscheidene ruimtelijk niveaus samentreft.

In **Hoofdstuk 5** werd een dynamisch toestandsvariabel (DTV) model ontwikkeld om optimale beslissingen hoe een gastheer te behandelen te voorspellen met gebruik van

kennis uit eerdere hoofdstukken en beschikbare literatuur. Ik veronderstelde dat de functie van gastheervoeden het verkrijgen van voedingsstoffen is die gebruikt kunnen worden voor het rijpen van eitjes, dat eirijping continu is en afhangt van de eitjesvoorraad, dat de overleving van de sluipwesp exponentieel verdeeld is, en dat sluipwespen hun gastheren willekeurig ontmoeten, autogeen zijn en onbeperkt toegang hebben tot niet-gastheer-voedsel voor het verkrijgen van energie voor onderhoud en activiteit. Ten eerste werd de optimale beslissing om de gastheer af te wijzen, er van te voeden of er een eitje in te leggen berekend met behulp van stochastisch dynamisch programmeren, gegeven de toestand van de sluipwesp (eitjesvoorraad en energieniveau), haar leeftijd en het gastheertype dat ze ontmoet. De optimale beslissing is de beslissing die het hoogste verwachte voortplantingssucces oplevert. Ten tweede werd het leven van een cohort sluipwespen gesimuleerd met behulp van de Monte Carlo aanpak om toetsbare voorspellingen te doen, zoals de optimale fractie gastheren waarvan per dag gevoed zou moeten worden. Beslissingen van gastheerbehandeling werden bestudeerd onder zes scenarios met behulp van gastheerdichtheid en levensverwachting van de sluipwesp. Van deze parameters werd verwacht dat ze een belangrijk effect hebben op de waarde van "straks voortplanten" en hun schattingen verschilden aanmerkelijk tussen laboratorium- en veldomstandigheden.

De belangrijkste voorspelling van het model is dat gastheervoeden maladaptief is onder de natuurlijke omstandigheden van lage gastheerdichtheid (0.015 cm^{-2}) en korte levensverwachting (gemiddelde levensduur van sluipwesp ongeveer 3 dagen). Voedingsstoffen uit het larvale stadium zijn toereikend om beperking van eitjes te voorkomen. Zowel gastheerdichtheid als de levensverwachting van de sluipwesp hebben inderdaad een positief effect op de optimale fractie gastheervoeden. Wespen die willekeurige beslissingen maken behalen slechts 35 tot 60 % van het levenslange voortplantingssucces van wespen die optimale beslissingen maken, afhankelijk van gastheerdichtheid. Elasticiteitsanalyses lieten zien dat het verband tussen gastheerdichtheid en de fractie acceptatie het gevoeligst is voor de relatieve behandeltijd van gastheervoeden en de temperatuur waarbij eirijping plaatsvindt. Het verband tussen gastheerdichtheid en de fractie gastheervoeden is het gevoeligst voor de relatieve behandeltijd van gastheervoeden en de opbrengst uit voeden met de slechtste gastheer. Parameters die een grote invloed hebben op het levenslange voortplantingssucces en dus geschiktheid zijn de levensverwachting van de sluipwesp en de overlevingskans van eitjes na leg (onafhankelijk van gastheerdichtheid), de ontmoetingskans op gastheren en lengte van actieve periode (bij lage gastheerdichtheid) en de eirijpingssnelheid en aantal gastheertypen (bij hoge gastheerdichtheid). De evolutie van gastheervoeden zou verklaard kunnen worden als de variatie in gastheerdichtheid of als geparasiteerde gastheertypen ingebouwd zou worden, of als er veldgegevens zouden zijn die aantoonen dat levensverwachting in het veld minder kort dan verondersteld is. Het inbouwen van variatie in loopsnelheid, resorptie of (snelle) afwijzing met de antennes zal waarschijnlijk geen verklaring kunnen geven voor de evolutie van gastheervoeden onder natuurlijke omstandigheden.

In **Hoofdstuk 6** toetste ik de voorspelling van het DTV model (Hoofdstuk 5) dat gastheervoeden maladaptief is onder de natuurlijke omstandigheden van lage gastheerdichtheid en korte levensverwachting. Verder toetste ik of het geclusterd voorkomen van gastheren een positief effect had op het gastheervoedgedrag, omdat deze ruimtelijke variatie in het veld was gevonden maar was genegeerd in het DTV model. Ten derde kwantificeerde ik de effecten van gastheerverdeling en sluipwespervaring op de verblijftijd van de sluipwesp op blaadjes en toetste of sluipwespen leerden om sneller weg te gaan na een gastheerontmoeting bij een uniforme gastheerverdeling dan bij een geclusterde gastheerverdeling. Dergelijk flexibel gedrag kan adaptief zijn in een omgeving zoals beschreven in Hoofdstuk 4, waar gastheerdichtheid varieert op een voorspelbare manier door het samentreffen van gastheren. Sluipwespen werden individueel geobserveerd in een driedimensionale opstelling van 21 blaadjes voor 10 uur per dag gedurende 6 dagen. In de ene behandeling bevatte elk blaadje 1 gastheer, overeenkomend met de gemiddelde gastheerdichtheid in het veld. In de andere behandeling bevatten blaadjes gemiddeld 1 gastheer maar de natuurlijke variatie werd geïncorporeerd door het exacte aantal te simuleren met het statistische model uit Hoofdstuk 4.

Gemiddeld ontmoetten wespen ongeveer 12 gastheren per dag in beide behandelingen en voeden ze ongeveer een keer per dag van een gastheer (11 % van de geaccepteerde gastheren). Een statistisch model liet zien dat na een dag of vier sinds het begin van de observatie de neiging tot gastheervoeden bij geclusterde gastheerverdeling de neiging tot gastheervoeden bij gelijkmatige verdeling oversteeg, onafhankelijk van de tijd sinds de laatste gastheeracceptatie. Een ander statistisch model liet zien dat gastheerontmoeting de neiging om een blaadje met 1 gastheer te verlaten deed *afnemen* als de tijd sinds de laatste gastheerontmoeting *kort* was, maar deed *toenemen* als de tijd sinds de laatste gastheerontmoeting *lang* was, onafhankelijk van gastheerverdeling. De tijd sinds de start van de observatie had een positief effect op de vertrekneiging. Deze resultaten suggereerden dat Skellams formule waarmee in het DTV model ontmoetingssnelheid werd berekend uit gastheerdichtheid (gemiddeld 1,4 ontmoeting per dag) de ontmoetingssnelheid onderschat. Hoewel variatie in gastheerdichtheid in de loop der tijd een positief effect op de neiging tot gastheervoeden had, lijkt het aanpassen van Skellams formule minstens zo belangrijk als het inbouwen van variatie in gastheerdichtheid. Verder gebruikt *E. formosa* de tijd sinds de laatste ontmoeting om te kiezen tussen blijven en weggaan na een ontmoeting met een gastheer. Dit maakt het voor de wesp mogelijk efficiënt te foerageren in zowel een omgeving met een gelijkmatige gastheerverdeling als in een omgeving met een geclusterde gastheerverdeling.

De waarde van "straks voortplanten" en dus de optimale strategie van gastheerbehandeling hangt sterk af van de levensverwachting van de sluipwesp. Er waren gegevens beschikbaar over de levensduur van *E. formosa* onder laboratoriumomstandigheden maar niet onder natuurlijke veldomstandigheden. Van predatoren, slecht weer of voedselgebrek werd verwacht dat ze de levensverwachting van de sluipwesp in het veld aanmerkelijk reduceren vergeleken met die in het relatief

veilige laboratorium. In de **Appendix** werden meerdere methodes getoetst om de leeftijd te bepalen van een in het veld gevangen sluipwesp om zo de levensverwachting van *E. formosa* en andere parasitoïden van wittevlies in het veld te kwantificeren en de hypothese te toetsen dat levensverwachting in het veld korter is dan in het laboratorium. Helaas bleek geen enkele techniek geschikt. *E. formosa* had wel meetbare fluorescerende bestanddelen en er was een effect van leeftijd op de mate van fluorescentie maar de ruis was te groot om van voorspellende waarde te zijn, zelfs wanneer temperatuur en wespgrootte als covariabelen werden meegenomen ter vermindering van de ruis. Bovendien werd fysiologisch gezien geen negatief maar positief verband verwacht. Er was geen effect van leeftijd op de mate van vleugelbeschadiging, waarschijnlijk omdat onder laboratoriumomstandigheden geen vleugelbeweging, predatoraanvallen of beschadiging door de habitat voorkwam. De methode van relatieve restleeftijd was ook ongeschikt omdat het aantal vrouwelijke parasitoïden van wittevlies in het veldmonster te klein was, geen nakomelingen als controle verkregen konden worden en er zelfs onder gecontroleerde omstandigheden een grote variatie tussen soorten was in overleving van commercieel geproduceerde parasitoïden van wittevlies. Ik heb dus geen bruikbare methode voor leeftijdsbepaling gevonden voor *E. formosa* of parasitoïden van wittevlies in het algemeen. De parameterschattingen voor levensverwachting in het veld blijven dus voornamelijk hypothetisch in modellen van het dilemma tussen nu en straks voortplanten.

De belangrijkste conclusie (**Hoofdstuk 7**) uit mijn onderzoek naar gastheerbehandeling in de parasitoïd *Encarsia formosa* is dat het gastheervoeden geëvolueerd zou kunnen zijn als een aanpassing aan een ruimtelijk en mogelijk temporeel heterogeen milieu. Het gedrag van de wesp geeft de wesp de mogelijkheid om om te gaan met variatie in gastheerdichtheid en -verdeling. Ik heb laten zien dat destructief gastheervoeden een evolutionair voordeel heeft over het non-destructief voeden met honingdauw. Wespen kunnen de winst uit gastheervoeden gebruiken om hun eilegsnelheid te verhogen. Dit kan voordelig zijn als wespen clusters met hoge gastheerdichtheden tegenkomen. Hoewel de gemiddelde gastheerdichtheid in het veld laag was vond ik verscheidene indices voor het samentreffen van gastheren. Eerdere studies wezen uit dat *E. formosa* geen gebruik maakt van de vluchtige stoffen die vrijkomen wanneer planten worden aangevallen door herbivoren zoals wittevlies. Hoewel deze stoffen zouden kunnen helpen om gastheren op afstand op te sporen, is het aantal gastheersoorten dat *E. formosa* aanvalt of het aantal plantensoorten dat haar gastheren aanvallen waarschijnlijk te groot voor een betrouwbare afstemming. Daardoor ervaren wespen waarschijnlijk lage gastheerdichtheden bij het verlaten van een geëxploiteerd cluster van hoge gastheerdichtheid. Het verlaten van een cluster van hoge gastheerdichtheid voordat het volledig geëxploiteerd is kan ook een adaptieve strategie zijn om de kans op hyperparasitisme (parasitering van parasitoïd door hyperparasitoïd) te verkleinen. Tijdens dergelijke karige periodes loont het om in anhydropie eitjes geïnvesteerd te hebben. Sluipwespen met hydropie eitjes hoeven zich niet destructief te voeden met gastheren en kunnen snel veel eitjes leggen maar sluipwespen met anhydropie eitjes kunnen eitjes resorberen. Dit geeft hun de mogelijkheid de

voedingsstoffen anders te gebruiken voor bijvoorbeeld onderhoud wanneer gastheren schaars zijn. De resultaten van het laatste experiment suggereerden dat wespen een vertrekmechanisme gebruiken dat gebaseerd is op de tijd sinds de laatste ontmoeting met een gastheer. Dit vertrekmechanisme en anhydrophe eitjes waarvoor destructief gastheervoeden nodig is maakt wespen flexibel en geeft ze de mogelijkheid om efficiënt bij verschillende gastheerverdelingen te foerageren.

Om de evolutie van gastheerbehandeling werkelijk te begrijpen liggen de belangrijkste hiaten in onze kennis bij het begrijpen van de fysiologische, biochemische en genetische mechanismen; het effect van ruimtelijke en temporele heterogeniteit in beschikbaarheid van gastheren op het risico van eilimiatie; en de levensverwachting van de sluipwesp in het veld. Volwassen vrouwelijke sluipwespen kunnen voedingsstoffen halen uit reserves van het larvale stadium, uit voeden met gastheerbloed, honingdauw en nectar en uit eiresorptie. Sluipwespen hebben voedingsstoffen nodig voor levensonderhoud, activiteit, eirijping en overleving; sommige voedingsstoffen die in eitjes worden geïnvesteerd verhogen mogelijk ook de levensvatbaarheid van de eitjes. Om dit complex van vraag en aanbod te begrijpen zijn in de eerste plaats meer kwalitatieve en kwantitatieve biochemische analyses nodig van de samenstelling van de verschillende voedselbronnen. Radioactief merken van voedingsstoffen kan laten zien hoe voedingsstoffen worden verdeeld over de verschillende organen en gebruikt worden in de tijd. Verder zijn veldgegevens nodig om de beschikbaarheid van voedselbronnen, de mogelijkheid voor wespen om ze te benutten en de temporele veranderingen in gastheerdichtheid en -verdeling te kwantificeren. Modellen zouden ontwikkeld moeten worden met het complex van vraag en aanbod van voedingsstoffen en de heterogeniteit in gastheerbeschikbaarheid om een parameterruimte te definiëren waarin gastheervoeden onder natuurlijke omstandigheden een adaptief kenmerk is.

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De kans om een eerste kruimeltje van de wetenschappelijke taart te proeven werd geboden door mijn promotoren Joop en Louise. Ik was erg gelukkig met de vrijheid waarmee ik mijn nieuwsgierigheid mocht loslaten op pure vragen zonder de hete adem in mijn nek van een op introductieschema's wachtende commerciële instelling. Verder was de combinatie van model-, lab- en veldwerk natuurlijk de ideale vorm voor een boeiend project waarin ik mijn intellect en creativiteit kon toetsen. Dat was voor mij een van de grote aantrekkingskrachten van het oorspronkelijke projectvoorstel, naast de fundamentele vraagstelling. Na afgrijselijke vakken als "Landbouworientatie" op de toenmalige Landbouwuniversiteit waren mensen als Louise, Jan Osse (Diermorphologie) en H.C.D. de Wit (Plantentaxonomie) degenen die mijn interesse voor fundamenteel biologisch onderzoek bevestigden en tot bloei brachten. Louise, jouw bruisende enthousiasme voor de ecologie werkt super motiverend. Verder heb je me vaak uit de diepte gesleurd om me ook op de horizon te wijzen. Joop, wij hebben elkaar pas tijdens het promotieonderzoek leren kennen. Het kostte me wat tijd om jouw efficiëntie bij te houden maar inmiddels begrijp ik wat ento zo'n productief en dynamisch lab maakt. Jouw kennis van het systeem en kritische geest waren van onschatbare waarde. Je was altijd erg snel in het becommentariëren van mijn manuscripten. Joop, Louise: bedankt!

Voor de meer directe en praktische begeleiding was ik veelal op mijn copromotor Lia aangewezen. Lia, bij jou moet ik meteen aan dat wonderlijke APL denken, waarmee je in vijf coderegels de hele wereld lijkt te kunnen beschrijven. In Pascal heb ik wat meer nodig maar dat begrijp ik tenminste. Je hebt me erg geholpen met het dynamisch programmeren, het vertalen van mijn biologische ideeën naar wiskundige formuleringen en het analyseren van het laatste experiment. Hartelijk dank daarvoor!

Entomologie was een inspirerend lab met veel jonge en gedreven mensen uit allerlei windrichtingen werkend aan even zo vele systemen. Ik heb veel geleerd van met name Matthijs, Bregje, Ties en Remco. Ook de aio- en parasietenlunches waren leerzame bijeenkomsten. Voor praktische vragen over wittevlies en *Encarsia* kon ik vaak bij Antoon, Yu Tong en Yvonne terecht. Frans, André en Leo verzorgden de wittevliegweek, wat mij veel tijd heeft opgeleverd. Richard organiseerde interessante seminars. Jetske bedacht en organiseerde met Frodo en Linde een boeiende en gezellige aio excursie door Duistland. Yde prepareerde de wittevliegen en determineerde de wespachtige creatuurtjes uit Costa Rica. Daarnaast zorgde hij als hoofd van de feestcommissie voor veel sociale evenementen op Entomologie rondom het jaarlijkse labuitje, met zeer geslaagde culturele avonden, sinterklaasmiddagen, en met Tibor

BBQ's in de proefvelden rond Wageningen. Kamergenoten Olga, Sara en Yu Tong hielden mij scherp door mij continu te verleiden tot koffie- of theeverslaving (wat is er toch zo "ongezellig" aan water?). Statistische ondersteuning en onderricht kwamen van Gerrit, onmisbare administratieve ondersteuning kwam van Ans, Ineke, Sabine, Alex, Wilma, Riet en Truus. Medewerkers van Tupea zorgden voor professioneel systeembeheer. Dick, Herman, Wouter en Henk hielpen bij allerlei ditjes en datjes. Dank aan allen plus iedereen die ik niet expliciet genoemd heb!

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Quisiero agradecer la gente en Costa Rica quien he encontrado durante mi trabajo de cambio. Gracias especial a Paul Hanson y Carolina Godoy para toda ayuda y espacio de trabajo, familia Arce-Hernandez y familia Tencio-Castro en Heredia y familia Hauter en La Angostura para la hospitalidad formidable y la gente de la Angostura para el tiempo "pura vida". Perdóneme mis faltas en español, pero es que hace alguno tiempo y escribir es todavía más difícil que hablar.

Koos, Bregje, Erik, Remco, Jetske, Miranda, Dennis, Sander, Marjon, Bart, Kamiel, Froukje, Wiesje, Jaap, Luca, Hans, Els, Rolf, Wendie en Arnold: bedankt voor de leuke uitjes, excursies en jullie vriendschap. Alessandro en leden van het Helikon, Bosch Blazers Ensemble, WSKOV en HWSO: bedankt voor de muzikale afwisseling en het warme kippenvel.

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Tot slot bedank ik Cindy, wiens hartelijkheid, optimisme, eigenwijsheid en liefde mijn buik kriebels en mijn leven een extra dimensie geven.

Curriculum vitae

Nederlands



Op 19 november 1973 werd ik als Jozef Martinus Stephanus (Joep) Burger geboren te Venray. Na het behalen van mijn VWO diploma in 1992 aan het Boschveldcollege te Venray begon ik mijn studie Biologie aan de toenmalige Landbouwniversiteit Wageningen. In september 1993 haalde ik mijn propedeuse waarna ik voor de specialisatie Populatie & Ecosysteem koos. Tijdens mijn eerste afstudeerproject bij Matthijs Vos en Louise Vet vergeleek ik verblijftijden tussen een generalistische en een specialistische *Cotesia* sluipwespsoort met behulp van elektronische gedragsregistratie in een semi-veld situatie. Voor mijn tweede afstudeerproject woonde ik met studiegenoot Bart Hoorens zes maanden in Burkina Faso (West-Afrika). Het aldaar uitgevoerde veldwerk onder begeleiding van Max Rietkerk en Pieter Ketner bevestigde het bestaan van patchiness in de vegetatie op verschillende schaalniveaus, zoals voorspeld in een model voor herbivoor-plant-bodem interacties in de Sahel. In een derde afstudeerproject bij Harmke van Oene simuleerde ik effecten van grote herbivoren op de vegetatiesuccessie van de Veluwe en deed ik ervaring op met gevoeligheidsanalyse, kalibratie en validatie van complexe modellen. Tot slot liep ik drie maanden stage bij Bill Murdoch en Sue Swarbrick aan de Universiteit van Californië in Santa Barbara (VS) waar ik gedragsbeslissingen van *Aphytis* sluipwespen in het laboratorium vergeleek met het gedrag in een citrusboomgaard. In september 1997 studeerde ik af.

Van november 1997 t/m mei 2002 was ik aangesteld als eerste-geldstroom onderzoeker in opleiding bij het Laboratorium voor Entomologie van Wageningen Universiteit. Onder begeleiding van Joop van Lenteren, Louise Vet en Lia Hemerik werkte ik aan het in dit proefschrift beschreven onderzoek naar de evolutie van gastheerbehandeling in de sluipwesp *Encarsia formosa*. Hiertoe deed ik manipulatie-experimenten naar de evolutionaire voordelen voor de sluipwesp van destructieve gastheerpredatie, maakte een kwantitatieve beschrijving van de natuurlijke habitat van de sluipwesp tijdens twee veldwerkperiodes in Costa Rica, ontwikkelde een optimaal-foerageermodel, en observeerde het gedrag en reproductieve succes van de sluipwesp onder semi-veldomstandigheden ter validatie van en aanvulling op het model.

English

On 19 November 1973 I, Jozef Martinus Stephanus (Joep) Burger, was born in Venray, the Netherlands. After I graduated secondary school in 1992 at Boschveld in Venray, I studied Biology at the then Wageningen Agricultural University, the Netherlands. I obtained my BSc in September 1993 after which I chose the specialisation Population & Ecosystem. During my first project with Matthijs Vos and Louise Vet I compared residence times between a generalist and specialist *Cotesia* parasitoid species using electronic behaviour recording in a semi-field set-up. For my second project I lived six months with fellow student Bart Hoorens in Burkina Faso (West Africa). The fieldwork there under the supervision of Max Rietkerk and Pieter Ketner confirmed the existence of vegetation patchiness at several spatial scales, as predicted by a model on herbivore-plant-soil interactions in a semi-arid African grazing system. In a third project with Harmke van Oene I simulated the effects of large herbivores on vegetation succession at Veluwe National Park and learned about sensitivity analysis, calibration and validation of complex models. Finally, I spent an internship with Bill Murdoch and Sue Swarbrick at the University of California Santa Barbara (USA) where I compared laboratory results on behavioural decisions in *Aphytis* parasitoids with the behaviour in a citrus orchard. In September 1997 I obtained my MSc.

From November 1997 through May 2002 I was appointed as a PhD student at the Laboratory of Entomology of Wageningen University, the Netherlands. Under the supervision of Joop van Lenteren, Louise Vet and Lia Hemerik I did research described in this thesis on the evolution of host-handling behaviour in the whitefly parasitoid *Encarsia formosa*. I performed manipulation experiments to study the evolutionary advantages to the parasitoid of destructive host feeding, made a quantitative description of the natural habitat of the parasitoid during two field seasons in Costa Rica, developed an optimal-foraging model, and observed the behaviour and reproductive success of the parasitoid under semi-field conditions to validate and complement the model.

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