Reproductive Biology of *Eretmocerus warrae* Naumann and Schmidt (Hymenoptera: Aphelinidae)

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Abstract

Eretmocerus warrae Naumann and Schmidt is a thelytokous and specialist parasitoid of the Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). Prior to this research, little information was available on its reproductive biology. Emergence of *E. warrae* occurs exclusively during the photophase and peaks during the first few hours of the photophase and then decreases rapidly afterwards. However, *E. warrae* adults remain active throughout 24 hours with host feeding peaking between 4 and 6 h and oviposition peaking between 10 and 14 h after lights on. With the increase of temperature from 15 to 30°C the average daily host feeding rates increase while the pre-oviposition period, longevity of adults and developmental period of immatures decrease. The maximum fecundity and host feeding of adults, and survival and emergence of offspring occur at 20 and 25°C. *E. warrae* successfully parasitises and feeds on all four nymphal instars of the greenhouse whitefly but eggs laid under the third to fifth stages of the fourth instar nymphs fail to complete development. *E. warrae* prefers the second and third instar nymphs for feeding and oviposition with higher survival rate of offspring. However, with the increase in host stage the parasitoid offspring gain more fitness with larger body size, higher egg load and longer longevity. Experienced females can discriminate between the parasitised and unparasitised hosts and avoid to superparasitise them when host density is high. However, the naïve females frequently lay eggs under the parasitised hosts. When initially deprived of food and hosts for 5 hours, *E. warrae* increases host feeding, fecundity and longevity. Females carry some mature eggs in their ovaries at emergence, and hence this is a pro-synovigenic species. Food and host deprived females can maintain eggs for up to two days while honey-fed females can keep eggs for up to 5-7 days. With the increase of host density the fecundity, parasitism, host feeding and longevity in *E. warrae* increase while the proportion of hosts fed on, parasitised and superparasitised decreases. Superparasitism increases with the increase in parasitoid density. Parasitoids emerging from singly parasitised hosts are larger, carry higher egg load and live longer than those from superparasitised hosts. Honey-fed parasitoids live 4-5 folds longer than those not provided with food or hosts after emergence. Honey-and host-fed parasitoids also live longer than those provided with hosts only. The findings from this study in relation to biological control of *T. vaporariorum* using *E. warrae*, e.g., mass-rearing or field release, are discussed.

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Chapter 1

General Introduction

1.1. Introduction

Whiteflies (Homoptera: Aleyrodidae) are highly polyphagous insect pests and feed on almost any terrestrial plant (van Lenteren et al. 1996). The most important pest species are the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and sweet potato whitefly, *Bemisia tabaci* (Gennadius), causing serious economic damage to agronomic, horticultural, and ornamental crops throughout warm regions and glass houses in temperate regions of the world (Byrne et al. 1990). In tropical regions with elevation above 500 m, *T. vaporariorum* is more common, whereas *B. tabaci* tends to be the predominant species below 500 m (Smith et al. 2000). *T. vaporariorum* was first found in the greenhouses in UK in 1856 and described in that year by Westwood (van Lenteren et al. 1996).

After World War II, newly introduced insecticides provided pest control on most greenhouse crops. However, within a few years, signs of pest resistance to a number of pesticides were observed, and interest in whitefly parasitoids increased in early 1970s (van Lenteren et al. 1996). It is well known that whitefly nymphs are sessile and susceptible to parasitism (Gerling et al. 1990); therefore, *T. vaporariorum* has been successfully managed in glasshouse systems with parasitoids (Vet et al. 1980). There are many species of parasitic wasps that attack whiteflies, including 34 from *Encarsia*, 14 from *Eretmocerus*, and several species from *Amitus* and *Metaphycus* (Zheng et al. 2005).

Eretmocerus spp. (Hymenoptera: Aphelinidae) are solitary and specialist parasitoids of whiteflies. Females deposit eggs between the venter of the host nymphs and the leaf surface. The early developmental stages of this genus are ectoparasitic while their later larval stages grow within the whiteflies (Gerling et al. 1998). Now there are about 65 *Eretmocerus* species described worldwide, of which 16 have been reported from *B. tabaci* and 6 from *T. vaporariorum* (Zolnerowich & Rose 2008). *E. eremicus* Rose and Zolnerowich is a successful biocontrol agent of the *B. tabaci* complex (Heinz & Parrella 1998; Hoddle et al. 1998), which primarily attacks *B. argentifolii* Bellows and Perring (Headrick et al. 1997, 1999). It was previously known

as *E. californicus* Howard, *E.* sp. nr. *californicus* or *E. haldemani* Howard (Rose & Zolnerowich 1997a). This parasitoid is native to southwestern deserts of California and Arizona in the United States (Rose & Zolnerowich 1997b; Headrick et al. 1997; Hoddle et al. 1998). It is being mass reared routinely on *T. vaporariorum* (Hoddle et al. 1999). However, it can also parasitise other whiteflies such as *T. abutiloneus* Haldeman and *B. argentifolii* (Hoddle et al. 1999).

Success of *Eretmocerus* spp. in the control of *T. vaporariorum* has been attributed largely to their high effectiveness in locating host patches and high parasitism rates (van Lenteren et al. 1996) and the short period required to commence oviposition after emergence (Qiu et al. 2004). *Eretmocerus* spp. can parasitise all immature stages of whiteflies except the egg but they prefer the second instar nymphs for oviposition (Headrick et al. 1995, 1996; Hoddle et al. 1999). Adult parasitoids can also kill whiteflies by probing the nymphs using ovipositors and consuming the haemolymph that subsequently exudes from the nymphs (Headrick et al. 1995). Host feeding is considered to be the most effective cause of the suppression of whitefly populations (Hoddle et al. 1999).

The New Zealand strain of *Eretmocerus warrae* Naumann and Schmidt is a thelytokous species. It was first found in greenhouses in Auckland, New Zealand, in 1997. Ten years later, this species was identified as *E. warrae* using DNA sequencing (Workman et al. 2008). During the present study, adult wasps were sent to the Natural History Museum, London, for identification, and were confirmed as *E. warrae* (A. Polaszek, pers. comm.).

1.2 Importance of whiteflies

In the late 1970's, 1156 species in 126 genera were recorded in the catalogue of the whiteflies of the world (Mound & Halsey 1978). The number of species and genera has been continuously increasing with 1556 species in 161 genera recorded in 2007 (Martin & Mound 2007) and 1551 species in 166 genera in 2008 (Evans 2008). They belong to three living subfamilies (Aleurodicinae, Aleyrodinae and Udamosellinae), and one fossil subfamily (Bernaeinae) (Martin & Mound 2007; Evans 2008). Only two whitefly species, *T. vaporariorum* and *B. tabaci*, occur in protected agriculture (Byrne et al. 1990).

Trialeurodes vaporariorum is one of the most important pests of glasshouse crops in many countries (Byrne et al. 1990). It can damage the plant by direct feeding, honeydew (excreta) contamination, and transmission of viral pathogens (Byrne & Bellows 1991). Some important diseases transmitted by the greenhouse whitefly are tomato infectious chlorosis virus (TICV), tomato chlorosis virus (ToCV), strawberry pallidosis associated virus (SPaV) and beet pseudo yellow virus (BPYV) (Jones 2003). Duffus (1965) also mentioned various virus diseases of cucumber, lettuce and ornamental crops transmitted by the greenhouse whitefly.

1.2.1 Host plants

Trialeurodes vaporariorum has been recorded to attack 72 genera in 35 families of plants (Table 1.1; Evans 2007).

Table 1.1 The host plants of *T. vaporariorum*

Family	**genera**
Alstroemeriaceae	*Alstroemeria* sp.
Apocynaceae	*Fernaldia pandurata*
Asclepiadaceae	*Gonolobus* sp.
Asteraceae	*Helianthus annuum*, *Ageratum* sp., *Arctium* sp., *Artemisia dracunculus*, *Aster ericoides*, *Sonchus olearaceus*, *Callistephus* sp., *Chrysanthemum* sp., *Dahlia* sp., *Dendranthema* sp., *Gerbera* sp., *Helichrysum* sp., *Lactuca serriola*, *Solidago* sp., *Solidaster* sp. and *Xanthium occidentale*
Bromeliaceae	*Ananas comosus*
Caryophyllaceae	*Dianthus* sp.
Chenopodiaceae	*Chenopodium ambrosioides*
Cucurbitaceae	*Cucumis sativus*, *Sechium edule* and *Citrullus colocynthis*
Ericaceae	*Rhododendron* sp.

Euphorbiaceae	*Manihot esculenta* and *Euphorbia geniculata*
Gentianaceae	*Gentiana* sp. and *Lisianthus* sp.
Geraniaceae	*Pelargonium* sp.
Goodeniaceae	*Scaevola* sp.
Guttiferae	*Hypericum* sp.
Iridaceae	*Crocosmia* sp. and *Iris* sp.
Labiatae	*Mentha* sp., *Ocimum* sp., *Origanum vulgare, Lavandula* sp., *Melissa* sp., *Perilla frutescens* and *Thymus vulgaris*
Lauraceae	*Persea americana* and *Laurus nobilis*
Malvaceae	*Abelmoschus esculentus, Hibiscus rosa-sinensis* and *alvastrum* sp.
Moringaceae	*Moringa oleifera*
Oleaceae	*Jasminum* sp.
Onagraceae	*Fuchsia* sp. and *Oenothera* sp.
Papilionaceae	*Phaseolus vulgaris*
Piperaceae	*Heckeria umbellate* and *Piper* sp.
Plumbaginaceae	*Limonium sinuatum*
Polemoniaceae	*Phlox* sp.
Ranunculaceae	*Paeonia* sp.
Rhamnaceae	*Ceanothus* sp.
Rosaceae	*Malus sylvestris*, *Physocarpus* sp. and *Rosa* sp.
Rubiaceae	*Bouvardia* sp.
Rutaceae	*Ruta graveolens*
Saxifragaceae	*Astilbe* sp.
Scrophulariaceae	*Veronica* sp.
Solanaceae	*Capsicum* sp., *Lycopersicon esculentum, Physalis ixocarpa*,

	Nicotiana glauca and *Solanum melongena*
Thymelaeaceae	*Daphne* sp.
Verbenaceae	*Duranta* sp.

1.2.2 Pesticide resistance in *T. vaporariorum*

Although *T. vaporariorum* is controlled successfully by applications of conventional insecticides, such as organophosphates, carbamates, and pyrethroids (Choi et al. 2003), their repeated use has led to resurgence and resistance of this pest (Omer et al. 1993), undesirable effects on non target organisms, and increase in the chance of human health problems and environmental degradation (Hayes & Laws 1991). The earliest report on occurrence of resistance by the greenhouse whitefly was found to be against Malathion and DDT in the United Kingdom (Wardlow et al. 1972). Studies demonstrated that *T. vaporariorum* also developed widespread resistance against buprofezin, teflubenzuron (IGR) and many other classes of insecticides in glasshouses, resulting in a serious constraint on the effective control of *T. vaporariorum* in many countries (e.g. Gorman et al. 2002). Recently, Gorman et al. (2007) reported the first confirmed case of neonicotinoid resistance, resulting in control failures in *T. vaporariorum*, and highlighted a need for careful vigilance to sustain the effectiveness of imidacloprid and related neonicotinoid insecticides. However, repeated use of any chemical pesticide for the control of whiteflies will inevitably lead to the development of pest resurgence and resistance.

1.3 Importance and relevance of this research

Eretmocerus warrae is a specialist parasitoid of *T. vaporariorum* (Polaszek, personal communication). Both parasitism and host feeding by *Eretmocerus* parasitoids can directly kill the hosts (Jervis & Kidd 1986), making them highly efficient for the control of greenhouse whiteflies. Knowledge on reproductive and feeding behaviour of a parasitoid is important for the understanding of its ability to suppress pest populations (van Alphen & Jervis 1996). However, little was known about the reproductive and feeding behaviour of *E. warrae* prior to the present study, making it difficult to use this

parasitoid to control *T. vaporariorum*.

The study of the reproductive and feeding behavior of *E. warrae* is relevant because:

1. Tomato, *Lycopersicon esculentum* Miller (Solanacae), is the most widely grown greenhouse crop throughout the world for fresh fruit market as well as for the processed food industries (Atherton & Rudich 1986). It was ranked fourth most significant fruit, making an important contribution to human nutrition during the 20th century (Davies & Hobson 1981). *T. vaporariorum* is one of the most important pests of tomato grown in greenhouses (Duffus et al. 1996).
2. *Eretmocerus warrae* is a specialist parasitoid of *T. vaporariorum*. It could be a highly efficient candidate for biological control of whiteflies (De Barro et al. 2000).

1.4 Aim and objectives

The aim of this study was to understand the reproductive and feeding behaviour of *E. warrae*, with three objectives:

1. to investigate the basic biology of *E. warrae*;
2. to determine the factors affecting the reproductive behaviour of *E. warrae*;
3. to investigate the factors affecting the host feeding behaviour of *E. warrae*.

Chapter 2

Literature Review

2.1 Introduction

This chapter reviews the current knowledge on whiteflies and their parasitoids that are relevant to my PhD studies. Special references are given to the known biology of *Eretmocerus* spp.

2.2 Classification of *E. warrae*

The genus *Eretmocerus* was first described by Haldeman in 1850 and *E. warrae* Naumann and Schimdt was first described from Australia in 2000 (De Barro et al. 2000).

Order: Hymenoptera

Superfamily: Chalcidoidea

Family: Aphelinidae

Subfamily: Aphelininae

Tribe: Eretmocerini

Genus: *Eretmocerus*

Species: *warrae*

2.3 General biology

All *Eretmocerus* undergo complete metamorphosis which includes four stages: egg, larva, pupa and adult. The life cycle of *E. warrae* lasts 17-26 days at 22 ± 1°C depending upon the host stage parasitised (Hanan, unpubl.).

2.3.1 Egg

The ovarian egg of *E. warrae* is obpyriform to reniform in shape but the newly deposited egg is oval in shape measuring 0.055-0.085 mm in length and 0.040-0.052

mm in width (Hanan, unpubl.) (Fig. 2.1a). The newly deposited egg is translucent and turns brown in 2-3 days. Similarly, the eggs of *E. eremicus* are obpyriform to reniform in shape and measuring 0.072 mm (range 0.065-0.085 mm) in length and 0.034 mm (range 0.029-0.038 mm) in width (Headrick et al. 1999). The eggs of other *Eretmocerus* species such as *E. mundus* are oval and transparent and turn brown on the next day after oviposition (Hafez et al. 1983).

2.3.2 Larva

There are apparently three larval instars in all aphelinids, and larvae tend to be homogeneous in shape and structure in many genera (Gerling 1966). However, the first instar larva shows a major morpho-biological plasticity, and the second instar is rather similar to the subsequent instar, but in endophagous species it shows reduction of the mandibles and of the respiratory system (Gerling 1966). The third instar larva is very uniform and similar to that of other allied families of Chalcidoidea (Viggiani 1984). *Eretmocerus* species are ectophagous in the first instar and endophagous in the latter instars (Gerling et al. 1998). The first instar larva of *E. warrae* is pear-shaped with its posterior part remaining in the chorion (Fig. 2.1b). The larva can be distinguished by the presence of lancet type (hook like) moving mouthparts fixed in the host venter for penetration (Gerling 1966; Gelman et al. 2005). Upon penetration into the host, host epidermal cells and possibly other immune system-derived cells are stimulated to form a capsule around the parasitoid larva (Gelman et al. 2005). Following the encapsulation process the larva moults to the second instar; then the capsule begins to disintegrate once the parasitoid moults to the third instar (Gerling et al. 1990). The second instar larva is circular in shape and has a dorso-ventrally depressed body (Fig. 2.1c). The shape of the third instar larva is oblong (Fig. 2.1d) and occupies the whole internal volume of the host.

2.3.3 Pupa

The body of the *Eretmocerus* pupa (Fig. 2.1e) is initially whitish and gradually turns to ripe lemon color in male and yellow in female.

2.3.4 Adult

Eretmocerus warrae is similar to the North American species *E. eremicus.* The only significant difference between the two is that *E. warrae* does not parasitise *B. tabaci* (Workman, Polaszec, personal communication). Adults of both species are tiny parasitic wasps (~1 mm in length) and females are light yellow or pale lemon yellow in colour, with green eyes and clubbed antennae (Fig. 2.1f) (Hoddle 2008). The head is light yellow or yellowish white and even paler than body, and ocelli red and legs pale yellow with dark tarsal claws. Males are rare in *E. warrae* and possess orange or yellow colour, darker than females (Rose & Zolnerowich 1997a; De Barro et al. 2000) and have longer, elbowed antennae (Hoddle 2008).

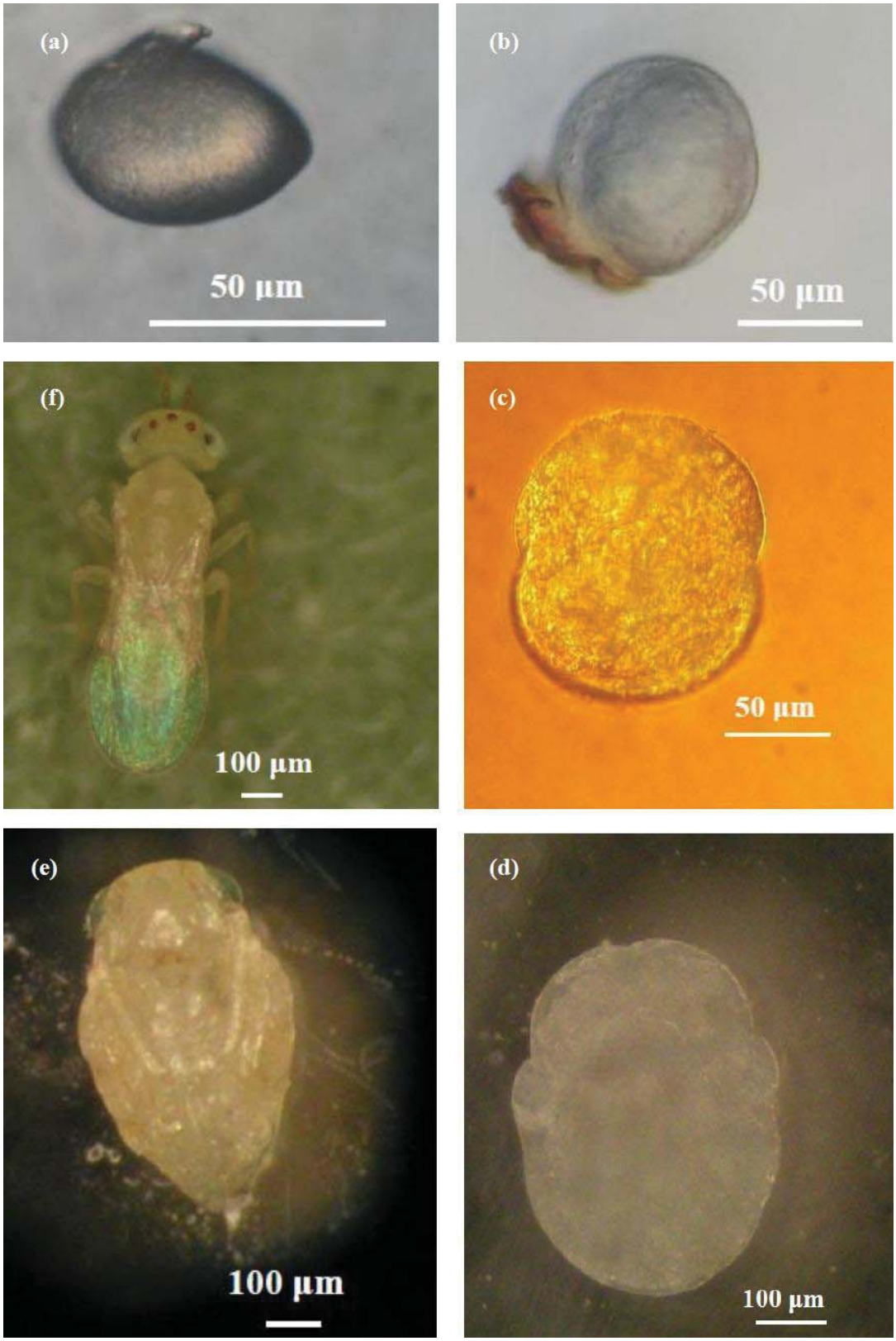

Figure 2.1 Life cycle of *E. warrae*: (a) egg, (b) first instar larva, (c) second instar larva, (d) third instar larva, (e) Pupa, and (f) adult. All pictures were taken in the present study.

2.4 History of *T. vaporariorum* and *E. warrae*

2.4.1 History of *T. vaporariorum* in New Zealand

Trialeurodes vaporariorum was first detected in New Zealand in 1889 (Cameron et al. 1989). Now there are about fourteen species of whiteflies known to occur in New Zealand. Eight of them are indigenous (native) and six introduced species. The latter six are resistant to many pesticides and difficult to control (Martin 1999).

2.4.2 Biological control history of whiteflies

Biological control, the use of living organisms to control pest populations, dates back to ancient times (Howarth 1991). Biological control is defined as "the actions of parasites, predators, and pathogens in maintaining another organism's density at a lower average than would occur in their absence" (DeBach 1964). This definition contains three different approaches: (a) classical biocontrol - the importation and release of exotic biocontrol agents, with the expectation that the agents will become established and further releases will not be necessary, (b) conservation - protection or maintenance of existing populations of biocontrol agents, and (c) augmentation - regular action to increase populations of biocontrol agents, either by periodic releases or by environmental manipulation (McFadyen 1998). The superior value of insect natural enemies in crops was recognised with the dramatic control of the cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae), in California in 1890, by *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) sourced from Australia (Cameron et al. 1989). This was one of the success stories that led to the world-wide acceptance of biological control by natural enemies, and considerable biological control research in New Zealand (Charles 1998).

The history of biological control of whiteflies is about 80 years. In 1926, a tomato grower drew the attention of Spreyer, the English entomologist, to the black pupae among the normal white nymphs of the greenhouse whitefly, which were subsequently identified as *Encarsia formosa* Gahan (van Lenteren et al. 1996). Within a few years, a research station in England supplied 1.5 billion *En. formosa* to about 800 nurseries, and during 1930 *E. formosa* was exported to mainland Europe, Canada, Australia and New Zealand (van Lenteren et al. 1996).

Among the hymenopteran parasitoids of whiteflies *Eretmocerus* spp. are

regarded as potentially important because they are primary parasites that attack Aleyrodidae (Rose et al. 1996). During the past two decades much research has been directed towards finding the efficient natural enemies of whiteflies (Qiu et al. 2004) and between 1984 and end of 2000 about 1500 papers were published on biological control of whiteflies (Naranjo 2001). The papers published between 2000 to 2003 were about 270, most of which are on the genera *Encarsia*, *Eretmocerus* and *Amitus* (Qiu et al. 2004).

2.4.3 Parasitoid species of *T. vaporariorum*

Evans (2008) listed 38 species of parasitoids in four families, parasitising the greenhouse whitefly (Table 2.1).

Table 2.1 Parasitoid species of *T. vaporariorum*.

Family	**Genus**	**Species**
Aphelinidae	*Encarsia*	*E. adusta*, *E. azimi*, *E. basicincta*, *E. bimaculata*, *E. californica*, *E. costaricensis*, *E. desantisi*, *E. formosa*, *E. guadeloupae*, *E. hispida*, *E. inaron*, *E. japonica*, *E. lutea*, *E. luteola*, *E. lycopersici*, *E. melanostoma*, *E. mineoi*, *E. nigricephala*, *E. noahi*, *E. oakeyensis*, *E. pergandiella*, *E. porteri*, *E. protransvena*, *E. quaintancei*, *E. sophia*, *E. synaptocera*, *E. tabacivora*, *E. tricolor*, *E. ustulata* and *Encarsia* sp.
	Eretmocerus	*E. californicus*, *E. eremicus*, *E. haldemani* (These three species are referred to as *E. eremicus* (Zolnerowich & Rose 2008)), *E. mundus*, *E. corni* and *E. warrae*
Eulophidae	*Euderomphale*	*Euderomphale* sp.
Platygastridae	*Amitus*	*A. arcturus* and *A. fuscipennis*
Pteromalidae	*Moranila*	*M. comperei*

2.4.4 The genus *Eretmocerus*

These aphelinids are minute parasitic wasps that rarely exceed 1 mm in length (Noyes & Valentine 1989). Their bodies are typically yellow, greyish, brown, black, or a combination of these colours. The name *Eretmocerus* refers to the prominent "oar-shaped" antennal clubs of females. The species of this genus are identified by the presence of an apically bifurcate seta near each propodeal spiracle (Hayat 1998) which is a unique characteristic of the genus *Eretmocerus* (Zolnerowich & Rose 2008).

Most aphelinids are primary parasitoids of the sternorrhynchous Homoptera (Aphidoidea, Aleyrodoidea, and Coccidea) (Noyes & Valentine 1989). In general, *Eretmocerus* spp. are considered to be solitary ecto/endo-parasitoids of whiteflies that parasitise the host nymphs externally and complete their life cycle inside the nymphs. Some species like *E. serius* Silvestri and *E. debache* Rose and Rosen are internationally accepted as effective biological control agents of citrus blackfly, *Aleurocanthus woglumi* Ashby, and bay berry whitefly, *Parabemisia myricae* Kuwana, respectively (Clausen & Berry 1932; Rose & Debach 1992).

2.5 Basic biology

2.5.1 Effect of temperature

Climatic factors are determinants of growth and development of an insect (Bale et al. 2002). Among these factors temperature is the most important that affects the biology, distribution and abundance of species (Pandey & Tripathi 2008). For example, a survey of more than 1,700 insect species in UK shows that nearly half of them are already affected by the climate change particularly by the global warming (Hance et al. 2007). In the tritrophic system direct effects of extreme temperature may lead to the extinction of at least some part of the system (Hance et al. 2007). For example, exposure of thelytokous *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) to extreme temperatures killed the bacteria *Wolbachia* sp., resulting in restoration of arrhenotokous reproduction of the parasitoid (Stouthamer et al. 1990).

Temperature affects the survival and rate of development of immatures, and longevity of both pest and parasitoid adults (Vet et al. 1980; Sharaf & Bhatta 1996; Schirmer et al. 2008; Pandey & Tripathi 2008). Several studies demonstrate that developmental time and longevity of parasitoids of the genus *Eretmocerus* are inversely

related to the temperature. For example, *E. eremicus*, *E. mundus*, and *E.* sp. nr. *furuhashii* on *B. tabaci* and *T. vaporariorum* have shorter developmental duration and longevity with increasing temperature (Sharaf & Bhatta 1996; Zilahi-Balogh et al. 2006; Qiu et al. 2004, 2007). Similarly, in other parasitoid species such as *En. formosa* on *B. tabaci*, *Trichogramma* spp. on *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) on *Aphis gossypii* Glover (Homoptera: Aphididae), and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) on *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) juvenile developmental period and adult longevity decrease as the temperature increases from 15 to 30°C (El-Ghany et al. 1990; Haghani et al. 2007; Hance et al. 2007; Schirmer et al. 2008; Shishehbor & Zandi-Sohani 2011). Variation in temperature also influences the host feeding, fecundity, parasitism rate and sex ratio of several aphelinid parasitoids. For example, host feeding, fecundity and parasitism rates of *E. eremicus* and *En. formosa* on *T. vaporariorum* increase when temperature increases from 20 to 24°C (Zilahi-Balogh et al. 2006). The sex ratio of *E.* nr. *furuhashii* on *B. tabaci* and *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) on *H. armigera* is significantly more female-biased between 17 and 27°C than at higher temperature (35°C) (Qiu et al. 2007; Pandey & Tripathi 2008).

Temperature also seems to have significant effect on host searching behaviour of female parasitoids (Sharaf & Bhatta 1996; Hance et al. 2007). In parasitoid *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae), females could not respond to volatile semiochemicals when emerged from chilled pupae (Hérard et al. 1988). Similarly, in the mymarid *Anaphes victus* Huber (Hymenoptera: Mymaridae), larval exposure to the temperature of 4°C reduces the capacity of females to detect the oviposition marks from conspecific females (van Baaren et al. 2005). Studies also suggest that temperature can affect pre-oviposition period of both pest and parasitoid. In *E. mundus* females, the pre-oviposition period is 2.8 days at 14°C and 1.6 days at 25°C (Sharaf & Bhatta 1996). However, in some *Eretmocerus* spp. no significant difference in pre-oviposition period is recorded at 20 and 29°C (Powell & Bellows 1992).

2.5.2 Diel activity patterns of insects

On this biosphere there are two predominant environmental rhythms - the earth's

daily cycle caused by its rotation about by its own axis and the annual cycle caused by rotation of earth around the sun (Ikeno et al. 2010). All animals have evolved a circadian clock in response to rhythms (light and dark) for their survival (Bradshaw & Holzapfel 2010). Bünning (1936) was the first to float the idea that both daily and seasonal activities are based on the endogenous circadian clocks (Danks 2005). These circadian rhythms are important regulatory processes that facilitate the organisms to envisage and get ready for the predictable changes in their environment (Stelzer et al. 2010). Thus, their common activities like flight, egg hatch, moulting, oviposition, feeding, pupation, and emergence are controlled by circadian rhythms (Saunder 1997).

Studies suggest that insects adjust their daily activities in synchrony with light (Saunder 1997; Tauber & Kyriacou 2001). For example, in the Parthenian beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) and the pink boll worm, *Pectinophora gossipiila* Saunders (Lepidoptera: Gelechiidae), oviposition occurs close to the end of the photophase (Pittendrigh & Minis 1964; Omkar et al. 2009) while in the ladybird beetles *Coccinella transversalis* Fabricius and *C. septempunctata* Linnaeus (Coleoptera: Coccinellidae), it takes place during the scotophase (Omkar et al. 2004).

In hymenopteran parasitoids emergence, oviposition and host feeding are usually rhythmic and governed by several endogenous and exogenous rhythms (Beck 1980; Walter 1988; van Lenteren et al. 1996; Saunders 1997). In most cases such rhythmicity is synchronised with mating (Nadel & Luck 1985; Fantinou et al. 1998) and oviposition for increasing the survival and reproductive fitness (Armstrong et al. 1996; Couch et al. 1997). In *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae), an egg parasitoid of *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae), the emergence of males occurs a few hours earlier than females, facilitating mating opportunities and thereby reducing the risk of females' death before reproduction (Fagerstrom & Wiklund 1982). In some parasitoid species host activity also plays an important role in their daily activity patterns. For example, in *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a parasitoid of weevil, *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), oviposition occurs during light and dark in synchrony with the host activity (feeding and oviposition) (Armstrong et al. 1996; Couch et al. 1997).

The study of diel behaviour in parasitoids is important because it may provide clues for elucidating the temporal occurrence of biological events such as emergence and oviposition in the field (Vogt & Nechols 1991). This information could be helpful

in determining the timings of spray and parasitoid application in the field and could provide basis for further studies on reproductive behaviour of insects (van Lenteren et al. 1992).

2.5.3 Developmental strategies of parasitoids

According to the earlier hypothesis, parasitoids can be divided into two groups: early succession colonisers and late succession colonisers. The former uses larval hosts and possesses high fecundity and large adult body size while the latter attacks pupal hosts and has low fecundity and small adult body size (Price 1973). In 1986, Askew and Shaw redefined this division according to the parasitoid developmental strategy and grouped the parasitoids into idiobionts and koinobionts.

The idiobiont parasitoids cease the development of their hosts after parasitisation (Mayhew & Blackburn 1999). The resources for the idiobiont parasitoids are limited because they paralyse their hosts before or during parasitisation (Vinson 1998). Idiobionts are either ectoparasitoids that feed externally on the hosts and paraslyse their hosts before parasitisation, or endoparasitoids that feed inside the host body and attack sessile host stages (Mayhew & Blackburn 1999; Pennacchio & Strand 2006).

In contrast, koinobiont parasitoids allow their hosts to feed and grow after parasitisation (Askew & Shaw 1986; Vinson 1998). Therefore, they can attack different size and age of hosts (Vinson 1998). Most koinobionts are endoparasitoids that attack immature stages of insects but some are ectoparasitoids that attack concealed sawflies, lepidopterans and spiders. These ectoparasitoids are restricted to two families, Ichneumonidae and Eulophidae and lay their eggs close to the hosts or attach their eggs to the hosts (Gauld 1988). Upon hatching their larvae start to feed on the hosts externally and inject the venom into the hosts which does not paralyse the hosts but prevents them from moulting (Nakamatsu & Tanaka 2004; Pennacchio & Strand 2006).

Generally, in order to maximize reproductive success and survival the parasitoids can regulate their own growth according to the development of their hosts and prevent them from pupation (Pennacchio et al. 1993; Beckage & Gelman 2004; Hu et al. 2003). For example, in *En. formosa*, although parasitisation occurs in the younger nymphal instars of its host *T. vaporariorum*, the second instar larva stops growing until

the host moults to the fifth stage of its final instar (Hu et al. 2002). Similarly, in *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae), when parasitisation occurs in younger larval instars of its host *Heliothis virescens* F. (Lepidoptera: Noctuidae), the first instar larva moults to the second instar only when the host moults to the fifth instar (Pennacchio et al. 1993). This permits the parasitoid to develop within the most nutritious host (Gelman et al. 2005).

Eretmocerus spp. possess unique developmental strategy (Gerling et al. 1991; Gelman et al. 2005). The early developmental stages are ectoparasitic and later larval stages are endoparasitic (Gerling et al. 1998). Females deposit eggs between the leaf surface and the venter of the host nymphs (Gerling et al. 1990). The first instar larvae wait and do not commence penetration until the host moults to the fourth instar. This permits the larvae to develop within the largest hosts with more nutrition and to avoid coping with the transformation of nymphal host tissues due to moulting (Gerling 1966; Gelman et al. 2005).

2.6 Host Feeding and Oviposition Behaviour

2.6.1 Host stage preference for feeding and oviposition

Host searching and foraging behaviour of parasitoids can be divided into three phases: (1) host selection, (b) host suitability and (c) host regulation (Vinson & Iwantsch 1980). After emergence, a female parasitoid may not be in a habitat surrounded by hosts (Vinson 1976). Hence, host location and selection of parasitoid are governed by various chemical and physical cues emanated from the hosts and host habitat (Vinson & Iwantsch 1980). Host suitability depends upon several factors such as host size, nutritional conditions, host defense mechanism and competition with other parasitoids (Vinson & Iwantsch 1980). However, to develop successfully parasitoids regulate their own and their hosts' growth by changing the physiology and ecology of their hosts (Vinson & Iwantsch 1980; Pennacchio et al. 1993; Beckage & Gelman 2004).

Moreover, many synovigenic parasitoid species use their hosts for oviposition as well as for feeding to get essential nutrients for egg maturation (Jervis & Kidd 1986), for example, *Eretmocerus, Aphytis, Aphelinus, Coccophagus* and *Encarsia* (Walter 1988; Heimpel & Collier 1996; Jervis & Copland 1996; Heimpel et al. 1997; Hoddle et

al. 1998; Qiu et al. 2004). In those species decisions of oviposition and host feeding preference are shaped by the stage and size of the host (Kidd & Jervis 1989). Several other factors such as host and parasitoid density, risk of predation, egg load, nutritional reserves, longevity and experience of female parasitoid can affect the parasitoid oviposition and feeding behaviour (Chan & Godfray 1993; Collier 1995; Heimpel & Rosenheim 1995). In addition to this, deprivation from food and host for a certain period of time also affects the host feeding in many parasitoids (Legner & Gerling 1967; Collier 1995). For example, in *En. sofia*, host and food deprivation for up to 6 hours increases the host feeding, resulting in increased parasitism and longevity of adult parasitoid (Zang & Liu 2009). However, in *E. melanoscutus* it increases host feeding and parasitism without affecting the longevity (Zang & Liu 2010). Host feeding can cause high levels of host mortality in the field (Jervis & Kidd 1986; Hoddle et al. 1999). It is expected that *E. warrae* may use smaller hosts for feeding as she often finds it more difficult to puncture older instars (Ardeh 2004; Kidd & Jervis 1989; Heimpel & Collier 1996).

The optimal foraging theory predicts that females prefer to feed on hosts which can increase their own fitness rather than of their offspring (Scheirs & de Bruyn 2002) while the optimal oviposition theory predicts that females prefer those hosts which can assure successful development of their offspring (Charnov 1976; Iwasa et al. 1984). The optimal host feeding theory suggests that parasitoids usually prefer to feed on lower quality hosts and oviposit on/under/in higher quality hosts due to nutrient richness, ensuring better chance of survival of their immatures (Chan & Godfray 1993; Kidd & Jervis 1991). Many parasitoid species such as *A. melinus* and *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae) feed mostly on smaller and lower quality hosts and oviposit on larger and higher quality hosts to enhance their offspring performance (Heimpel & Rosenheim 1995; Lauzière et al. 2001). To better understand the evolutionary adaptation of host stage preference behaviour of a parasitoid investigation into the optimal foraging, oviposition and host feeding is important (Kidd & Jervis 1991; Scheirs & de Bruyn 2002).

Eretmocerus spp. can parasitise all nymphal stages of whiteflies and show a propensity to oviposit under the second and third instar nymphs (Foltyn & Gerling 1985; Tawfik et al. 1979; Headrick et al. 1995; 96; Hoddle et al. 1999; Qiu et al. 2004; Urbaneja & Stansly 2004). However, crawlers (before it becomes sessile first instar

nymph) and fourth-instar nymphs in the pharate adult stage are not acceptable for oviposition (Gerling et al. 1998; Ardeh 2004). The first instar larvae wait for whitefly nymphs to develop to the fourth instar before they penetrate the hosts (Gelman et al. 2005), suggesting that the parasitoids may prefer to lay eggs under older nymphal instars of the greenhouse whitefly.

2.6.2 Host discrimination behaviour and superparasitism of *E. warrae*

In the evaluation of parasitoids for biological control, several aspects have to be considered. Host discrimination is the ability of a female parasitoid to distinguish unparasitised hosts from parasitised ones and to reject the latter for egg laying (Salt 1934). Host discrimination has several advantages to the parasitoid, for example, it saves foraging time and prevents wastage of the egg and host (Bakker et al. 1985).

Superparasitism, the allocation of more than one egg under the host, was considered to be a mistake by parasitoids in the past but now it is believed to be an adaptive behaviour of female parasitoids (Speirs et al. 1991). Generally, superparasitism reduces the survivorship, size, fecundity and longevity of the parasitoids (Potting et al. 1997; Jones et al. 1999; Chong & Oetting 2006). The avoidance of superparasitism is adaptive when sufficient unparasitised hosts are available for parasitism. However, under certain conditions such as in host depleted patches, superparasitism is an adaptive strategy where individual parasitoids have to compete with others for hosts (Visser et al. 1990, 1992a).

Many parasitoids have the ability to discriminate the parasitised host from the unparasitised one with the help of markers (reviewed by Van Alphen & Visser 1990). These markers can be the pheromones left by the ovipositing female under or on the host and within the visited patch (Fatouros et al. 2005). Moreover, physiological changes in the host haemolymph induced by the parasitoid progeny (van Lenteren et al. 1978) or physical changes of the host surface may provide cues for host discrimination (Fatouros et al. 2005).

In some parasitoid species, time and experience are important parameters to judge their discriminating ability, for example, *Nemeritis canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) do not distinguish parasitised hosts if the time interval between the first and the second oviposition is more than 48 hours (Hubbard et al.

1987). Naïve *E. eremicus* does not discriminate parasitised hosts until she oviposits under an unparasitised host (Ardeh 2004). Understanding the discrimination and superparasitism behaviour of a parasitoid may improve the efficiency of the parasitoids in biological control programmes.

2.7 Factors Affecting Reproductive Fitness of *E. warrae*

2.7.1 Egg load

The egg load is the number of mature eggs a female insect bears (Minkenberg et al. 1992). Theoretical analyses predict that egg load affects many aspects of oviposition behaviour of insects (Iwasa et al. 1981; Parker & Courtney 1984; Mangel 1989). A female with higher egg load searches more vigorously, thereby encounters and accepts more hosts for oviposition per foraging period, spends less time in handling the hosts and deposits larger clutches (gregarious species) (Minkenberg et al. 1992; Heinz & Parrella 1990). For example, in *Diglyphus begini* Ashmead (Hymenoptera: Eulophidae), the search intensity and oviposition rate increase when the female has higher egg load (Heinz & Parrella 1990). Exploration of the egg load dynamics of a foraging insect would help understand its oviposition behaviour (Minkenberg et al. 1992).

2.7.2 Host stage

Host age and size are important factors that affect the fitness and size of emerging offspring (Hu et al. 2002, 2003). Both theoretical and empirical models explain that parasitoid offspring body size has a positive relationship with the host size at parasitism (Godfray 1994; Jervis & Copland 1996). The larger females live significantly longer and have higher egg load than smaller ones (Eijs & van Alphen 1999). They are also more capable of searching and dispersal than smaller ones (Kazmer & Luck 1995).

It is a general concept that parasitoid juvenile development is shorter and body size is larger when larger hosts are parasitised than when smaller hosts are parasitised (Zaviezo & Mills 2000; Qui et al. 2004, 2007) because the larger hosts are richer in nutrition to support the rapid growth and larger body of emerging offspring (Mackauer & Sequeira 1993; Hu et al. 2003).

2.7.3 Host density

Density dependence is an essential component of host-parasitoid association which determines the stability of this system (Wang & Ferro 1998). Parasitoid response to varying host density (functional response) is one of the important aspects of evaluating the effectiveness of a natural enemy (Holling 1959). Holling (1959) proposed three types of functional response of parasitoids. In type I response, a parasitoid attacks a host at a constant rate which increases linearly when host density increases; in type II response, parasitoid attack rate increases non-linearly to increasing host density, and in type III response, a sigmoid response increases initially, and then decreases with increasing host density.

The hymenopteran parasitoids can increase their fecundity and alter the sex ratio of their progeny in response to host density (Waage 1982; Wang & Keller 2005). For example, *Aphidius ervi* Haliday (Hymenoptera: Aphelinidae), a parasitoid of pea aphid, parasitises higher number of hosts and allocates higher proportion of female progeny in response to higher host densities (He et al. 2006). However, in the parasitoid species that use their hosts for oviposition and feeding decision to oviposit or host feed is manipulated by several factors depending upon the host and parasitoid itself (Jervis & Kidd 1986; Heimpel & Collier 1996; Lauzière et al. 1999). Some studies suggest that host feeding is density-dependent and the proportion of hosts fed upon by the parasitoid decreases with increasing host density (Kidd & Jervis 1989). Other studies demonstrate that host feeding is density-independent (van Lenteren et al. 1978; Sahragard et al. 1991). However, oviposition is density-dependent and parasitoids incline to oviposit greater number of hosts at increasing host densities (Lauzière et al. 1999).

Investigation into the functional response would help us better understand host-parasitoid interactions (Hassell & Waage 1984) and can be useful in assessing the potential of a particular parasitoid in maintaining the host population (Murdoch & Briggs 1996).

2.7.4 Parasitoid density

Functional response of parasitoids can be affected by both parasitoid and host densities in the field (Mills & Lacan 2004). Parasitism rates are usually determined by the host density a parasitoid encounters (Tripathi & Singh 1991; Bellamy et al. 2004).

However, in the field, an individual parasitoid is not a single foraging parasitoid, rather, it frequently encounters with other foraging parasitoids, which involves the probability of competition among them (Chong & Oetting 2006). Therefore, an individual parasitoid response at a given host density can be affected by the parasitoid density (Mills & Lacan 2004; Chong & Oetting 2006).

Many studies have demonstrated that the parasitoid density has significant effect on the parasitism and feeding by parasitoids (Hoddle et al. 1999; Jones et al. 1999; Mills & Lacan 2004). In general, parasitoids increase their host feeding and decrease percent parasitism when released at low parasitoid-host ratio, for example, in *E. eremicus* and *En. formosa*, percent parasitism decreases by almost 24% when higher numbers of these are released at the low host density (Hoddle et al. 1999).

Although an individual parasitoid response to host density is lower when parasitoids are searching in groups, parasitoids parasitise more hosts as a whole. For example, *Trichogramma minutum* Riley and *Anagyrus kamali* Mours (Hymenoptera: Encyrtidae) parasitise more hosts when foraging in groups (Montoya et al. 2000; Mills & Lacan 2004). Furthermore, parasitoids superparasitise more hosts when a number of competitors are foraging in the same patch (Montoya et al. 2000). Superparasitism also affects the reproductive fitness of emerging progeny (Jones et al. 1999; Chong & Oetting 2006). Understanding the relationship between the parasitoid and its host density could improve mass rearing techniques and recommend release rates against its host.

2.7.5 Effect of food supply

In many hymenopteran parasitoids, longevity and in some cases fecundity are influenced by a range of factors, such as temperature, host stage, host density, host plant species and presence of food (Sahragard et al. 1991; Leatemia et al. 1995; Jones & Greenberg 1998; Greenberg et al. 2000; Qiu et al. 2005; Hardin et al. 2008). Among these factors, availability of food sources appears to be the most practical and economical means of promoting longevity (McDougall & Mills 1997). Numerous studies have demonstrated that sugar sources and host haemolymph have a significant effect on the longevity of many parasitoids. For example, *E. debachi* (Kuwana) and *E. mundus* (Mercet) live significantly longer when fed with saccharose and honey (Sengonca et al. 1994; Ghahari et al. 2005). *E. eremicus* also lives significantly longer

when fed with carbohydrate diets (Hardin et al. 2008).

Food supply such as honey may provide proovigenic species with nutrients to increase their longevity (Thompson 1999) and synovigenic species to increase both longevity and fecundity (Heimpel & Collier 1996). Jervis & Kidd (1986) also suggested that even the species that feed on host haemolymph to obtain essential nutrients still need sugar as their main source of energy during the adult stage. For example, a synovigenic *A. melinus* fed with honey increases its longevity as well as fecundity (Heimpel & Collier 1996).

Sugar limitation may occur in nature, which can temporarily or permanently limit the reproductive success of parasitoids in agricultural systems (Evans & England 1996). Therefore, understanding the effects of supplementary food on adult parasitoids' reproductive fitness would help optimise mass-rearing techniques and field application of a parasitoid in biological control programmes.

CHAPTER 3

General Biology

3.1 General introduction

The New Zealand strain of *E. warrae* is thelytokous (no males) (De Barro et al. 2000), which was first found in greenhouses in Auckland, New Zealand, in 1997 (Workman et al. 2008). However, not much information on the biology of this parasitoid is available. This chapter provides information on general methodology applied throughout this research and reports the general biology of *E. warrae*, including the effects of temperature on growth, development and reproduction; diurnal rhythms of emergence, oviposition and host feeding, and developmental strategy in each host instar.

3.2 General methodology

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

3.2.1 Materials

Plants, plastic trays and plastic pots. Tomato, *Lycopersicon esculentum* Mill. cv. Money Maker, was used as a host plant for the greenhouse whitefly, *T. vaporariorum*. The seeds were sown in 60 cell plastic trays with each cell (4.5 cm diameter × 4.5 cm high) filled with potting mix (15% N, 8.4% P and 10.8% K). Three- to 4-week-old plants were transplanted into plastic pots (4 cm diameter × 6 cm in height). Whole plants or leaves/branches of different size and age were used for the laboratory cultures/experiments. The branches were kept in transparent plastic containers with water to avoid wilting.

Plastic Petri dishes. Experiments were conducted in plastic Petri dishes (5.5 cm diameter × 1.3 cm high) having 1% agar solution to keep the leaf fresh.

Plastic containers. A branch of a tomato plant infested with parasitised and unparasitised pupae was cut off from the plant and placed in a transparent plastic

container (3 cm diameter × 6 cm height) filled with water via a hole (0.5 cm in diameter) cut in the centre.

Breeding cage for T. vaporariorum. An aluminium framed cage (64 cm in length × 45 cm in width × 40 cm in height) with fine metal screen (0.2 × 0.2 mm) on the back and both sides and perspex on the top and front and aluminium alloy on the bottom was used for oviposition of *T. vaporariorum* (Fig. 3.1).

Figure 3.1 Breeding cage for *T. vapararіorum*.

Breeding cage for E. warrae. Perspex cages (30 cm in length × 30 cm in width × 30 cm in height) with fine metal screen (12 cm in diameter) on top for ventilation were used for oviposition of *E. warrae* (Fig. 3.2).

Figure 3.2 Breeding cages for *E. warrae*.

Glass vials. The parasitised pupae were individually kept in glass vials (1.5 cm in diameter × 5 cm in height) with a 0.5 cm mesh covered hole in lids for emergence.

Microscopes. A stereomicroscope (Leica MZ12, German) equipped with a micrometer eyepiece (Fig. 3.3) was used for measuring body size and dissecting the newly emerged parasitoid adults. A compound microscope (Olympus, GH, Japan) equipped with transmitted light and a micrometer eyepiece was employed for counting the egg load in newly emerged *E. warrae*.

Monitor. The parasitoid's behaviour was recorded using a camera (JVC, Japan) attached to the stereomicroscope, which was connected to a Samsung video cassette recorder (DVD-V530, Korea). The images were viewed on a Panasonic color monitor (TC-21T1Z, Japan) (Fig. 3.3).

Figure 3.3 System used for behaviour observation and recording.

3.2.2 Environmental conditions

All experiments were carried out at 22 ± 1°C and 60 ± 5% RH, with a photoperiod of 16:8 h light:dark (lights on from 09:00 to 01:00 and off from 01:00 to 09:00). Lighting was provided by high frequency broad-spectrum biolux tubes (Osram, Germany).

3.2.3 Procedures

Breeding colony of T. vaporariorum. The colony of *T. vaporariorum* was initiated with about 1000 pupae obtained from BioForce Ltd, Auckland, New Zealand, and maintained in the breeding cage. A plant was placed into the cage for egg laying. After 24 hours the plant was removed and kept in a separate room. When whiteflies became pupae, the infested branches were cut off from the plants and placed into transparent plastic containers filled with water via a hole. Those containers were kept in another breeding cage for maintenance of the colony.

Breeding colony of E. warrae. The colony of *E. warrae* was initiated with about 500 parasitised pupae obtained from BioForce Ltd, Auckland, New Zealand. The emerging parasitoids were directly released into the breeding cages with plants/branches infested with the second and third instar nymphs of *T. vaporariorum*. When the parasitised nymphs reached the pupal stage, they were harvested and placed in glass vials for emergence, and used for experiments and colony maintenance.

Eggs laid. To determine the number of eggs laid by an *E. warrae* female under a whitefly nymph, the nymph was turned over under the stereomicroscope 24 h after exposure to the parasitoid and the number of parasitoid eggs was counted.

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

3.2.4 Definitions

Number of parasitism. The number of whitefly nymphs parasitised by *E. warrae*.

Parasitism rate. The percentage of whitefly nymphs parasitised by *E. warrae.*

Superparasitism. The number of whitefly nymphs with two or more *E. warrae* eggs under each.

Fecundity. The total number of eggs laid by *E. warrae*.

Reproductive fitness. The host feeding, fecundity, and longevity of adult parasitoids, and body size, egg load and longevity of their progeny.

3.2.5 Statistical analyses and reported values

All analyses were made using SAS 9.2 (SAS Institute, Cary, NC, USA). Rejection level was set at $P < 0.05$. Unless stated otherwise, all reported values are means ± SE.

3.3 Diurnal Rhythms of Emergence, Host Feeding and Oviposition of *E. warrae*

3.3.1 Introduction

Diurnal rhythms are important regulatory processes which enable the organisms to foresee and to get ready for expected changes in their environment (Sandrelli et al. 2008; Stelzer et al. 2010). Many behavioural, developmental and physiological events displayed by insects are controlled by endogenous circadian rhythms, which, in many cases, are modulated by external factors (Saunder 1997). The knowledge of parasitoids' emergence, oviposition and feeding rhythms is fundamental for understanding the ecology and evolution of their reproductive strategies, which in turn contributes to the development and implementation of biological control programmes (He et al. 2004).

Understanding diurnal activity patterns of a parasitoid is vital to the field and laboratory application of that parasitoid. For example, the knowledge about the peak emergence and oviposition period of the parasitoids helps determine the appropriate time of pesticide application without disturbing parasitoid activity (van Lenteren et al. 1992). So far, no information on diurnal rhythms of *E. warrae* is available. In the present study, the diurnal rhythms of adult emergence, feeding and oviposition of *E. warrae* were investigated in the laboratory. The information generated will provide a basis for further investigations on its feeding and oviposition behaviour.

3.3.2 Materials and methods

3.3.2.1 Emergence

To observe the circadian emergence rhythm of *E. warrae*, two bioassay rooms were set up: a normal light regime in which photophase was set from 08:00 to 24:00 hours and a reverse-light regime in which scotophase was set from 10:00 to 18:00 hours. High-frequency broad-spectrum biolux tubes (Osram, Germany) were used as light source. Observations in the scotophase were made under red photographic safe lamps (Phillips, Greensboro, NC).

To obtain parasitised whitefly nymphs, a fresh tomato leaf infested with about 80-100 second instar nymphs was placed onto an agar-based Petri dish. One newly

emerged parasitoid was released into the Petri dish and allowed to stay for 24 h. The parasitoid was then moved to another Petri dish containing the same number of whitefly nymphs and allowed to stay for 24 h. This process was repeated until she died. When nymphs had developed to the pupal stage, they were collected and kept singly in glass vials in the same bioassay room. Adult emergence was observed hourly in the entire photophase in the normal-light regime room, and in the entire scotophase in the reverse-light regime room. Twenty parasitoids were used in each room.

3.3.2.2 Oviposition and feeding

To determine circadian oviposition and feeding rhythms of *E. warrae*, the light regimes in the two bioassay rooms were set up as above. One newly emerged parasitoid was released into a Petri dish containing a fresh tomato leaf infested with 20 second instar whitefly nymphs. The parasitoid was allowed to oviposit for 2 h (first oviposition and feeding period), and then moved into another Petri dish containing the same number of nymphs for 2 h (second oviposition and feeding period). This procedure was repeated until eight oviposition and feeding periods in the photophase and four oviposition and feeding periods in the scotophase were completed. Ten parasitoids were tested in each light regime. As *E. warrae* place their eggs between the venter of the whitefly nymph and leaf surface (Qiu et al. 2007), all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope after each oviposition period. The oviposition and host feeding patterns were determined by counting the number of eggs laid and host feeding by the parasitoid in each oviposition and host feeding period. Host feeding was recorded if the nymph body fluid was found to have escaped as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs (Vet et al. 1980; Viggiani 1984). The period between emergence and first oviposition was recorded as the pre-oviposition period.

3.3.2.3 Statistical analyses

Data on hourly emergence and the number of eggs laid and hosts fed per oviposition and feeding period were not normally distributed even after transformation and thus analysed using the non-parametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons (Zar 1999). ANOVA was used to examine

the difference in the mean number of eggs laid and hosts fed per 2 h period between the photophase and scotophase.

3.3.3 Results

3.3.3.1 Emergence

No emergence was observed in the scotophase. In the photophase, adult emergence was significantly higher between 2 and 3 h after lights on and then significantly decreased ($\chi^2 = 80.00$; df = 5; $P < 0.0001$) (Fig. 3.4). No adults emerged 7 h after lights on.

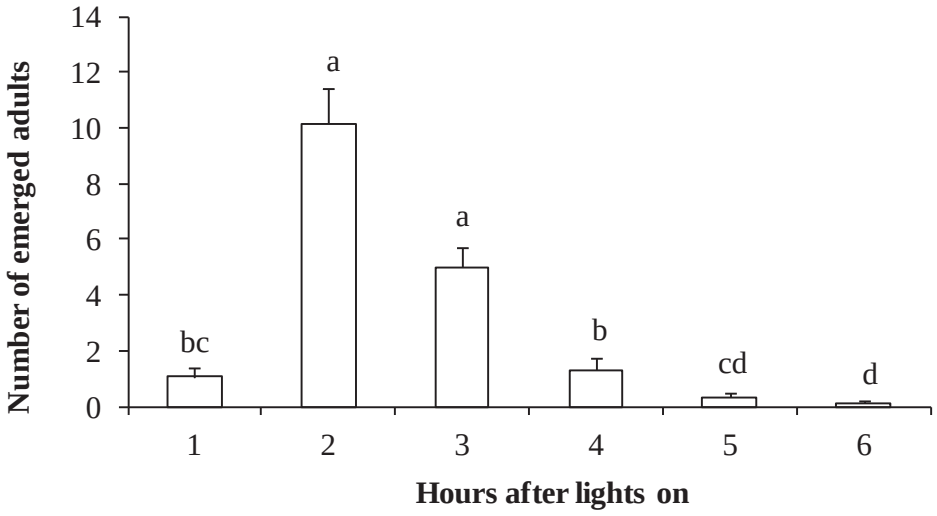

Figure 3.4 Emergence of *E. warrae* in the photophase. Columns with the same letters are not significantly different ($P > 0.05$).

3.3.3.2 Oviposition and feeding

In the photophase, the number of host feedings by *E. warrae* tended to be greater between 4 and 6 h after lights on than at other periods of the photophase but no significant difference was detected between feeding periods ($\chi^2 = 6.84$; df = 7; $P > 0.05$) (Fig. 3.5). In the scotophase, the number of hosts fed by *E. warrae* was significantly greater just before light on ($\chi^2 = 13.16$; df = 3; $P < 0.01$) (Fig. 3.5).

Parasitoids laid significantly more eggs between 10 and 14 h after lights on than in other periods of the photophase ($\chi^2 = 19.30$; df = 7; $P < 0.01$) (Fig. 3.5). In the

scotophase, although more oviposition was detected in the first 2 h after lights off, no significant difference was found between oviposition periods (χ^2 = 5.74; df = 3; P > 0.05) (Fig. 3.5). There was no significant difference in the mean number of eggs laid and hosts fed per period between the photophase and scotophase (F = 1.55 and 0.79 for number of eggs laid and hosts fed, respectively; df = 1, 12; P > 0.05). The pre-oviposition period of *E. warrae* was 7.20 ± 1.27 h.

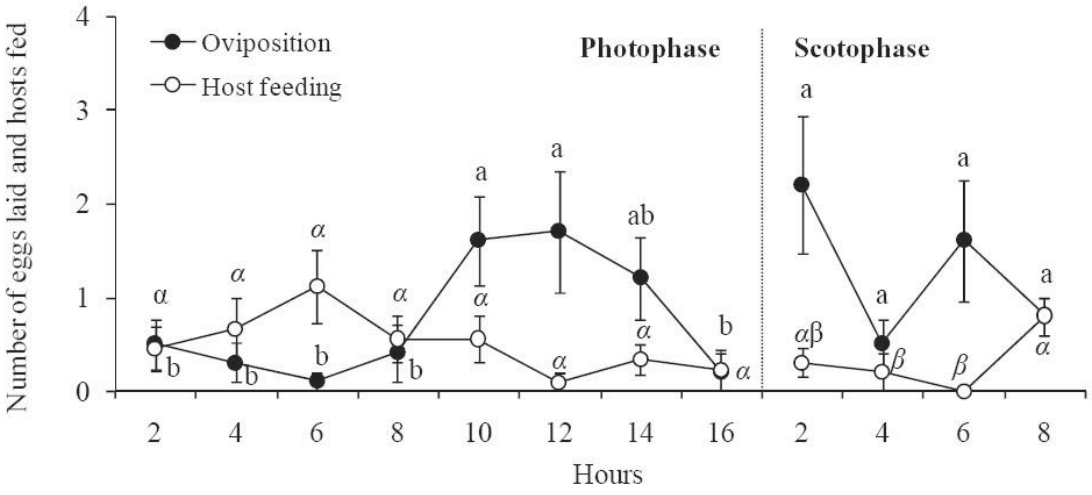

Figure 3.5 The number of eggs laid and hosts fed on by *E. warrae* throughout the photophase and scotophase. Means (± SE) followed by the same English letters within the oviposition line and the same Greek letters within the host feeding line are not significantly different (P > 0.05). Data from the photophase and scotophase were analysed separately.

3.3.4 Discussion

Diurnal rhythm is characteristic of the majority of important processes of insect life, such as adult emergence, migration, feeding and oviposition (Jonušatte & Buda 2002). Results of this study indicate that emergence occurred exclusively during the photophase, peaking during the first few hours of the photophase and then decreasing rapidly afterwards (Fig. 3.4). It is suggested that the onset of light may act as a signal of adult emergence. Fantinou et al. (1998) suggest that in *T. busseolae* Gahan, a solitary egg parasitoid of various lepidopterans, emergence during early photophase probably coincides with more favourable conditions for their survival, as in the morning field

temperature is lower and humidity is higher than the rest of the day. Furthermore, *E. warrae* emergence early in the morning (Fig. 3.4) may facilitate maximum oviposition in the afternoon (Fig. 3.5).

My results also show that *E. warrae* is active throughout 24 hours, suggesting that oviposition and host feeding by *E. warrae* are not controlled by an endogenous oscillator or exogenous factor (i.e. the light); rather, the parasitoid may respond to cues from the host (Couch et al. 1997). These properties may enable *E. warrae* to act successfully as an agent in the biological control of the greenhouse whitefly.

Jervis & Kidd (1986) suggest that the primary role of host feeding is to secure nutrients necessary for egg maturation and studies have demonstrated that host feeding can promote parasitoid egg production (Briggs et al. 1995; Giron et al. 2004; Burger et al. 2005). The main host feeding of *E. warrae* occurred before the oviposition peak in the photophase, suggesting that host feeding supplies nutrients for egg maturation.

The findings of this study have implications in the laboratory mass rearing and field release of *E. warrae*. For example, pre-emerged *E. warrae* (i.e. beige pupal colour) should be placed in the greenhouse early in the morning so that with the light signal, parasitoids emerge and begin to feed on whiteflies to promote oviposition. Furthermore, this information can also be useful to estimate parasitoid emergence and oviposition peak periods which will help avoid greenhouse management practices such as pesticide application during that period.

3.4 Effect of Temperature on Reproductive Fitness of *E. warrae*

3.4.1 Introduction

Among the abiotic factors temperature is the most important that influences life history, distribution and abundance of both parasitoids and pests (Pandey & Tripathi 2008). Extreme temperatures, either low or high, can directly reduce the survival, hinder development and lower the reproductive rate of insects (Bale et al. 2002; Pandey & Tripathi 2008). Variation in temperature is one of the most important causes of dramatic changes in insect abundance (Cornell & Hawkins 1995). In general, higher temperature results in higher growth rates and shorter developmental time in parasitoids (Sharaf & Bhatta 1996) because of the direct effect of temperature on metabolic rates (Nylin & Gotthard 1998).

Temperature also affects fecundity, feeding and total activity of both pests and parasitoids (Vet et al. 1980; Sharaf & Bhatta 1996; Schirmer et al. 2008). For example, in *E. eremicus* longevity and developmental periods are shorter at 32°C and longer at 15°C (Greenberg et al. 2000). Likewise, other *Eretmocerus* spp. perform well at moderate temperatures of 20-25°C (Powell & Bellows 1992; Zilahi-Balogh et al. 2006; Urbaneja et al. 2007). Variation in temperature can also have significant effects on the pre-oviposition period of parasitoids, which increases with the decrease in temperature and *vice versa* (Sharaf & Bhatta 1996).

Though *E. warrae* is thelytokous (Hanan et al. 2009, 2010), some studies suggest that when the females of thelytokous species are exposed to higher temperature of 30°C and over, males are produced (Pijls et al. 1996). So far, no work has been conducted on the biology of *E. warrae* at different temperatures. The objectives of this study were therefore to investigate the effects of different temperatures ranging from 15 to 30°C on the general biology, particularly longevity, fecundity, parasitism rate and host feeding of *E. warrae*.

3.4.2 Materials and methods

3.4.2.1 Experimental conditions and insects

Experiments were conducted at four constant temperatures (15, 20, 25 and 30 ±

1°C). Different bioassay rooms were set to carry out these experiments with each room having one of the mentioned temperatures with 16 h light:8 h dark, and 60 ± 5% R.H. The parasitoids used for these experiments emerged from *T. vaporariorum* that were parasitised as the second instar nymphs. The second instar nymphs were used as hosts in this study.

3.4.2.2 Host feeding, fecundity, parasitism and longevity of adults

One parasitoid that emerged at a given temperature (<1 h after eclosion) was released into a Petri dish containing a fresh tomato leaf infested with 100 nymphs, allowed to stay for 24 h, and then moved into another Petri dish with the same number of test insects. This process was repeated until she died. Host feeding was recorded if the nymph body fluid was found. To record the fecundity and parasitism of *E. warrae* half of the nymphs were turned over and numbers of eggs laid by the parasitoid at a given temperature were counted. The longevity of adult parasitoids was also recorded. There were 10 replicates (parasitoids) for each temperature treatment.

3.4.2.3 Survivorship, emergence and developmental period of immatures

The remaining half of the nymphs from the above experimental setup were reared at a given temperature for determination of survivorship, emergence and developmental period of immatures. Juvenile survival and adult emergence rates at each temperature were calculated as the number of larvae that pupated/the number of eggs laid and the number of adults that emerged/the number of pupae, respectively. Emergence was recorded daily to determine survival, emergence and juvenile developmental period of *E. warrae* at each temperature. The emerging parasitoids were examined under the microscope to determine whether males could be produced at different temperatures.

3.4.2.4 Pre-oviposition period

To determine the pre-oviposition period of *E. warrae* the experiment was set up with four temperature treatments as described above. One newly emerged (0 h old) parasitoid was released into a Petri dish containing a tomato leaf infested with 20

second instar whitefly nymphs. The parasitoid was allowed to oviposit for 2 h (first oviposition period), then moved into another Petri dish containing the same number of test insects (second oviposition period). This procedure was repeated until eight oviposition periods were completed. All nymphs in the Petri dishes were turned over to determine the presence or absence of eggs under the stereomicroscope after each oviposition period. The period between emergence and the first oviposition was recorded as the pre-oviposition period. Ten replicates (parasitoids) were tested at each temperature treatment.

3.4.2.5 Statistical analyses

A goodness-of-fit test was used to test the data distribution. Data for developmental period were not normal even after transformation, and thus analysed using the nonparametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons (Zar 1999). All other data were normally distributed, and thus analysed by one-way ANOVA followed by Tukey's test. The percentage data were arcsine transformed before analysis but non-transformed means are presented here.

3.4.3 Results

3.4.3.1 Host feeding, fecundity, parasitism, and longevity of adults

The total number of eggs laid, hosts parasitised and fed on by *E. warrae* was significantly higher at 20 and 25°C ($F = 29.14$, 21.50 and 28.21, for number of eggs laid, hosts parasitised and fed on, respectively; $df = 3,36$; $P < 0.0001$) (Figs. 3.6A-C).

The average daily parasitism rates were also significantly higher at 20 and 25°C ($F = 61.65$; $df = 3,36$; $P < 0.0001$) (Fig. 3.6D).

The average daily host feeding rates significantly increased with the increase of temperature from 15 to 30°C while the longevity of adult *E. warrae* significantly decreased ($F = 168.07$ and 49.93, for host feeding rates and longevity, respectively; $df = 3,36$; $P < 0.0001$) (Figs. 3.6E & F).

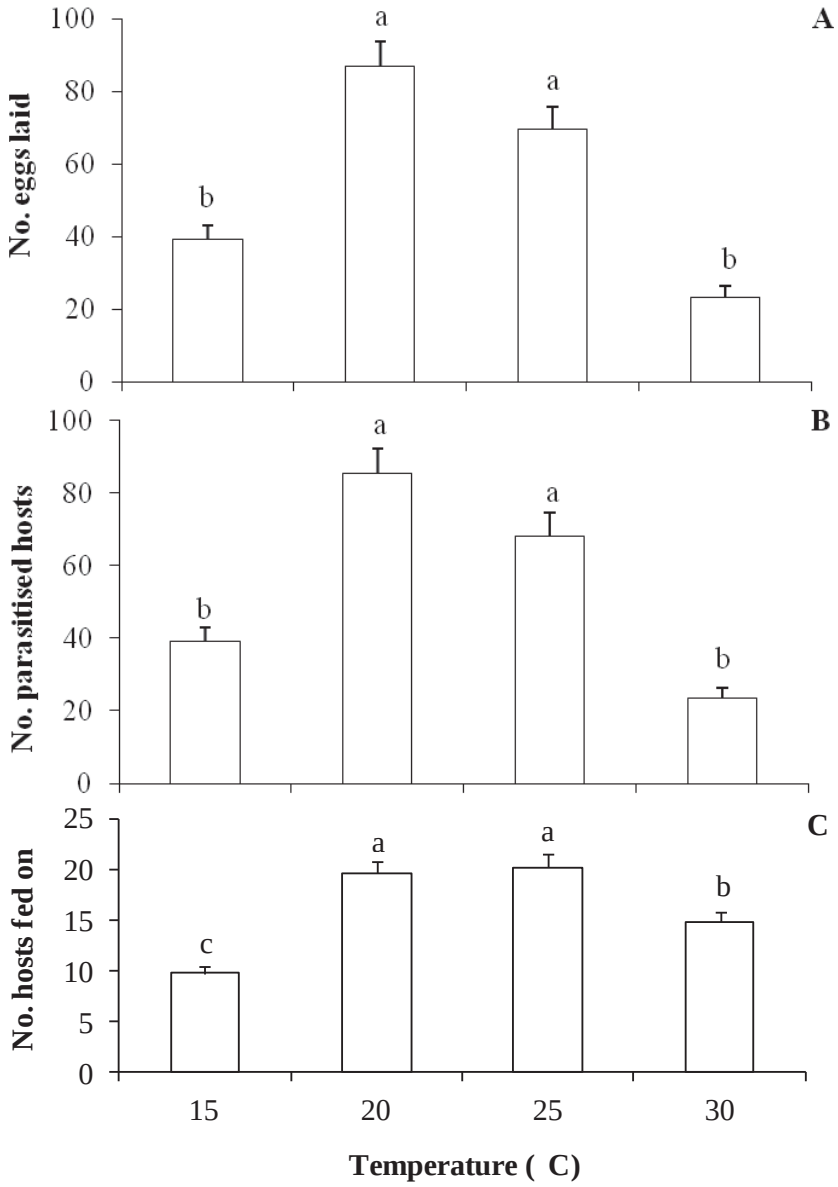

Figure 3.6 Effect of temperature on the total number of eggs laid (A), hosts parasitised (B), and hosts fed on (C) in *E. warrae*. Columns with the same letters are not significantly different (P > 0.05).

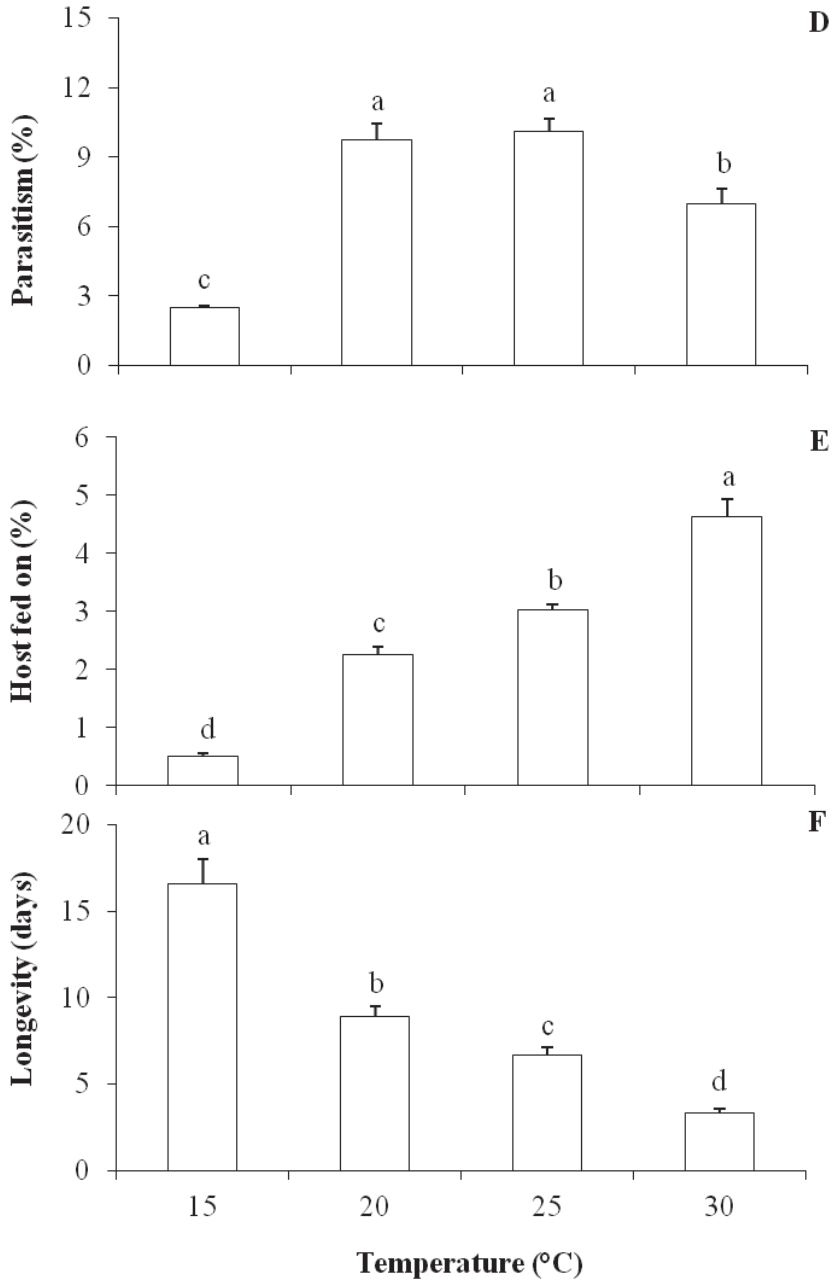

Figure 3.6 (continues) Effect of temperature on average daily parasitism rate (D), host feeding rate (E), and longevity (F) in *E. warrae*. Columns with the same letters are not significantly different (P > 0.05).

3.4.3.2 Survivorship, emergence and developmental period

The survival and emergence rates of *E. warrae* were significantly higher at 20 and 25°C (F = 22.28 and 27.31 for survival and emergence rates, respectively; df = 3,36; P < 0.0001) (Figs. 3.7A & B). The developmental period of immatures of *E.*

warrae significantly decreased with the increase of temperature from 15 to 30°C (χ^2 = 485.13; df = 3; P < 0.0001) (Fig. 3.7C). However, no males emerged at any of the test temperatures.

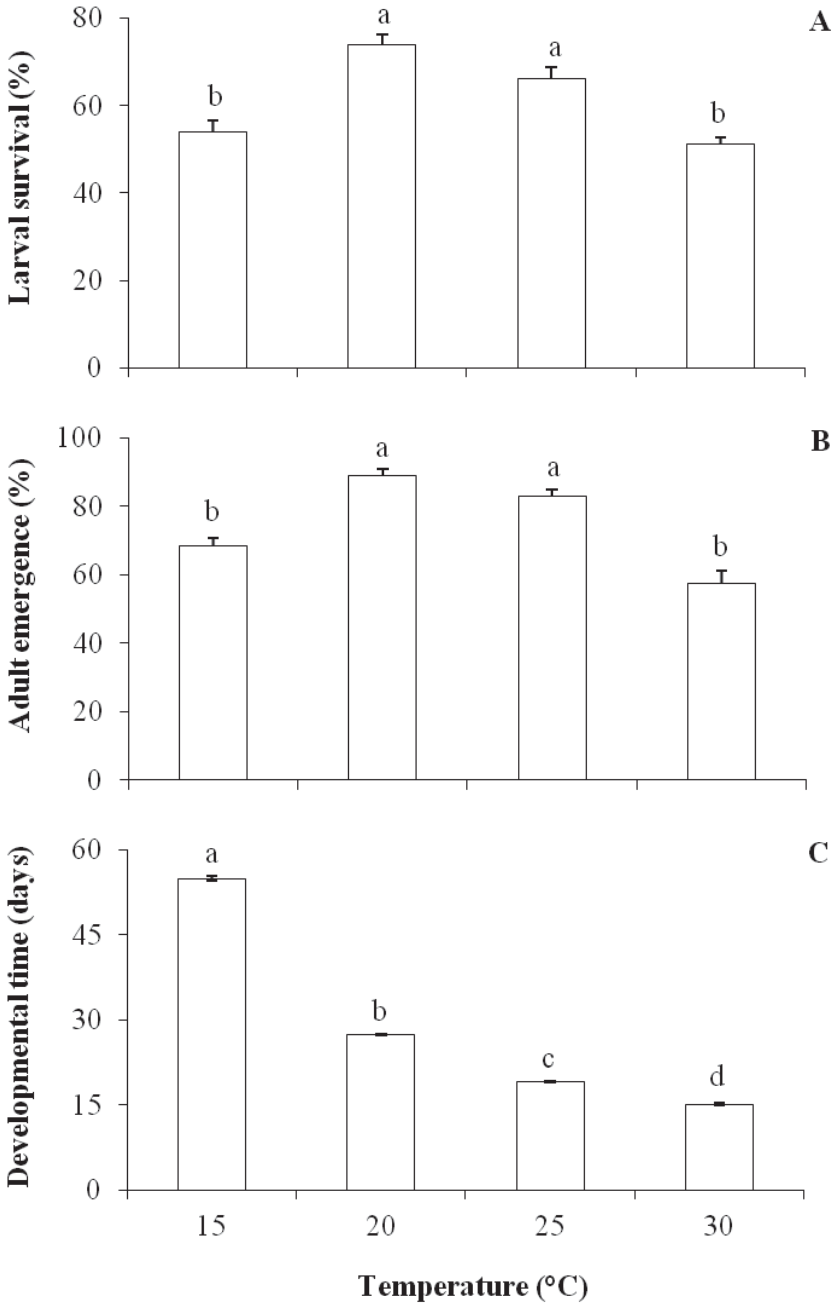

Figure 3.7 Effect of temperature on survival rate (A), emergence rate (B), and developmental time (C) in *E. warrae*. Columns with the same letter are not significantly different (P > 0.05).

3.4.3.3 Pre-oviposition period

The pre-oviposition period of *E. warrae* significantly decreased with the increase of temperature from 15 to 30°C ($F = 8.70$; $df = 3,36$; $P < 0.001$) (Fig. 3.8).

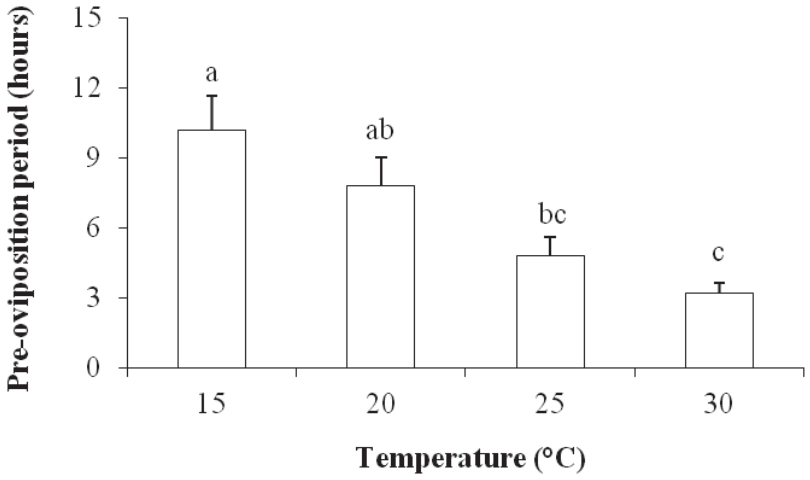

Figure 3.8 Effect of temperature on pre-oviposition period in *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$).

3.4.4 Discussion

Temperature significantly affects the developmental time, survival and longevity of many parasitoid species (Greenberg et al. 2000; Sharaf & Bhatta 1996; Enkegaard 1993). In the present study, developmental time and longevity of *E. warrae* significantly decreased with the increasing temperature. These results suggest that increased heat at higher temperatures accelerates the development of parasitoids. Insects need a certain amount of heat energy to moult from one instar to another (Gordan 1999). Similar results can be found in *E. eremicus* (Greenberg et al. 2000) and *E. mundus* (Sharaf & Bhatta 1996). Present results also show that the daily feeding rates of *E. warrae* increased with the increase in temperature. This suggests that with the increase in temperature most enzyme actions increase, which has significant effect on metabolism and ultimately on feeding and growth rates of an organism (Howe 1967; Tomberlin et al. 2009). Similarly, in *Trichogramma turkestanica* Meyer, with the increase in temperature feeding rates significantly increase and longevity significantly decreases (Hansen & Jensen 2002).

In a majority of insects, physiological processes such as respiration, digestion and circulation work properly within a suitable range of temperatures (Chapman 1998), which lead to higher fecundity (Luciano et al. 2003). My study shows that, although at 30°C *E. warrae* completed its development in significantly shorter time, maximum fecundity and survival rate occurred at 20 and 25°C. This dramatic fall in fecundity at 30°C suggests that *E. warrae* population growth may be checked when environmental temperature reaches this high. These results also imply that temperatures of 20 to 25°C are optimal for *E. warrae* oviposition and survival. Moreover, pre-oviposition period of *E. warrae* decreased with the increase in temperature. Significant reduction in pre-oviposition period may be attributed to the accelerated development of oocytes, resulting in early maturity of ovules (Bonhag 1958). Similar results have been reported in the pear rust mite, *Epitrimerus pyri* (Nalepa) (Acari: Eriophyidae) (Bergh 1994), *E. mundus* (Sharaf & Bhatta 1996), and *B. Argentifolii* (Greenberg et al. 2000).

The present results also reveal that significantly lower survival and adult emergence rates occurred at 15 and 30°C. This suggests that these upper and lower extreme temperatures are detrimental to the growth and propagation of *E. warrae*. At lower temperatures, the decreased survival can be attributed to the incomplete development of the embryo while the low emergence can be ascribed to the low degree of activity of the wasp inside the pupae, preventing adult emergence from the pupal case. Similarly, in mealy bug *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) eggs show reduced embryonic development such as segmentation and development of eyes at 15°C (Chong et al. 2008). However, the low survival of *E. warrae* at higher temperature can be attributed to the physiological stresses such as changes in lipids and imbalance of the rates of digestion and circulation (Chapman 1998). It is also possible that at higher temperature mortality occurs due to the desiccation because at higher temperature, the animal loses more moisture to the air due to sweating, resulting in dehydration of the animal (Fields 1992). Similar reports can be found for *E. eremicus* (Greenberg et al. 2000) and *E. mundus* (Sharaf & Bhatta 1996).

The life history of koinobionts parasitoids is also profoundly affected by that of their hosts (Harvey & Strand 2002). *T. vaporariorum* is the only host of *E. warrae* (De Barro et al. 2000), which is extremely sensitive to the higher temperature (Greenberg et al. 2000). The higher temperature causes the higher levels of mortality of *T. vaporariorum*, reaching 98% at 32°C (Greenberg et al. 2000). In the present study, the

higher level of mortality of *E. warrae* that occurred at higher temperature may be due to the higher level of mortality of its host at higher temperature. Similar results were also reported by Greenberg et al. (2000) on *E. eremicus* reared on *T. vaporiorum* and *B. argentifolii*.

Present studies have confirmed that the temperature has a significant effect on fecundity, host feeding, developmental time and survival of *E. warrae*. The temperatures of 20 and 25°C were found to be the most suitable for the fitness of adult *E. warrae* and the development and survival of its progeny. The information generated here can be useful for the design of mass-rearing protocols, which will eventually help make decisions for augmentative releases of *E. warrae*. Moreover, *E. warrae* lives significantly longer at 15°C, suggesting that when the hosts are rare or temporarily absent in a mass-rearing or biological control programme, the increase in longevity at this temperature may enable the parasitoid to survive until they find the suitable host.

3.5 Oviposition and Developmental Strategy of *E. warrae* in Greenhouse Whitefly

3.5.1 Introduction

Unlike insect predators which usually consume several preys before reaching maturity, parasitoids rely on the resources provided by a single host (Harvey et al. 2004). As a result, parasitoids use different strategies to manipulate their hosts to complete their development (Harvey et al. 2004). Generally, in order to maximize reproductive success and survival the parasitoids can regulate their own growth according to the development of their hosts and prevent the hosts from pupation (Pennacchio et al. 1993; Beckage & Gelman 2004). For example, in *En. formosa*, no matter which instar is parasitised, the parasitoid second instar larva waits for its host, *T. vaporariorum*, to reach the fifth stage of the fourth instar before it moults to the third instar (Hue et al. 2002). Similarly, the larvae of *C. nigriceps* never moults to the second instar until its host *H. virescens* has developed to the fifth instar (Pennacchio et al. 1993).

Eretmocerus spp. possess unique developmental strategy to increase survival and development of their juveniles (Gerling 1966; Gerling et al. 1991; Gelman et al. 2005). Females deposit eggs between the leaf surface and the venter of the host nymph (Gerling et al. 1990). The first instar larva, after hatching, waits for the host to moult to the fourth and last instar before penetration into the host (Gerling et al. 1990). This permits the parasitoid to develop within the largest host with more nutrition, and at the same time, to avoid the transformation of nymphal host tissues due to moulting (Foltyn & Gerling 1985; Gerling et al. 1991; Gelman et al. 2005). Thus, early developmental stages of *Eretmocerus* are ectoparasitic while the later larval stages grow within the host (Gerling et al. 1991, 1998). As a result, the parasitoid development can be divided into three stages: (1) egg, (2) the first instar larva from hatch to penetration, which occurs beneath the whitefly nymph, and (3) the period from the time of penetration to adult emergence which occurs within the host or its remnants (Gelman et al. 2005).

Previous studies have shown that *Eretmocerus* species can parasitise all nymphal stages of whiteflies but prefer the second and third instar nymphs (Tawafik et al. 1979; Foltyn & Gerling 1985; Headrick et al 1995; 96; Hoddle et al. 1999; Urbaneja & Stansly

2004; Qiu et al. 2004, 2007). However, little is known about the preference for stages (1-9) of the fourth instar nymphs of whiteflies by the parasitoids.

Prior to the present study little information is available on the oviposition and development strategy of *E. warrae*, making it difficult to develop strategies for effective mass-rearing and field application of this parasitoid. The objectives of the present study were thus to investigate: (1) the parasitoid egg and first instar larval mortality (external mortality) under each host instar; (2) the parasitoid mortality after the penetration into each host instar (internal mortality); (3) the time when the first instar larva starts to penetrate the host in relation to the host stage parasitised; (4) the time when the permanent developmental arrest of its host occurs; and (5) the stages of the fourth instar whitefly nymphs under which parasitoid eggs could be laid.

3.5.2 Materials and methods

3.5.2.1 Egg mortality (external mortality)

To determine *E. warrae* egg mortality under each instar, experiments were conducted separately on all nymphal instars (from the first to fourth) of *T. vaporariorum*. For each replicate, one parasitoid (<12 h) was released into a Petri dish with a fresh tomato leaf infested by 100 test nymphs, and allowed to stay for 24 h. The nymphs exposed to parasitoids were reared in the Petri dishes. In *Eretmocerus* parasitoids, egg period is 3 days (De Barro et al. 2001; Gelman et al. 2005; present study). Therefore, 3 days after oviposition all the nymphs in the Petri dishes were turned over to record the egg mortality under each instar. The hatched egg is distinguished by the moving mouthparts of the first instar larva with its posterior body remaining in the egg shell (Gerling 1966) while the egg that remains pale is considered as un-hatched (De Barro et al. 2001). Ten parasitoids were used for each host instar.

3.5.2.2 Mortality of the first instar larvae (external mortality)

This experiment was designed to determine the first instar *E. warrae* larval mortality under each host instar. The experimental setting was the same as the above but 3 days after oviposition, all nymphs in Petri dishes were turned over and observed under the microscope daily until the parasitoid larvae penetrated into the host. The data on mortality were recorded under nymphs of each instar. Ten parasitoids were used for

each host instar.

The first instar larvae of *Eretmocerus* parasitoids have to wait for whitefly nymphs to develop to the fourth instar before penetration (Foltyn & Gerling 1985; Gerling et al. 1991; Gelman et al. 2005). To investigate the survival rate of the first instar parasitoid larvae under and/or outside the body of the host nymphs after egg hatching, four treatments were set up after turning over the nymphs: (1) the first instar parasitoid larva at original place (nymph was turned over and removed), (2) the first instar larva remained on the leaf but covered with the nymph not touching the host (the nymph was turned over and again placed over the larva), (3) the first instar larva remained on the leaf but covered with moist tissue paper, and (4) the nymph was turned over and the larva was removed and placed on moist tissue paper. For each treatment one 1-d-old parasitoid was released into a Petri dish containing a leaf infested with 100 nymphs of greenhouse whiteflies and allowed to stay for 24 hours, after which time, she was removed and Petri dishes with parasitised nymphs were kept in the controlled temperature room. As the egg period of *Eretmocerus* is 3 days (Gelman et al. 2005), 3 days after parasitisation, all nymphs in Petri dishes were turned over until 90 first instar parasitoid larvae were obtained for each treatment. Larval survival was observed hourly until they died.

3.5.2.3 Mortality of the second and third instar larvae, and pupae (internal mortality)

This experiment was designed to determine the second and third instar larval and pupal mortality (internal mortality) in each host instar. The experimental setting was the same as the above but 50 nymphs were turned over randomly to record the total number of eggs laid. The remaining 50 nymphs were reared in the Petri dish until the parasitoids pupated. The pupae (when the parasitoid pupates, the colour of the parasitised nymph starts to change from half white to beige colour) (Hoddle 2008; Personal observation) were kept in the glass tubes in the same bioassay room until adult emergence. The mortality of the second and third instar larvae was determined by subtracting the average mortality of the eggs and the first instar larvae recorded in Sections 5.2.2.1 & 5.2.2.2. The pupal mortality rates were determined by calculating the number of adults that emerged divided by the numbers of pupae. Ten parasitoids were

used for each host instar.

3.5.2.4 Period between oviposition under and penetration into the hosts

This experiment was designed to determine the time when the first instar *E. warrae* larvae began to penetrate the host. The experimental setting was the same as mentioned in Section 3.3.2.1. As the first instar parasitoid larva is the only stage that has mandibles suitable to puncture insect tissues (Gerling 1966), 3 days after oviposition, 20 parasitised nymphs were randomly selected from each dish by turning over the nymphs each day to record the time (days) when the first instar larvae started to penetrate the hosts. Data were recorded on the time (days) taken from oviposition to start of penetration in relation to each host instar parasitised.

3.5.2.5 Host developmental arrest

The experimental setting of this experiment was the same as the above but a parasitoid (< 12 hour old) was released into a Petri dish with a fresh tomato leaf infested with 20 nymphs of whiteflies. The parasitoid was allowed to stay in the Petri dish for 24 hours, after which time she was removed and the nymphs exposed to parasitoids were reared in the Petri dish. According to Gelman et al. (2005) parasitised hosts cannot develop to the sixth (pharate adult) stage of the fourth instar no matter which instar nymph was parasitised. Following oviposition, Petri dishes with parasitised nymphs were checked daily to record the developmental progress of each parasitised host. In preliminary studies, it was also found that the parasitised hosts do not develop to the sixth (pharate adult) stage of the fourth instar nymphs and changes their colour from half-white to beige when the larva pupates. Therefore, when a nymph reached the fifth stage of the fourth instar, it was turned over and dissected to determine the time when the parasitoid arrested host development in each host instar. Ten parasitoids were used for each host instar.

3.5.2.6 Host stage acceptance for oviposition (choice test)

To determine which stage of the fourth instar nymphs a parasitoid accepted for oviposition, the first five (nymphal stages) and the sixth to seventh (pharate adult)

stages (total 7 stages) were used in this experiment. The nymphs on the leaf were counted under the stereo microscope and excessive nymphs were removed using an entomological pin. Subsequently, a parasitoid (< 12 hour old) was introduced into an agar based Petri dish containing a fresh tomato leaf infested with 35 individuals (5 of each test stage) of whitefly. The parasitoid was allowed to stay in Petri dish for 12 hours because one stage takes less than 24 hours (Gelman et al. 2002). Twelve hours later, she was removed and all nymphs in Petri dishes were turned over to determine the host stages under which eggs were laid. Ten parasitoids were used in this experiment.

3.5.2.7 Host stage acceptance for oviposition (non-choice test)

The experimental design was the same as the above in Section 3.5.2.6 but in each replicate, a parasitoid was offered 40 nymphs of one stage of the fourth instar. Twelve hours later, she was removed and half of the nymphs in Petri dishes were turned over to determine the host stages under which eggs were laid. The remaining half were reared to record the parasitoid development. Ten parasitoids were tested for each of the seven stages.

3.5.2.8 Statistical analyses

A goodness-of-fit test was used to test the data distribution. Data for the mortality of the parasitoid eggs and larvae, and host stage acceptance for oviposition (non-choice) were normally distributed, and thus analysed by one-way ANOVA followed by Tukey's test (Zar 1999). All other data were not normally distributed even after transformation and thus analysed using the nonparametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons. The percentage data were arcsine transformed before analysis but non-transformed means were presented here.

3.5.3 Results

3.5.3.1 External and internal mortality of parasitoids

Similar egg and internal (second instar larvae and pupae) mortality rates were recorded in all test host instars (F = 1.26 and 0.57 for mortality of egg and for the

second/third instar larvae, respectively, df = 3,36, P > 0.05; χ^2 = 0.46, df = 3, P > 0.05 for pupae; Table 3.1). However, the parasitoid first instar larval mortality rates were significantly higher under the first and fourth instar nymphs than under the second and third ones (F = 24.07; df = 3, 36; P < 0.0001; Table 3.1).

The first instar larvae covered with nymphs (not touching the larvae) lived significantly longer than those in other treatments with no significant difference found between larvae covered with moist tissue and those left open on the original site (χ^2 = 164.61; df = 3; P < 0.0001) (Fig. 3.9).

Table 3.1 Parasitoid mortality (%) with respect to host stage parasitised.

Host stage	Parasitoid stage			
	Egg	1st instar	2nd/3rd instar	Pupae
1st	14.10 ± 1.23a	19.39 ± 1.22a	8.26 ± 1.82a	11.04 ± 3.26a
2nd	12.44 ± 1.24a	10.78 ± 0.93b	6.75 ± 1.58a	9.42 ± 2.47a
3rd	11.42 ± 0.88a	9.49 ± 0.99b	6.19 ± 1.73a	8.59 ± 2.40a
4th	11.31 ± 1.10a	23.17 ± 2.11a	8.93 ± 2.23a	8.25 ± 2.81a

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

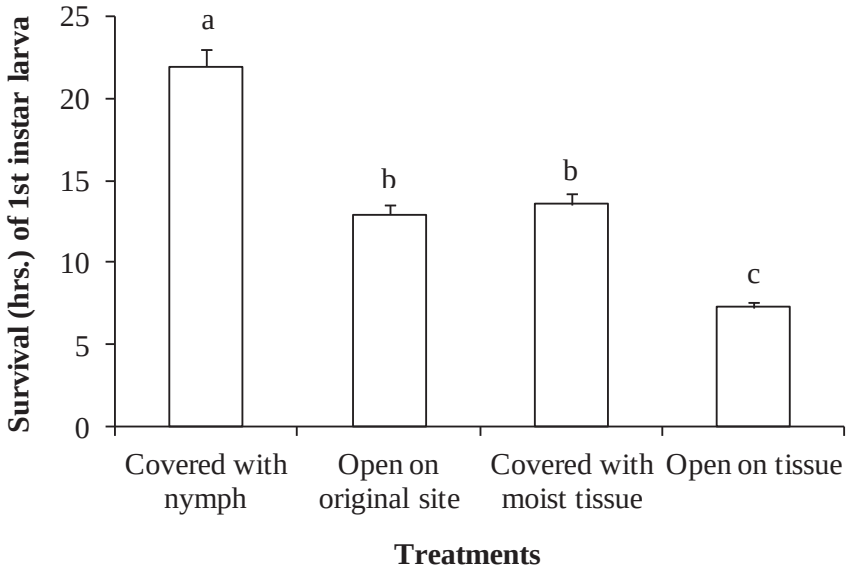

Figure 3.9 Survival of the parasitoid first instar larvae after hatching. Columns with the same letter are not significantly different (P > 0.05).

3.5.3.2 Period between oviposition under and penetration into the hosts

The parasitoid stayed significantly longer under the host if the younger hosts were parasitsed (χ^2 = 130.44; df = 3; P < 0.0001) (Fig. 3.10).

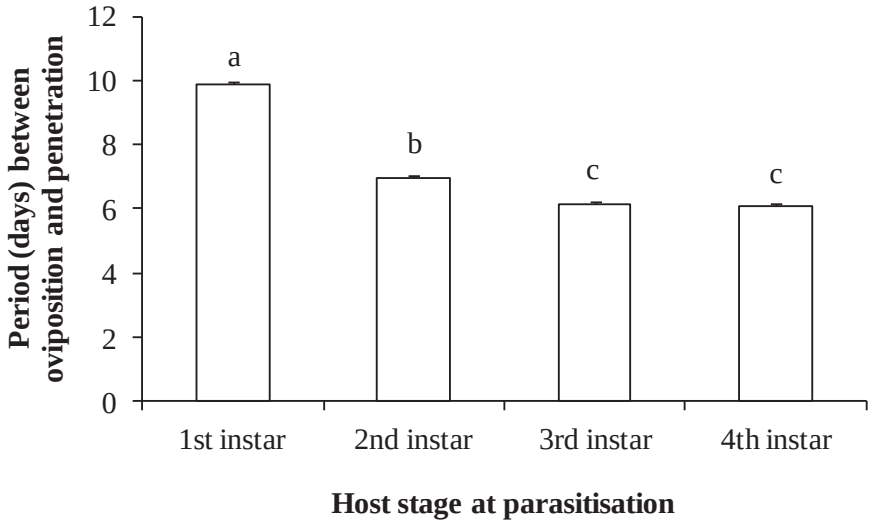

Figure 3.10 Mean period between oviposition and penetration in *E. warrae* under different stages of hosts. Columns with the same letters are not significantly different (P > 0.05).

3.5.3.3 Host developmental arrest

Regardless of host stages parasitised, the parasitoid larvae induced permanent host developmental arrest when the hosts reached the fifth stage of the fourth instar. Therefore, the host developmental arrest occurred significantly sooner if older hosts were parasitised ($\chi^2 = 389.26$; df = 3; $P < 0.0001$) (Fig. 3.11).

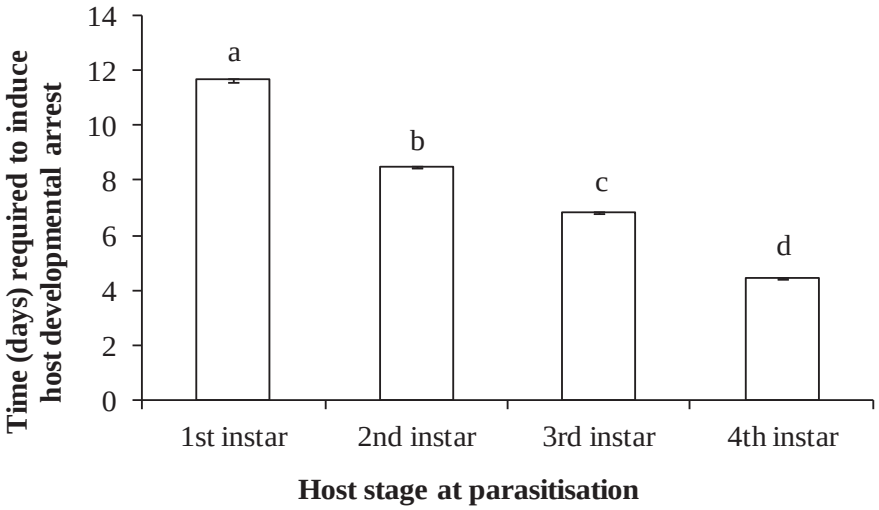

Figure 3.11 Host arrest caused by *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$).

3.5.3.4 Host stage acceptance for oviposition

When offered with different stages of the fourth instar nymphs, *E. warrae* successfully parasitised whitefly nymphs of up to the fifth stage. However, in both choice and non-choice tests significantly more eggs were laid under the first and second stages than under other stages ($\chi^2 = 38.11$, df = 4, $P < 0.0001$ for choice test; $F = 35.78$, df = 4,45, $P < 0.0001$ for non-choice test) (Fig. 3.12). No egg was laid under the sixth and seventh (pharate adult stages) of the fourth instar nymphs and no parasitoid successfully developed from the third, fourth and fifth stages of the fourth instar nymphs.

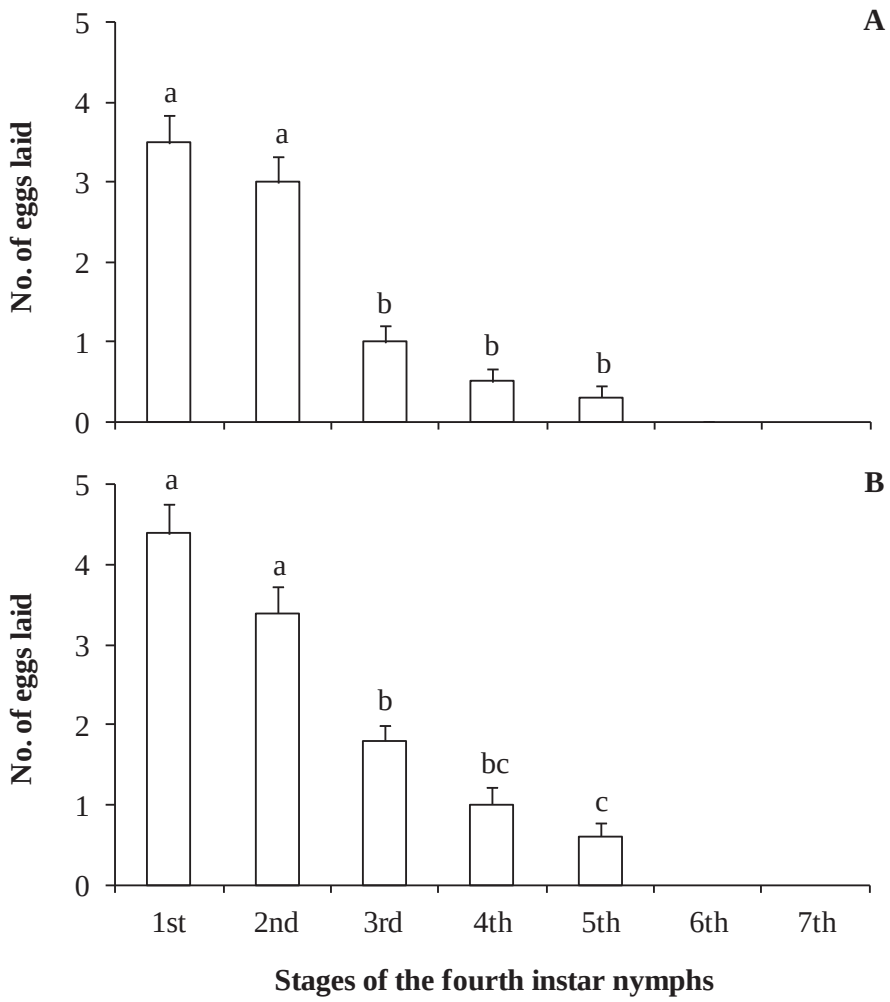

Figure 3.12 Oviposition of *E. warrae* under the different stages of the fourth instar nymphs in the choice (A) and non-choice test (B). Columns with the same letters are not significantly different ($P > 0.05$).

3.5.4 Discussion

The present results indicate that the parasitoid first instar larval mortality was significantly higher when parasitism occurred under the first and fourth instar nymphs, whereas similar mortality rates of the second and third instar larvae, and pupae occurred in all host instars (Table 3.1). The significant higher mortality under the first instar nymph can be attributed to the delay in the penetration process (Fig. 3.10) because the first instar parasitoid larva, after hatching, has to wait for the host to become the fourth instar before penetration (Gerling 1966; present study). It is also possible that *E. warrae* first instar larva suffers starvation due to waiting, resulting in higher mortality under the

first instar host nymphs. The higher mortality of the parasitoid first instar larvae under the fourth instar nymphs may be due to the transformation of the whiteflies into the pharate adult stage where it is difficult for the parasitoid to penetrate the host. Since the second and third instar larvae, and pupae develop inside the hosts, similar mortality in these stages may be due to the similar environment available for them. Similarly, in *En. formosa*, mortality rate is higher when parasitism occurs in the first and fourth instar host nymphs of whitefly (Nechols & Tauber 1977).

In the present study, the parasitoid first instar larvae survived significantly longer when covered with the host nymphs (not touching the larva) (Fig. 3.12), suggesting that the parasitoids may have survived on the honey dew that pours out from the body of the host. In contrast, shorter survival on open moist tissue suggests that the larvae die due to the dehydration and starvation.

In order to survive and develop successfully parasitoids arrest their hosts' development when the latter reach a specific stage (Beckage & Gelman 2004). In the present study, regardless of host instars parasitised, *E. warrae* arrested whitefly development before it developed to the sixth (pharate adult) stage of the fourth instar. These results suggest that *E. warrae* does not affect the whitefly development until it reaches the fifth stage of the fourth instar.

My results show that the parasitoid laid significantly fewer eggs under stages 3-5 of the fourth instar nymphs and no egg was laid under the pharate adult stages because the parasitoid could not complete development in the pharate adult stages. Similarly, *E. mundus* never oviposits under the late fourth instar or pharate adult hosts (Ardeh 2004; Gelman et al. 2005). However, to the best of my knowledge, the present study is the only one that reports the inability of *Eretmocrus* to develop within the pharate adult stages. Moreover, all whitefly adults emerged from the nymphs parasitised at 3-5 stages of the fourth instar hosts, leaving the dead parasitoid larvae in the puparia. This suggests that if the parasitoid egg hatches under the pharate adult stages of whitefly, the parasitoid is incapable of penetrating the host's body and ultimately unable to prevent the host from emerging. It is also possible that the pharate adult structures i.e., wings and thicker cuticle make it difficult for the parasitoid to penetrate. Similarly, larva of *Telenomus heliothidis* Ashmead (Hymenoptera: Scelionidae) cannot develop if parasitism occurs in 65 hours old eggs of *H. virescens*, and when the host embryo becomes a pharate first instar larva (Strand 1986).

Based on the present results, I conclude that both second and third instar nymphs of whiteflies are the most suitable stages for the oviposition and development of *E. warrae*. These findings have implications for mass-rearing and laboratory culture of *E. warrae* as the use of the second and third instar whitefly nymphs would increase the production of *E. warrae*, which, in turn, reduce the rearing costs of *E. warrae*.

CHAPTER 4

Host Feeding and Oviposition Behaviour

4.1 General Introduction

Host stage preference by a parasitoid significantly affects its survival and reproductive fitness (Godfray 1994). A parasitoid encountering hosts of different size selects a suitable host that maximizes its reproductive output and foraging efficiency (Speirs et al. 1991; Ueno 1997). Moreover, females of synovigenic parasitoid species such as *A. melinus* and *E. mundus* use their hosts for oviposition as well as feeding (Heimpel & Collier 1996; Ardeh 2004). In these parasitoids, propensity to host feeding depends upon several factors, including deprivation from food and host for a certain period of time (Legner & Gerling 1967; Heimpel & Collier 1996). In most host feeding parasitoids, host feeding renders the hosts unsuitable for oviposition due to the repeated drilling (Jervis & Kidd 1986). Consequently, a female has to make a decision on which host to oviposit under and which host to feed on. Optimal host-feeding models based on dynamic programming predict that due to the difficulty of puncturing older instars, parasitoids usually prefer to feed on smaller hosts and oviposit on/under/in larger and higher quality hosts which ensure better survival of their future generation (Jervis & Kidd 1986). Understanding feeding and oviposition behaviour of a parasitoid may provide a strong base for successful biological control programmes (Jervis & Kidd 1986; Lewis et al. 1990; Godfray 1994).

Another behaviour of parasitoids that needs attention is their ability to discriminate between parasitised and unparasitsed hosts (van Lenteren et al. 1978; reviewed by van Alphen & Visser 1990). Many parasitoid species such as *E. eremicus* and *E. mundus* are able to discriminate parasitised hosts from unparasitised ones (Ardeh 2004; Buckner & Jones 2005). With this discriminative ability they can reject a substantial amount of parasitised hosts, and they superparasitise as an adaptive strategy when host densities are low (van Alphen et al. 1992; van Dijken & Waage 1987). However, to avoid competition among the sibs, it would be advantageous to the parasitoid to discriminate between the eggs laid by herself and those of a different female (Visser et al. 1990). Investigations into the discriminative ability of a parasitoid may improve its mass rearing techniques in biological control programmes.

The aim of this chapter was to investigate the feeding and oviposition preference behaviour of *E. warrae* with regard to host stage. This chapter reports the superparasitism and ability of *E. warrae* to discriminate unparasitised hosts from parasitised ones. This chapter also reports the effect of different duration of food and host deprivation on the feeding, fecundity and longevity of *E. warrae*.

4.2 Host Stage Preference for Oviposition and Feeding

4.2.1 Introduction

Knowledge of host stage preference behaviour of a potential biological control agent is fundamental to a successful biological control programme (Lewis et al. 1990; Godfray 1994; Ardeh 2004). Many studies have shown that the host selection by parasitoids is determined by the host size (He et al. 2005; Da Rocha et al. 2007; Karut 2007) because host size often represents the quantity of the resources available for the parasitoid development (Godfray 1994; Hu et al. 2002; Harvey et al. 2004; He et al. 2005). Larger hosts possess more resources and produce larger offspring (Da Rocha et al. 2007; Karut 2007). Therefore, in solitary parasitoids, host stage preference is considered to be the critical factor, maximising their reproductive fitness (Da Rocha et al. 2007).

According to optimal foraging theory a parasitoid encountering hosts of different size should select suitable hosts that maximise her own reproductive output such as fecundity rather than that of her offspring while optimal oviposition theory predicts otherwise (Scheirs & Bruyn 2002). For parasitoids that use their hosts for both feeding and oviposition, theory of optimal host feeding strategies predicts that they usually prefer to feed on lower quality hosts and oviposit on/under/in higher quality hosts, ensuring better chance of survival of their immatures (Chan & Godfray 1993; Kidd & Jervis 1991). In many parasitoid species host feeding is specific to a particular host stage (Kidd & Jervis 1991; Godfray 1994; Heimpel & Rosenheim 1995), for example, *A. melinus* uses younger hosts more frequently for host feeding.

Previous studies on various *Eretmocerus* spp. show that they can parasitise all whitefly nymphs except for crawlers (1st instar nymphs) and the pharate adult stage of the 4th instar nymphs but prefer the 2nd and 3rd instar nymphs (Tawfik et al. 1979; Foltyn & Gerling, 1985; Headrick et al 1995, 96; Gerling et al. 1998; Hoddle et al. 1999; Qiu et al. 2004; Urbaneja & Stansly 2004). The 1st instar larvae wait for the whitefly nymphs to moult to the 4th instar before penetrating (Gelman et al. 2005), suggesting that *E. warrae* may prefer to lay egg under the later instar nymphs which will protect the 1st instar larvae from external factors.

The objectives of this study were to investigate oviposition and feeding

behaviour of *E. warrae* to determine its host stage preference under choice and non-choice conditions. Information generated would help optimise methods for mass production and field release of *E. warrae.*

4.2.2 Materials and methods

4.2.2.1 Choice experiment

To evaluate the host stage preference behaviour by *E. warrae*, four host stages from the 1st to 4th instar nymphs of whiteflies were tested in this experiment. To obtain the above mentioned stages, a tomato plant was placed in a rearing cage with 50-100 whitefly adults for 9-10 days. Those infested plants were then kept in another rearing cage for 10 days to obtain all four nymphal stages. The infested leaf parts (4.5 cm × 5.5 cm) were cut off from the plant and placed into an agar based Petri dish. Greenhouse whitefly nymphs were observed under the stereo microscope. The older (2nd, 3rd and 4th instars) nymphs were identified on the basis of their body size and appearance while the 1st instar nymphs identified by their flat appearance (Gelman et al. 2002). Ten individuals of each host stage of whiteflies were left in the Petri dish (a total of 40 nymphs) while the excessive nymphs removed using an entomological pin. A parasitoid (<12 h old) was introduced into the Petri dish and observed for 1 h. The oviposition and feeding preference behaviour was recorded using a recording system (Section 3.2.1, Fig. 3.3). Parasitoids were used only once. Twenty replicates were performed for this test.

The following oviposition and feeding behaviour of *E. warrae* was recorded:

(a) Encounter - when parasitoid contacted the host physically;

(b) Acceptance - the parasitoid examined a host with its antennae and tried to probe it for egg laying or feeding;

(c) Host searching - the parasitoid walked on the leaf in search of hosts;

(d) Antennating and circling - the parasitoid touched the host with the tips of the antennae and circled round the nymph;

(e) Oviposition probing - the parasitoid inserted its ovipositor between the nymph and leaf surface;

(f) Feeding probing - the parasitoid inserted its ovipositor repeatedly into the host

vasiform orifice for making a wound;

(g) Host feeding - the parasitoid fed on the haemolymph (blood) that exuded from the wound resulting from repeatedly probing with the ovipositor;

(h) Grooming - the parasitoid repeatedly brushed her ovipositor with her forelegs and then rubbed the legs together;

(i) Resting - the parasitoid stood still and became motionless.

After each replicate, all the nymphs probed by the parasitoid were turned over to verify the parasitism (presence of parasitoid eggs) under the stereomicroscope. The number of hosts fed upon by the parasitoid was also recorded. The duration and proportion of time a parasitoid spent on behaviour from (c) to (i) were recorded. The proportion of acceptance (no. acceptance/no. encountered), oviposition probing (no. oviposition probing/host density), parasitism (no. parasitism/host density) and host feeding (no. hosts fed/host density) was calculated.

4.2.2.2 Non-choice experiment

The experimental design was the same as the above in choice test experiment with the exception that a parasitoid was offered 40 nymphs of the same stage on a leaf part in a Petri dish. Ten parasitoids for each host stage were tested.

4.2.2.3 Statistical analyses

A goodness-of-fit test was used to test the data distribution. Data on probing for oviposition, host feeding and parasitism, mean probing for feeding, antennation and total time spent on each instar in choice test, and the proportion of time spent on probing for host feeding, feeding on host fluid, and total time spent on each instar in non-choice test, were not normally distributed even after transformation. These data were analysed using non-parametric Kruskal Wallis Test (KWT) followed by Dunn's procedure for multiple comparisons. Other data were normally distributed and thus analysed using ANOVA followed by the Tukey's test. Before ANOVA, data on the number of encounters in both choice and non-choice test were square-root transformed and all percentage data were arcsine transformed but non-transformed means were presented here.

4.2.3 Results

4.2.3.1 Host stage preference behaviour observation

The parasitoid showed the following behaviour in sequence after being introduced onto the tomato leaf surface: walking, encountering the hosts, walking away or examining the hosts with antennae, and accepting and probing them for oviposition or feeding. Before repeating the process, she also had such behaviour as grooming, resting and resumption of walking. She accepted the nymph at the first contact after introduction on the leaf surface but later she might reject it. She usually laid eggs under the hosts and accepted all nymphal instars for oviposition and feeding. For feeding she punctured the vasiform orifice of the hosts with her ovipositor. She normally probed the nymph repeatedly for two to four times to make a wound, and each time she showed the following sequence of behaviours: antennation, circling, probing the wound, grooming and then either feeding on host fluid or making another wound.

4.2.3.2 Choice test

In the choice test the parasitoids were significantly more likely to encounter the 4th instar nymphs than younger ones ($F = 7.61$; $df = 3,76$; $P < 0.001$) (Table 4.1). However, compared to the youngest and oldest nymphs, the intermediate hosts were significantly more likely to be accepted for host feeding and oviposition ($F = 27.08$; $df = 3,76$; $P < 0.0001$), probed for oviposition ($\chi^2 = 38.93$; $df = 3$; $P < 0.0001$) and host feeding ($\chi^2 = 9.61$; $df = 3$; $P < 0.01$), and parasitised ($\chi^2 = 49.66$; $df = 3$; $P < 0.0001$) (Table 4.1).

Table 4.1 Oviposition and host feeding behaviour of *E. warrae* in response to different stages of *T. vaporariorum* in the choice test.

Host instar	Number of encounter	Acceptance (%)	Probing for oviposition (%)	Parasitism (%)	Probing for feeding (%)
1st	5.40 ± 0.45c	27.78 ± 4.01b	15.50 ± 2.66b	9.00 ± 1.91b	1.00 ± 0.68b
2nd	6.25 ± 0.43bc	62.17 ± 3.91a	33.00 ± 2.31a	27.50 ± 1.61a	4.50 ± 1.35a
3rd	7.45 ± 0.41ab	49.75 ± 3.55a	31.50 ± 2.10a	25.00 ± 1.54a	3.50 ± 1.12a
4th	8.45 ± 0.64a	18.53 ± 2.10b	13.50 ± 1.66b	8.50 ± 1.50b	1.00 ± 0.68b

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

There was no significant difference in mean antennation/circling duration on any nymphal stage ($F = 1.01$; df = 3,36; $P < 0.05$) (Fig. 4.1A). However, the mean duration of probing for oviposition and host feeding was significantly longer in older (3rd and 4th instars) nymphs than in younger ones ($F = 40.23$, df = 3,36, $P < 0.0001$ for oviposition; $\chi^2 = 11.83$, df = 3, $P < 0.01$ for host feeding) (Figs. 4.1B & C). The mean total time spent by a parasitoid was significantly longer on intermediate hosts than on the younger and older ones ($\chi^2 = 17.16$; df = 3; $P < 0.0001$) (Fig. 4.1D).

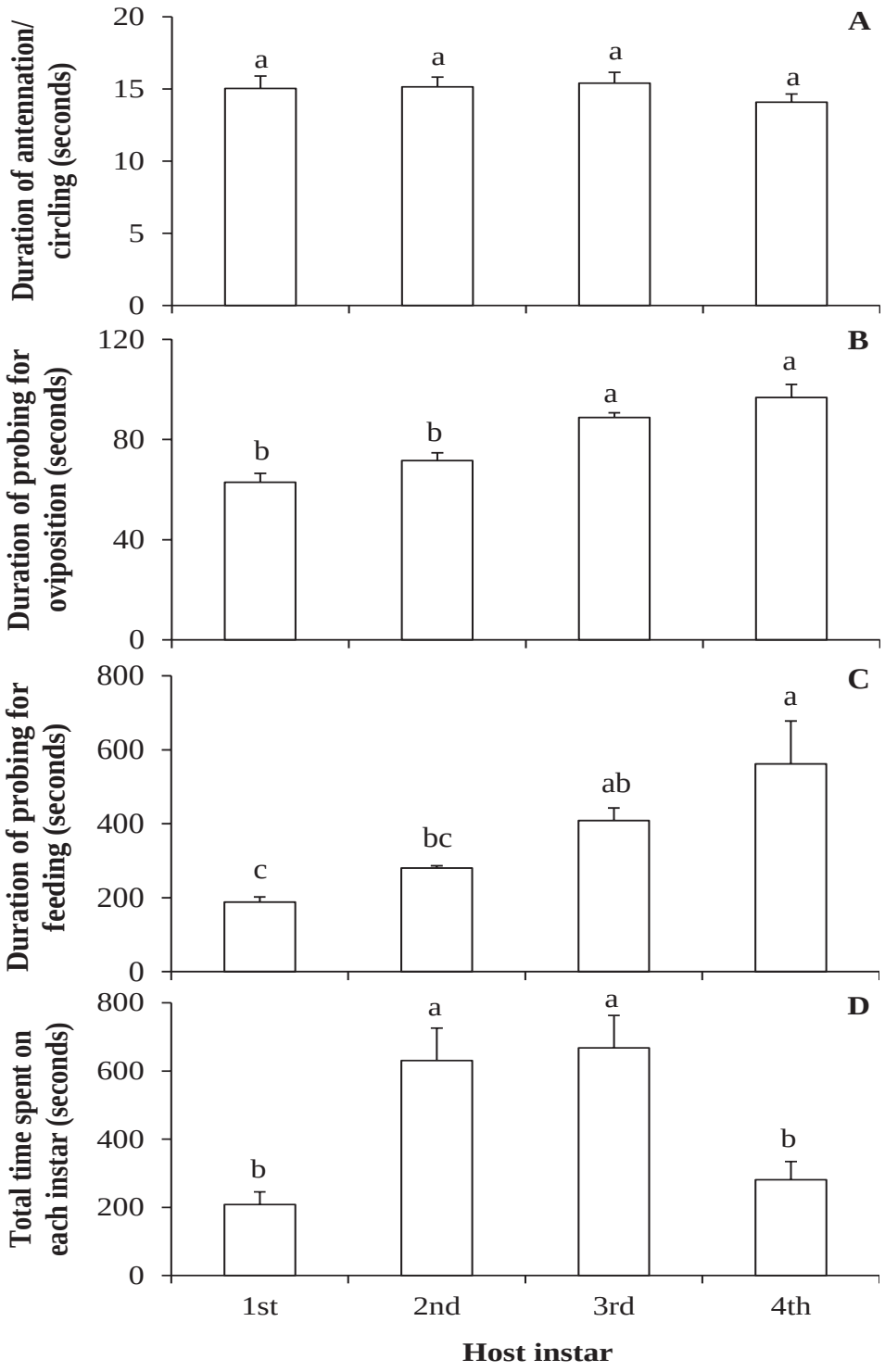

Figure 4.1 Mean duration of antennation and circling (A), probing for oviposition (B), probing for feeding (C), and the total time spent on each instar of whiteflies (D) in the choice test of *E. warrae* behaviour. Columns with the same letters are not significantly different ($P > 0.05$).

4.2.3.3 Non-choice test

In the non-choice test the parasitoids were also significantly more likely to encounter the 4th instar nymphs than younger ones ($F = 5.50$; $df = 3,36$; $P < 0.01$) (Table 4.2). However, compared to the younger and older nymphs, the intermediate hosts were significantly more likely to be accepted for host feeding and oviposition ($F = 23.23$; $df = 3,36$; $P < 0.0001$), probed for oviposition ($F = 3.05$; $df = 3,36$; $P < 0.05$) and host feeding ($\chi^2 = 10.56$; $df = 3$; $P < 0.01$), and parasitised ($F = 10.88$; $df = 3, 36$; $P < 0.0001$) (Table 4.2).

Table 4.2 Oviposition and host feeding behaviour of *E. warrae* on different stages of *T. vaporariorum* in the non-choice test.

Host instar	Number of encounter	Acceptance (%)	Oviposition probing (%)	Parasitism (%)	Probing for feeding (%)
1st	21.90 ± 2.69ab	34.22 ± 3.20b	18.75 ± 2.74ab	09.50 ± 1.43b	0.50 ± 0.67b
2nd	15.60 ± 1.28b	58.84 ± 3.26a	20.00 ± 2.33a	16.50 ± 1.59a	2.25 ± 0.59a
3rd	15.40 ± 1.71b	57.32 ± 3.85a	19.00 ± 1.91a	15.25 ± 1.15a	2.00 ± 0.56a
4th	25.50 ± 1.52a	21.94 ± 4.18c	12.25 ± 1.55b	07.75 ± 0.79b	0.50 ± 0.67b

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

The parasitoids spent significantly more time in searching when provided with older and younger hosts than with intermediate ones ($F = 10.50$; $df = 3,36$; $P < 0.0001$) but similar time in antennating and circling ($F = 1.48$; $df = 3,36$; $P > 0.05$), probing for oviposition ($F = 1.30$; $df = 3,36$; $P > 0.05$) and grooming ($F = 0.99$; $df = 3,36$; $P > 0.05$) on all host stages (Table 4.3). However, they spent significantly more time in probing for host feeding ($\chi^2 = 11.50$; $df = 3$; $P < 0.05$) and feeding on the host fluid in the intermediate ones ($\chi^2 = 12.15$; $df = 3$; $P < 0.05$). They allocated significantly more time in resting on younger nymphs than on older ones ($F = 11.46$; $df = 3,36$; $P < 0.0001$) (Table 4.3).

Table 4.3 Time allocation (%) of *E. warrae* on different behaviour on different stages of *T. vaporariorum* in the non-choice test.

Host instar	Searching	Antennation/ circling	Probing for Oviposition	Probing for host feeding	Host feeding	Grooming	Rest
1st	52.50 ± 5.01a	5.19 ± 0.75a	14.37 ± 2.15a	0.82 ± 0.55b	1.60 ± 1.37b	11.90 ± 2.16a	13.63 ± 3.00a
2nd	40.00 ± 3.54a	4.39 ± 0.46a	15.48 ± 1.85a	5.80 ± 1.30ab	14.75 ± 4.07a	13.06 ± 0.97a	6.72 ± 1.51a
3rd	30.17 ± 5.38b	3.79 ± 0.38a	18.63 ± 1.06a	8.75 ± 2.03a	14.65 ± 4.11a	15.12 ± 1.68a	8.97 ± 1.47a
4th	64.00 ± 3.53b	3.61 ± 0.33a	15.43 ± 1.69a	2.29 ± 1.52b	1.73 ± 1.18b	11.62 ± 1.94a	1.33 ± 0.49b

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

No significant difference was found in mean antennation/circling on any nymphal stage ($F = 0.21$; $df = 3,36$; $P > 0.05$) (Fig. 4.2A). However, the mean duration of probing for oviposition and host feeding was significantly longer in older (3rd and 4th instars) nymphs than in younger ones ($F = 18.60$; $df = 3,36$; $P < 0.0001$ for oviposition; $F = 14.26$; $df = 3,13$; $P < 0.01$ for host feeding) (Figs. 4.2B & C). The total time spent by a parasitoid was significantly longer on intermediate hosts than on the youngest and oldest ones ($\chi^2 = 19.08$; $df = 3$; $P < 0.01$) (Fig. 4.2D).

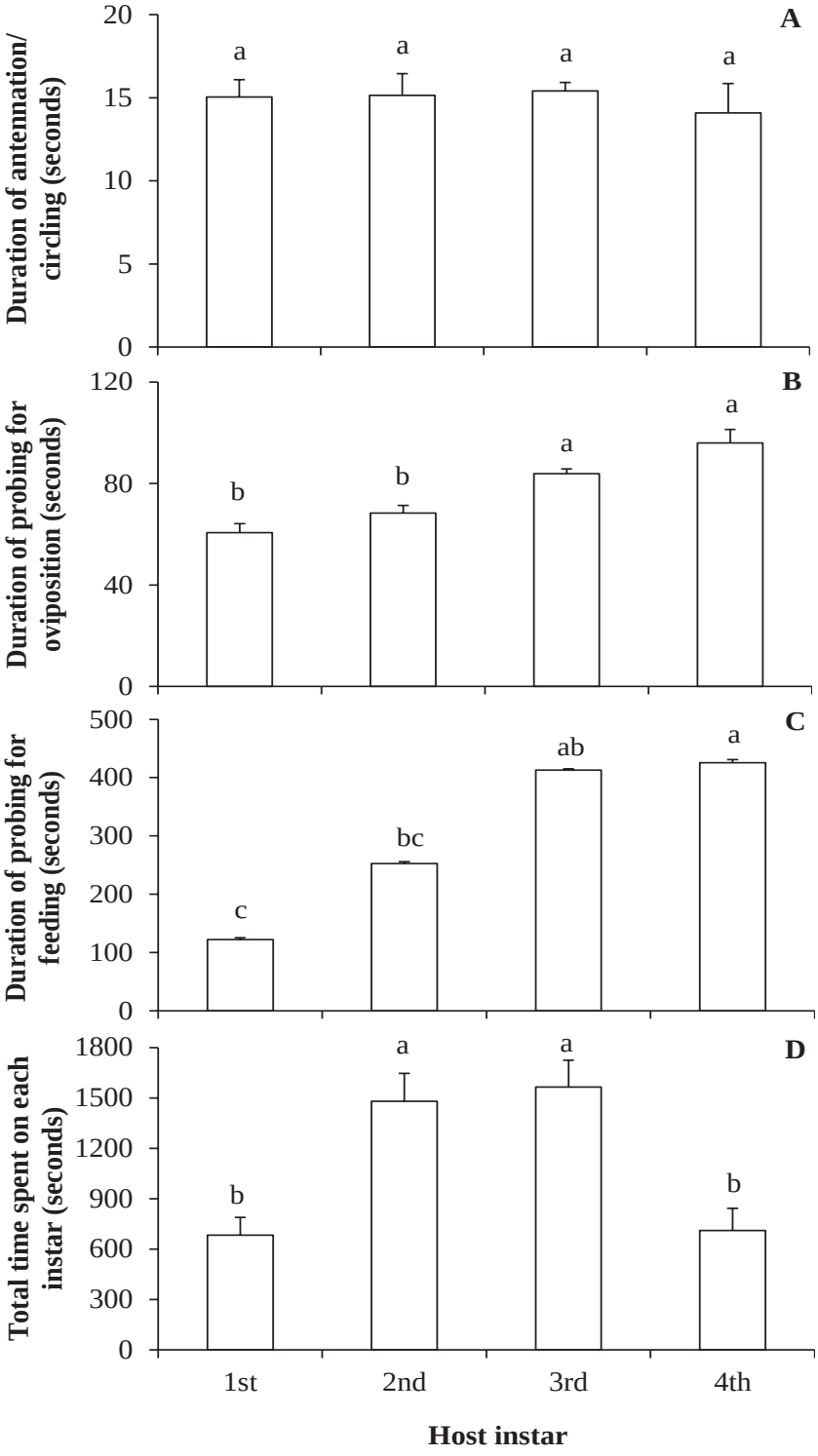

Figure 4.2 Mean duration of antennation/circling (A), probing for oviposition (B), probing for host feeding (C) and total time spent on each instar (D) by *E. warrae* in the non-choice test. Columns with the same letters are not significantly different (P > 0.05).

4.2.4 Discussion

Some parasitoid species do not respond to the host until they physically contact it (van Lenteren et al. 1976; Drost et al. 2000). In the present study, *E. warrae* did not respond to the host until she touched the host with her antennae, suggesting that the parasitoid uses her sensory organs to recognize the host (van Roermund & van Lenteren 1995). Parasitoids' chance of finding a host correlates significantly with the host size (van Lenteren et al. 1976). Present results also show that in both choice and non-choice tests, *E. warrae* encountered and found the larger hosts (4th instar nymphs) more often, suggesting that the parasitoids perceive larger hosts more easily than smaller ones. This agrees with the findings of van Lenteren et al. (1976) on *En. formosa*. It is also possible that the larger hosts emit more odours (semiochemicals) from their body (van Roermund & van Lenteren 1995; Ardeh 2004) so that the parasitoids are more likely to find them.

In both tests, the parasitoids parasitised significantly more intermediate hosts than younger and older ones (Tables 4.1 & 4.2). These results suggest that the oviposition preference correlates with the host stage that maximises her offspring fitness. Although the oldest (4th instar) hosts contain more resources for development and fitness of her offspring (Godfray 1994; Hu et al. 2002; Harvey et al. 2004), *E. warrae* preferred the 2nd and 3rd instars because of higher survival and reproductive fitness of her offspring developing from these nymphs (Hardy et al. 1992; Murdoch et al. 1997; Qui et al. 2004, 2005; Unpubl. data).

In *Eretmocerus* spp. host feeding results in killing of the hosts, therefore, the theory of optimal host feeding strategies suggests that a parasitoid should feed on and oviposit under different host stages (Kidd & Jervis 1991). However, in contrast to the suggested theory, my results show that in both non-choice and choice tests *E. warrae* successfully parasitised and fed on all four instars of nymphs offered but preferred the 2nd and 3rd instars for feeding and egg laying. This suggests that the harder exoskeleton of the 4th instar nymphs and less fluid and lower quality of the 1st instar nymphs prevent the parasitoid from using these instars for feeding (Kidd & Jervis 1991; Ardeh 2004; Personal observation).

In many parasitoids, time is a crucial factor which can be used to determine their host stage preference (Drost et al. 1999). In the present study, though the mean time for

probing was significantly longer in older nymphs, the total time spent by *E. warrae* was significantly longer on the 2nd and 3rd instar nymphs (Figs. 4.1C & 4.3E). This may be because *E. warrae* parasitises and feeds on significantly more 2nd and 3rd instar nymphs (Tables 4.1 & 4.2), resulting in longer time spent on these instars.

The findings of this study reveal that *E. warrae* shows an adaptive preference toward the 2nd and 3rd instar nymphs for oviposition and feeding which may result in higher survival of both adult and its progeny. In order to improve mass rearing techniques *E. warrae* should be reared and released in the field on the 2nd and 3rd instar whitefly nymphs.

4.3 Superparasitism and Host Discrimination Behaviour in *E. warrae*

4.3.1 Introduction

Host discrimination is the ability of a parasitoid to distinguish an unparasitised host from parasitised one and to reject the latter for egg laying (Salt 1934). Such ability is considered to be useful for survival of progeny (Doutt 1959). It also saves the foraging time and reduces the risks associated with the host defence, e.g., encapsulation (reviewed by van Alphen & Visser 1990). Although this ability is widespread among hymenopteran parasitoids, superparasitism is common in nature (van Lenteren et al. 1978; Bakker et al. 1985; reviewed by van Alphen & Visser 1990; Fatouros et al. 2005).

Superparasitism, previously considered to be maladaptive by parasitoids, is now believed to be an adaptive behaviour (Speirs et al. 1991). In solitary parasitoids, superparasitism can delay the progeny development, increase the larval mortality, and result in poor offspring fitness (e.g. Potting et al. 1997; Vet et al. 1994; Jones et al. 1999). However, the evolutionary stable strategy (ESS) predicts that under certain conditions solitary parasitoids switch from rejecting parasitised hosts to superparasitise them (Visser et al. 1992b). This can be adaptive for conspecifics under a wider range of conditions due to the probability of elimination of the non-sibling competitor from the parasitised host (Visser et al. 1992b). However, in case of superparasitism by the same female, siblings compete for the resources (Weisser & Houston 1993). Therefore, the same female should always avoid to superparasitise but in host depleted patches and in the presence of other different females, it can be her adaptive strategy to superparasitise (Visser et al. 1992a; Weisser & Houston 1993). In that case, it is highly likely that the host parasitised by her can be attacked by another foraging parasitoid. Therefore, allocating more than one egg under the same host can enhance the possibility that the survivor would be of her own offspring (reviewed by van Alphen & Visser 1990).

In many parasitoid species, the female is able to distinguish between the hosts already parasitised by her and those by other females with the help of markers (reviewed by van Alphen & Visser 1990). These markings may be pheromones deposited by the ovipositing females (Fatouros et al. 2005), physiological changes in the host haemolymph induced by the parasitoid progeny (van Lenteren et al. 1978) or

physical changes in the host surface acting as external markers (Fatouros et al. 2005).

In mass rearing programmes the parasitoids are often reared under crowded conditions (Waage & Godfray 1985), resulting in frequent superparasitism. The objectives of this study were to investigate superparasitism and host discrimination ability of *E. warrae*, which may lead to improving the use of this parasitoid efficiently for the management of whiteflies. Moreover, I also examined whether *E. warrae* used external or internal markers to discriminate parasitised hosts.

4.3.2 Materials and methods

To determine whether *E. warrae* recognised hosts parasitised by herself or by a different female, an experiment was set up with six treatments (Table 4.4) with host densities of 10 or 15 hosts/parasitoid. Only the second instar nymphs were used in this experiment with ten parasitoids for each treatment. For each treatment, a leaf/leaflet infested with the test number of host nymphs was placed into a Petri dish, and a map was drawn to describe the distribution of the hosts. Subsequently, a naïve parasitoid (1st oviposition) was released into the Petri dish and was observed until she probed five nymphs. Parasitoid behaviour was recorded using a recording system (see Section 3.2.1, Fig. 3.3). When she probed a nymph, the location of the nymph was marked on the map. After the female had probed five nymphs, she was removed and all the probed nymphs were gently turned over to confirm the presence of an egg. Then nymphs with eggs underneath were marked on the same map. After 1 or 24 hours, the same experienced parasitoid (SEP), a naive parasitoid (NP) or a different experienced parasitoid (DEP), was released into a Petri dish to record its ability to discriminate parasitised hosts in the first oviposition. She was observed until she probed five nymphs. At the end of the experiment all the probed nymphs were turned over to assess the host discrimination and superparasitism of *E. warrae*.

The following behaviours of the parasitoids in the first and second ovipositions were recorded:

(a) Encounter - the parasitoid meets a host physically;

(b) Rejection of host - the parasitoid walks away from a host after encounter without probing;

(c) Superparasitism – the parasitoid lays eggs under the parasitised hosts;

(d) Total time – time taken by parasitoids to probe five nymphs.

Table 4.4 Six treatments used in the experiment.

First oviposition	Second oviposition	Time interval between the 1st and 2nd ovipositions
NP	NP	After 1 hour
NP	SEP	After 1 hour
NP	DEP	After 1 hour
NP	NP	After 24 hours
NP	SEP	After 24 hours
NP	DEP	After 24 hours

SEP = same experienced parasitoid; NP = naïve parasitoid; DEP = different experienced parasitoid.

4.3.3 Statistical analyses

A goodness-of-fit test was used to test the distribution of data prior to analysis. Data for superparasitism rate after 1 hour at density of 10 hosts, number of encounters, rejection rate and total time spent by each parasitoid to probe five nymphs were normally distributed, and thus analysed using ANOVA followed by the Tukey's test. All other data were not normally distributed even after transformation and thus analysed using the nonparametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons (Zar 1999).

4.3.4 Results

No parasitoid could 100% discriminate between the parasitised and unparasitised nymphs at both host densities. However, at a density of 10 hosts, SEP parasitised significantly lower proportion of parasitised nymphs than did NP and DEF

(after 1 hour: F = 8.64; df = 2,27; P < 0.001; after 24 hours: χ^2 = 11.19; df = 2; P < 0.01) (Table 4.5). At the density of 15 hosts, significantly lower number of ovipositions in parasitised nymphs was performed by the SEP and DEP than NP 1 and 24 hours after parasitisation (χ^2 = 8.89 and 9.44 for 1 hour and 24 hours, respectively; df = 2; P < 0.01) (Table 4.5).

The host density had significant effect on host discrimination and superparasitism of *E. warrae*, with significantly lower number of eggs being allocated by the SEP, NP and DEP in parasitised hosts at the host density of 15 nymphs than at that of 10 nymphs (after 1 hour: χ^2 = 5.18, 12.01 and 12.14 for the SEP, NP and DEP, respectively; df = 1; P < 0.05; after 24 hours: χ^2 = 7.32, 10.56 and 11.56, for the SEP, NP and DEP, respectively; df = 1, P < 0.001).

Table 4.5 Superparasitism (%) of *E. warrae* at different host densities.

Time interval	Parasitoid type	Host density	
		10 hosts	15 hosts
1 hour	SEP	22.00 ± 3.09b	10.50 ± 3.53b
	NP	44.50 ± 3.37a	24.00 ± 1.94a
	DEP	35.50 ± 3.76a	14.50 ± 2.91b
24 hours	SEP	24.50 ± 1.89b	12.50 ± 3.44b
	NP	45.50 ± 3.69a	25.50 ± 2.52a
	DEP	39.00 ± 4.98a	16.00 ± 3.82b

For time interval of 1 or 24 hours, means (± SE) followed by the same letters in columns are not significantly different (P > 0.05). Data from the time interval of 1 and 24 hours were analysed separately. SEP = same experienced parasitoid; NP = naïve parasitoid; DEP = different experienced parasitoid.

At the density of 10 hosts SEP, NP and DEP rejected significantly more hosts in the second oviposition than they did in the first oviposition (after 1 hour: F = 20.86, 18.35 and 12.56 for the SEP, NP and DEP, respectively; df = 1,18; P < 0.002; after 24

hours: F = 13.89, 8.62 and 6.76 for the SEP, NP and DEP, respectively; df = 1,18; P < 0.01) (Fig. 4.3A). The parasitoids encountered significantly more number of hosts in the second oviposition than in the first oviposition (after 1 hour: F = 23.92, 17.31 and 13.20 for the SEP, NP and DEP, respectively; df = 1,18; P < 0.001; after 24 hours: F = 15.97, 19.89 and 13.20 for the SEP, NP and DEP, respectively; df = 1,18; P < 0.01) (Fig. 4.3B). Eventually, parasitoids spent significantly more time in probing five nymphs during the second oviposition than in the first oviposition (after 1 hour: F = 7.52, 7.31 and 8.87 for the SEP, NP and DEP, respectively; df = 1,18; P < 0.01; after 24 hours: F = 6.83, 8.70 and 6.58 for SEP, NP and DEP, respectively; df = 1,18; P < 0.01) (Fig. 4.3C).

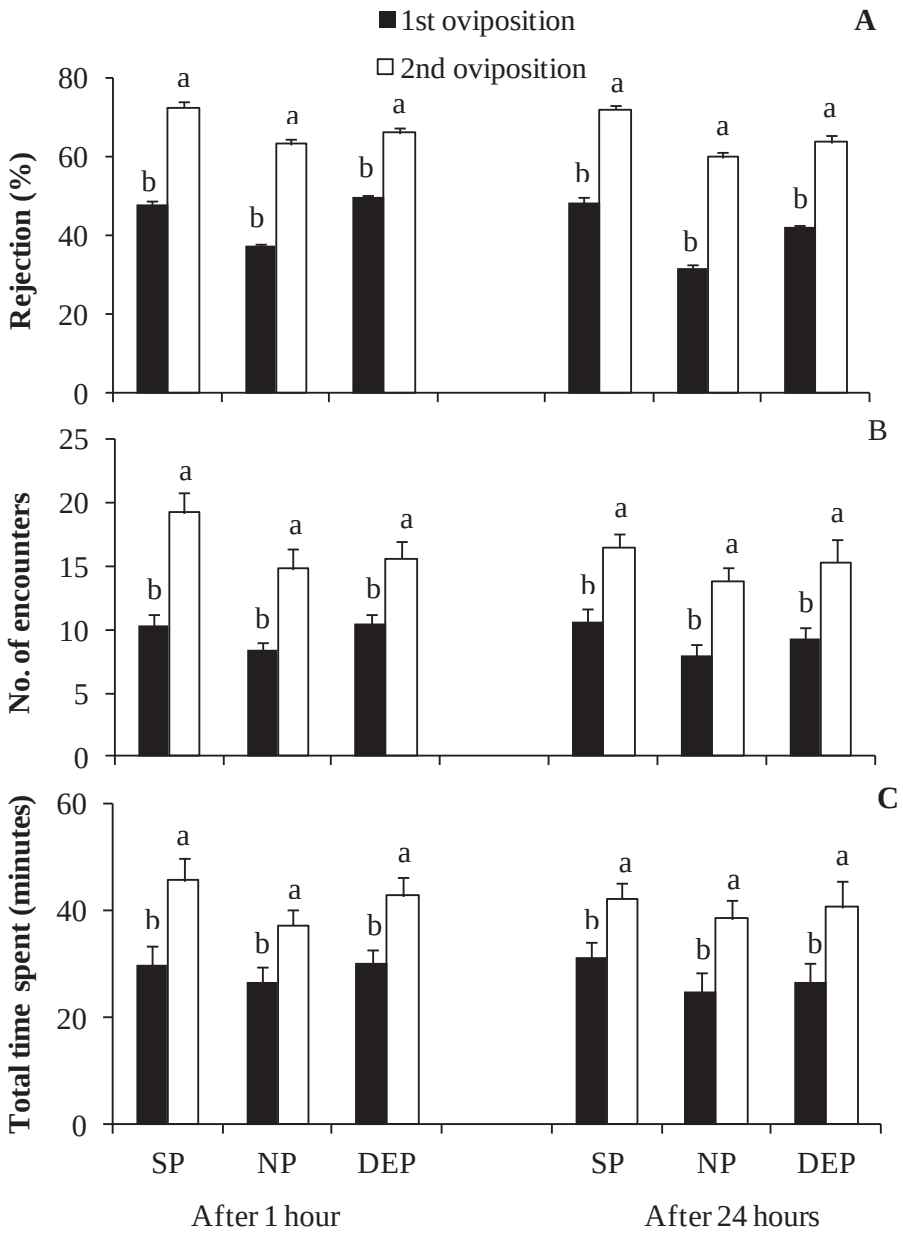

Figure 4.3 Rejection rate (A), number of encounters (B) and total time spent (C) by *E. warrae* in the first and second oviposition at the density of 10 hosts after 1 and 24 hours of oviposition. SEP = same experienced parasitoid; NP = naïve parasitoid; DEP = different experienced parasitoid. Columns with the same letters in each category are not significantly different (P > 0.05).

4.3.5 Discussion

In solitary parasitoids host discrimination is never absolute (van Lenteren et al. 1978; Bakker et al. 1985). In the present study, *E. warrae* superparasitism rate was significantly higher at the lower host density than at the higher host density (Table 4.5). This suggests that superparasitism is common under the situations when a limited number of hosts is available to the parasitoid for oviposition. Moreover, at the lower host density *E. warrae* self-superparasitism might be adaptive and advantageous to the parasitoid when the probability of a host being attacked by another parasitoid is high. Laying two or more eggs in a host parasitised by herself increases the possibility of survival of her offspring from that host (reviewed by van Alphen & Visser 1990). However, it is always an evolutionary stable strategy (ESS) for a parasitoid to employ con-specific (different) superparasitism when she senses the presence of other parasitoids in the same patch (reviewed by van Alphen & Visser 1990).

The present study shows that superparasitism rate by the naïve *E. warrae* was significantly higher than the experienced ones (Table 4.5), indicating that the naïve parasitoids have limited capacity to discriminate between the parasitised and unparasitised hosts. Ardeh (2004) also report that the naïve *E. eremicus* and *E. mundus* frequently lay eggs under the parasitised hosts. Godfray (1994) suggests that if the number of hosts available for oviposition is less than the potential egg load of the parasitoid, it can be advantageous for the parasitoid to superparasitise the host. *E. warrae* is a pro-synovigenic species, which emerges with a high number (30-35) of mature eggs (Section 5.5.3.2, Table 5.2). Therefore, the higher egg load of naïve parasitoids may encourage them to lay eggs under the parasitised hosts, as the higher egg load in the parasitoids increases the probability of superparasitism (Keasar et al. 2006). It is also possible that when they find only parasitised hosts at the first encounter, they secure at least some of the offspring in the first visiting patch by superparasitisation (Bakker et al. 1985). Therefore, the significant higher superparasitism rate of *E. warrae* at lower host density may be due to the higher probability of females encountering parasitised hosts.

Many parasitoid species often deposit marking pheromones as an indication to themselves and other females that the host has been parasitised (Salt 1934; van Alphen & Visser 1990; Ardeh 2004; Buckner & Jones 2005). My study also suggests that *E. warrae* females mark the host after oviposition. Buckner & Jones (2005) report that *E.*

mundus applies the chemicals (dimethylalkanes) to its host *B. argentifolii*, which prevent other parasitoids to use the same host again.

In most parasitoids time is a limiting factor, which can be applied to determine the parasitoid's willingness to accept the host for oviposition (Bakker et al 1985; Drost et al. 1999). In the present study, *E. warrae* spent significantly more time in the second oviposition than in the first oviposition (Fig. 4.3C). The longer searching time in the second oviposition may be attributed to the higher rejection rate and greater number of hosts being encountered by the parasitoids (Figs. 4.3A & B). It is possible that when the density of unparasitised hosts is lower, the second parasitoids spend longer time in host searching rather than accepting the parasitised hosts for oviposition. Several studies have also suggested that when parasitoids are forced to stay in a patch with low density of unparasitised hosts for a long time, they utilize the patch with lower marginal value, resulting in longer search time and higher superparasitism (van Lenteren et al. 1978; Bakker et al 1985; van Alphen & Visser 1990; Montoya et al. 2000).

Results of the present study have increased our knowledge of superparasitism and host discrimination behaviour of *E. warrae*, which is an important aspect for evaluating the efficiency of a biological control agent (Ardeh 2004). For example, when a sufficient number of hosts is available for oviposition, host discrimination can be an adaptive strategy of *E. warrae* which enables a female to avoid superparasitism and thus reduce the food competition among her offspring. However, when a limited number of hosts is available, particularly when the host patch has been utilized by other females, superparasitism will allow at least some of her offspring to survive.

4.4 Host Feeding and Oviposition Behaviour of *E. warrae* After a Certain Period of Host and Food Deprivation

4.5.1 Introduction

Many aphelinid genera, such as *Eretmocerus*, *Aphytis*, *Aphelinus*, *Coccophagus*, and *Encarsia*, are considered to be important biological control agents of whiteflies through their reproductive as well as host-feeding activities (Hoddle et al. 1998; Qiu et al. 2004; Zang & Liu 2008). These parasitoids are non-concurrent destructive host feeders and superior biological control agents to non-host feeders (Jervis & Kidd 1986; Jervis & Copland 1996). In these parasitoids tendency to host feeding increases with (1) declining egg load (Heimpel & Rosenheim 1995), (2) declining nutritional reserves and (3) increasing probability of survival (Chan & Godfray 1993; Heimpel & Rosenheim 1995). Apart from these factors, food and host deprivation for a certain period of time affects the host feeding and oviposition behaviour and egg resorption in many parasitoids (Legner & Gerling 1967; Collier 1995). For example, in *Coccophagus bartlettz* Annecke & Insley (Hymenoptera: Aphelinidae) egg resorption occurs after 10 days of host deprivation (Walter 1988). In *E. sofia*, host and food deprivation up to 6 hours increases the host feeding, parasitism and longevity of adults (Zang & Liu 2009). However, in *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammitidae), it reduces parasitism but does not affect longevity (Fleury & Bouletreau 1993).

Host shortage is likely to occur in the field and laboratory, which can affect the efficiency of a parasitoid as a biological control agent. Understanding an optimal duration of food and host deprivation of a parasitoid would help improve its host feeding and parasitising efficiency. So far, no information on the optimal duration of food availability and host deprivation of *E. warrae* is available. The objectives of the present study were to determine the effect of food availability and host deprivation after a certain period of time on host feeding and oviposition behaviour, and fecundity and longevity of *E. warrae*.

4.4.2 Materials and methods

4.4.2.1 Effect of food and host deprivation on host feeding and oviposition behaviour and longevity

To determine whether and how certain duration of food availability and host deprivation after emergence affected feeding and oviposition behaviour of *E. warrae*, seven treatments were set up: 0, 5, 10, 15, 20 and 25 hours of food deprivation, and 24 hours of honey feeding before being used for the experiment. Ten parasitoids (replicates) were used for each treatment. For each replicate, one parasitoid was released into a Petri dish with a fresh leaf infested by 100 2nd instar nymphs, allowed to stay for 24 h, and then moved into another Petri dish containing the same number of nymphs. This process was repeated until she died. The fecundity and parasitism of *E. warrae* were determined by turning over all the nymphs and counting the number of eggs laid in each Petri dish. Host feeding was recorded if the nymph body fluid was found to have effused as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs. The longevity of adult parasitoids was also recorded.

4.4.2.2 Effect of food and host deprivation on egg resorption

To determine the effects of food and host deprivation on egg resorption of *E. warrae*, two experiments were set up. In the first experiment, nine treatments were set up: 0, 5, 10, 15, 20, 25, 48 and 72 hours of food deprivation, and 24 hours of honey feeding of parasitoids before being dissected for the experiment. In treatments 1 to 8, the parasitoids were reared individually in glass vials without food and host while in the treatment 9, the parasitoids were reared individually in Petri dishes and fed with 10% honey solution. Honey solution was provided in a cotton wick inserted into the Petri dish through a 1 cm hole in the lid. For each treatment, a newly emerged parasitoid (0 h after emergence) was released into a Petri dish and dissected after 0, 5, 10, 15, 20, 25, 48 and 72 hours of food and host deprivation, and 24 hours of honey feeding. They were dissected in 70% alcohol on a slide under the stereomicroscope. One drop of 1% acetocarmine was added to the alcohol and allowed to stand for 3 to 5 minutes for staining. The chorion of mature eggs prevents the stain but immature eggs absorb the stain (Edward 1954). The number of mature eggs in the ovaries was then counted under a compound microscope to record the effects of food and host deprivation and 24 hours

honey feeding on egg resorption in *E. warrae*. Ten parasitoids were dissected for each treatment.

In the second experiment, 120 parasitoids (0 h after emergence) were reared individually in Petri dishes and fed with 10% honey solution. The honey solution was provided in a cotton wick as described above. Ten parasitoids were then selected randomly and dissected daily for up to ten days. They were dissected in 70% alcohol as described above. Those parasitoids which died naturally during the experiment were discarded from the experiment. The number of mature eggs in the ovaries was then counted under a compound microscope to record the effects of honey feeding on egg development and resorption in *E. warrae*.

4.4.2.3 Statistical analyses

A goodness-of-fit test was used to test data normality. All data were normally distributed, and thus analysed using ANOVA followed by Tukey's test.

4.4.3 Results

4.4.3.1 Effect of food and host deprivation on host feeding and oviposition behaviour and longevity

Food and host deprivation for a certain period of time significantly affected the longevity and host feeding behaviour of *E. warrae* (Figs. 4.4 & 4.5). The results show that the parasitoids deprived of food and hosts for 5 hours laid significantly more eggs, lived significantly longer and fed on and parasitised significantly more hosts ($F = 33.69$, 44.27, 32.95 and 33.86, for number of eggs laid, longevity, number of hosts fed on and parasitised, respectively; $df = 6,63$; $P < 0.0001$) (Fig. 4.4). However, no significant difference was found in the longevity between the parasitoids deprived of food and hosts for 5 hours and those fed with honey for 24 hours (Fig. 4.4D).

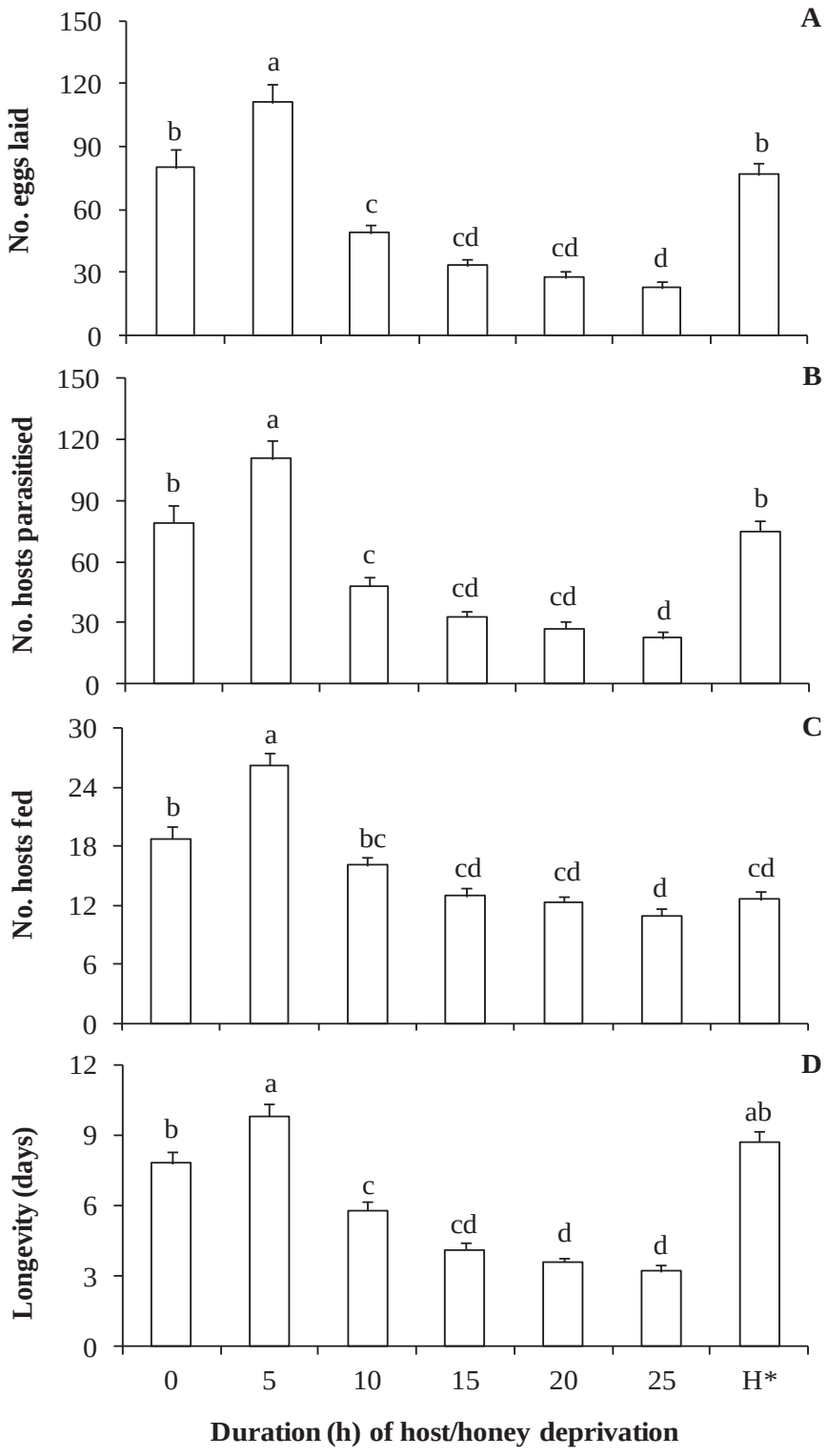

Figure 4.4 Effect of host and food deprivation on the total number of eggs laid (A), hosts parasitised (B), hosts fed (C), and longevity (D) in *E. warrae*. Columns with the same letters are not significantly different (P > 0.05). H* = 10% honey feeding for 24 hours.

The average daily host feeding rate significantly increased with the increase in food and host deprivation period from 5 to 25 hours while it was significantly lower in 24-hour honey-fed parasitoids ($F = 16.20$; $df = 6,63$; $P < 0.0001$) (Fig. 4.5A). However, the parasitism rate was significantly higher in the parasitoids deprived of food and host for 5 hours followed by the newly emerged parasitoids and 24-hour honey-fed parasitoids ($F = 6.79$; $df = 6,63$; $P < 0.0001$) (Fig. 4.5B).

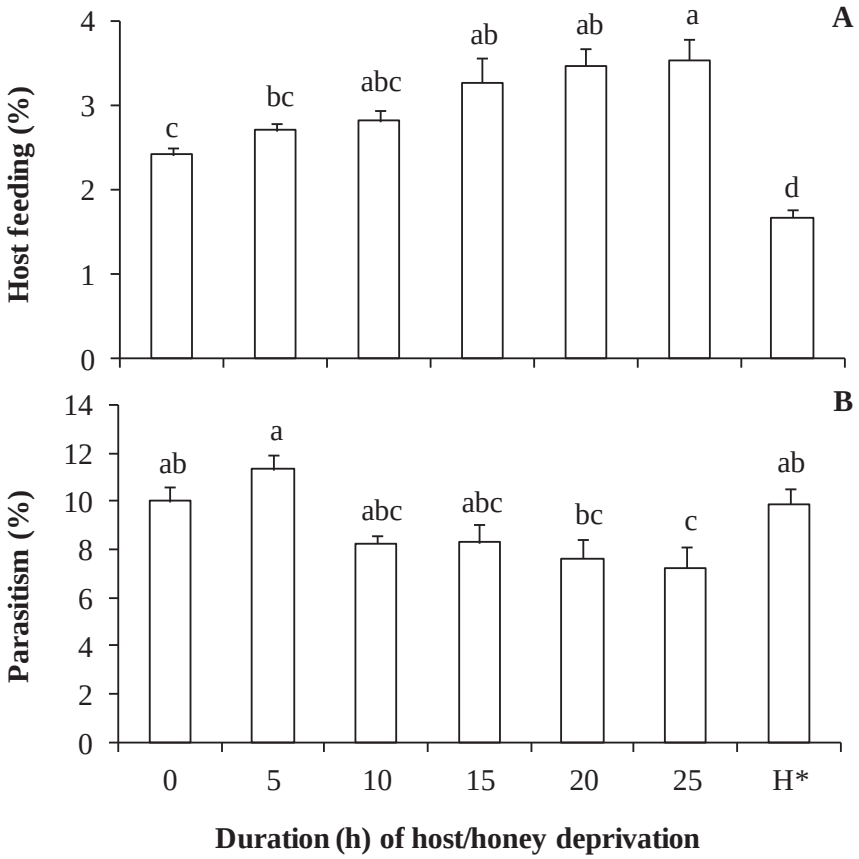

Figure 4.5 Effect of host and food deprivation on the average daily host feeding (A) and parasitism (B) in *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$). H* = 10% honey feeding for 24 hours.

4.4.3.2 Effect of food and host deprivation on egg resorption

No significant egg resorption was detected after up to 2 days of food and host deprivation but the number of mature eggs significantly decreased after 3 days of food and host deprivation in *E. warrae* ($F = 4.75$; $df = 8,81$; $P < 0.0001$) (Fig. 4.6). However,

in honey fed parasitoids, the number of mature eggs significantly decreased after 6 days of host deprivation ($F = 15.55$; $df = 9,89$; $P < 0.0001$) (Fig. 4.7).

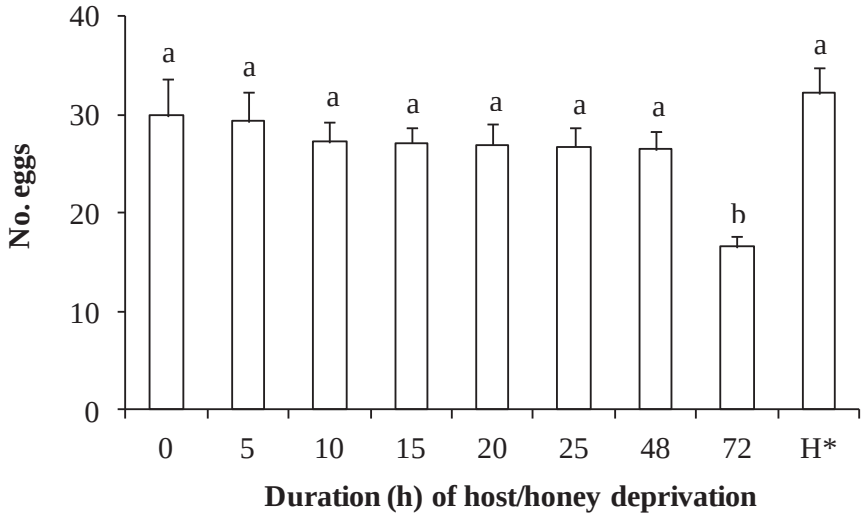

Figure 4.6 Effect of host and food deprivation and 24 hours honey feeding on egg load in *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$). H* = 10% honey feeding for 24 hours.

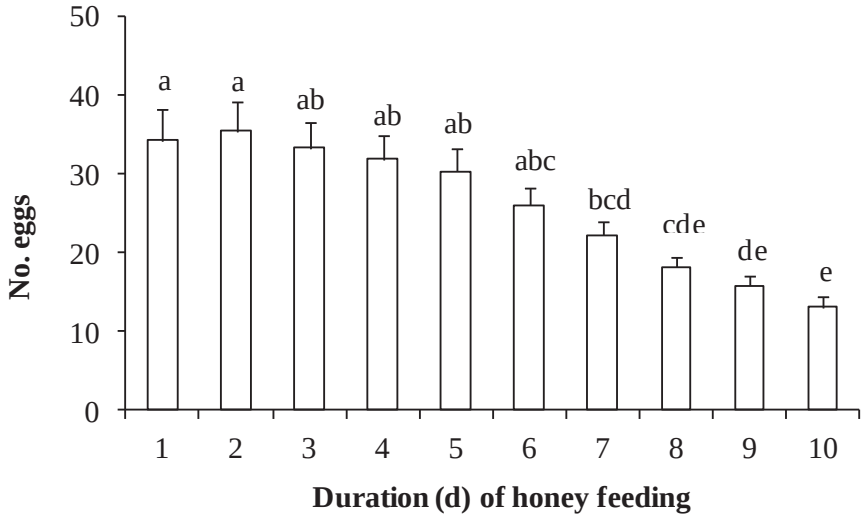

Figure 4.7 Effect of honey feeding on egg resorption in *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$).

4.4.4 Discussion

Many insects modify their feeding behaviour in response to food and host deprivation for a certain period of time (Legner & Gerling 1967; Hickman et al. 2001). The present study indicates that *E. warrae* fed on significantly more hosts when deprived of food and hosts for 5 hours. These results suggest that food and host deprivation for 5 hours is the optimum period for *E. warrae* host feeding. Similar results are reported for *E. melanoscutus* (Zang & Liu 2010).

Numerous studies have demonstrated that host feeding has a linear relationship with the fecundity and longevity of parasitoids (Jervis & Kidd 1986; Heimpel & Collier 1996; Giron et al. 2004). In the present study, fecundity, parasitism, and longevity of *E. warrae* also significantly increased if it was deprived of food and hosts for 5 hours, suggesting that the increased host feeding may have a positive impact on the fecundity and longevity. Feeding on host haemolymph by the parasitoids provides lipids and carbohydrates necessary for higher fecundity and greater longevity of adult parasitoids, such as in *Eupelmus vuilletti* (Crowford) (Hymenoptera: Eupelmidae) (Giron et al. 2004).

The present study also indicates that although after >5h of food and host deprivation the daily host feeding rates significantly increased (Fig. 4.5), the longevity and consequently fecundity and parasitism significantly decreased in *E. warrae* (Figs. 4.4A, B & D). Similarly, *T. brassicae* reduces parasitism when starved for 4 days (Fleury & Bouletreau 1993). These results imply that more than 5 hour starvation period is not favourable for *E. warrae* longevity, resulting in overall reduced fecundity. Sahragard et al. (1991) also demonstrated that decreased fecundity of *Dicondylus indianus* Olmi (Hymenoptera: Dryinidinae) is a positive function of decreased longevity.

In pro-ovigenic or pro-synovigenic species, certain time of food and host deprivation has serious consequences because egg resorption may occur during that period of deprivation (Jervis & Kidd 1986). However, in the present study, no significant differences were found in egg load of *E. warrae* after up to two days of host deprivation and 24 hours of honey feeding (Fig. 4.6). These results suggest that *E. warrae* can maintain its egg load for up to this period of deprivation, and honey feeding does not increase its egg production. However, when fed with 10% honey solution with

no hosts, egg resorption was detected after 5-7 days, suggesting that in the presence of honey *E warrae* maintains its egg load up to 5-7 days. Likewise, Walter (1988) found that *C. Bartlettz* retains its egg load for up to 10 days when fed with honey.

In conclusion, host feeding behaviour of *E. warrae* is influenced by initial food/host deprivation period after emergence. Food and host deprivation for 5 h is found to be the optimal period for host feeding, fecundity and longevity in *E. warrae*. To enhance biological control efficiency of *E. warrae*, emerging parasitoids may be kept without food and hosts for 5 hours and then released in the field or laboratory. Moreover, when hosts are rare or temporarily absent in a mass-rearing or biological control programme, *E. warrae* can be reared on honey solution for 5-7 days without egg depletion.

CHAPTER 5

Factors Affecting Reproductive Fitness of *Eretmocerus warrae*

5.1 General Introduction

In hymenopteran parasitoids, reproductive success such as fecundity and longevity of adults, and development, survival, and reproductive fitness of their offspring is determined by a number of factors (Sahragard et al. 1991; Mills & Lacan 2004; Tripathi & Singh 1991; Bellamy et al. 2004; Hu et al. 2003; He et al. 2006). Among these, host stage (Jones & Greenberg 1998), parasitoid and host density (Sahragard et al. 1991; Mills & Lacan 2004; Chong & Oetting 2006) and supply of food (Leatemia et al. 1995; McAuslane & Nguyen 1996; Hardin et al. 2008) are the most important.

Host stage and size reflect the quality of the hosts for parasitoid development (He et al. 2006; Karut 2007; Da Rocha et al. 2007). Generally, larger hosts contain more resources and give rise to larger offspring than do smaller ones, and thus are usually preferred by parasitoids for oviposition (Godfray 1994; Hu et al. 2002; Harvey et al. 2004). Many studies show that large body size of emerging progeny has strong positive correlation with life history parameters such as reproductive success and longevity (Jervis & Copland 1996; Murdoch et al. 1997; Harvey et al. 2004). Larger females bear more mature eggs and live longer due to the greater energy reserves (Harvey et al. 2004). As a result, host stage choice is considered an important determinant of parasitoid fitness (Hågvar & Hofsvang 1991).

Both host and parasitoid densities influence the reproductive potential of parasitoids such as fecundity and parasitism (Hoddle et al. 1999; Montoya et al. 2000; Mills & Lacan, 2004; Chong & Oetting 2006). In general, increasing host density positively affects parasitism and progeny fitness (He et al. 2006) while increasing parasitoid density negatively influences adult fecundity and progeny fitness (Jones et al. 1999; Chong & Oetting 2006). Studies also suggest that increasing host density increases the parasitoid-host encounter rate which has a positive effect on the fecundity and host feeding of parasitoids (Sahragard et al. 1991). However, increasing parasitoid density increases superparasitism, which has significantly negative effect on the reproductive fitness of parasitoid offspring (Jones et al. 1999; Chong & Oetting 2006).

In many hymenopteran parasitoids, longevity and fecundity are also influenced by the presence of food (Jervis & Kidd 1986). For example, *E. eremicus* lives significantly longer when fed with carbohydrate diets (Hardin et al. 2008). Some parasitoid species that feed on hosts may require sugars as their main source of energy during the adult stage. For example, provision of honey and host significantly increases longevity and fecundity of *A. melinus* (Heimpel & Collier 1996).

The aim of this chapter was to provide critical information on the effect of different factors on the reproductive potential of *E. warrae*, with the following objectives: (1) to determine the effects of host and parasitoid densities on adult fecundity, host feeding and longevity, and progeny fitness; (2) to examine the effects of host stages on adult fecundity, host feeding and longevity, and development, survival, and reproductive fitness of offspring, and (3) to investigate effects of food supply on fecundity, host feeding and longevity of adult *E. warrae*.

5.2 Functional Response of *Eretmocerus warrae* under Different Host Densities

5.2.1 Introduction

It is an important objective of ecologists that study parasitoids to establish the attributes that make them successful biological control agents (Fernandez-arhex & Corley 2003). Among these attributes parasitoids' response to varying host density, the functional response, is an important aspect of evaluating the effectiveness of a natural enemy (Holling 1959). Three types of functional response of parasitoids to their host density are proposed by Holling (1959): (1) a parasitoid attacks its host at a constant rate which increases linearly with the increase in host density; (2) a parasitoid attack rate increases non-linearly with increasing host density; and (3) a sigmoid response where attack rate initially increases, then decreases and subsequently increases with the increase in host density, reaching an upper asymptote. The functional response plays a vital role in understanding the host-parasitoid dynamics (Hassell & Waage 1984) and the shape of its response can be useful in assessing the potential of a particular parasitoid in biological control programmes (Murdoch & Briggs 1996).

Hymenopteran parasitoids can increase their fecundity and alter the sex ratio of their progeny in response to host density (Waage 1982; Wang & Keller 2005). For example, *A. ervi*, a parasitoid of pea aphid, parasitises a higher number of hosts and allocates a higher proportion of female progeny in response to higher host densities (He et al. 2006). However, in parasitoid species that use their hosts for both oviposition and feeding (Jervis & Kidd 1986; Heimpel & Collier 1996), their decision to oviposit or feed is governed by several factors depending upon the host and parasitoid itself (Lauzière et al. 1999). Egg load, maturation delay and life expectancy are parasitoid-related while host quality and quantity are host-related factors (Sahragard et al. 1991; Rosenheim & Rosen 1992; Heimpel & Collier 1996). Some studies suggest that host feeding is density-dependent and the proportion of hosts fed upon by parasitoid decreases with increasing host density (Kidd & Jervis 1989). However, other studies demonstrate that host feeding is density-independent (van Lenteren et al. 1996; Sahragard et al. 1991) and oviposition is density-dependent where parasitoids respond positively to increasing host densities (Lauzière et al. 1999).

According to the classification of Jervis and Kidd (1986), *E. warrae* is a pro-synovigenic parasitoid that has destructive host feeding behaviour. At emergence, its ovaries contain a limited number of mature eggs; therefore, in order to mature oocytes, it must feed on the host haemolymph (Heimpel & Collier 1996; Heimpel et al. 1997). The objectives of this study were to investigate the influence of host density on the fecundity, host feeding, parasitism, superparasitism and longevity of *E. warrae*, information of which would help determine its release rates against whiteflies.

5.2.2 Material and methods

5.2.2.1 Experiments

To determine whether and how host density affected host feeding, fecundity, parasitism and longevity of *E. warrae*, I set up seven host densities: 20, 40, 60, 80, 100, 120 and 140 second instar nymphs of *T. vaporariorum*. Ten females (ten replicates) were used for each treatment. For each replicate, one female parasitoid (< 12 h) was released into a Petri dish with a fresh leaf infested by a test number of nymphs, allowed to stay for 24 h, and then moved into another Petri dish containing the same number of nymphs. This process was repeated until she died. As *E. warrae* place their eggs between the venter of nymphs and leaf surface (Hanan et al. 2009, 2010), all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope. The daily and lifetime oviposition and host feeding patterns were determined by counting the number of eggs laid and hosts fed upon by the parasitoid. Host feeding was recorded if the nymph body fluid was found to have effused as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs (Hanan et al. 2010). The longevity of adult parasitoids was also recorded.

5.2.2.2 Statistical analyses

A goodness-of-fit test was used to test data normality. All data were normally distributed, and thus analysed using ANOVA followed by Tukey's test. The percentage data were arcsine transformed prior to analysis but non-transformed means were given.

5.2.3 Results

The results show that with the increase of host density the parasitoids laid significantly more eggs, lived significantly longer, and fed on and parasitised significantly more hosts ($F = 23.01, 8.44, 16.81$ and 27.83, for number of eggs laid, longevity, number of hosts fed on and parasitised, respectively; $df = 6,63$; $P < 0.0001$) (Fig. 5.1).

The host feeding, superparasitism and parasitism rates were significantly higher in lower host densities of 20 and 40 nymphs than in higher host densities of 60-140 nymphs ($F = 82.34, 55.12$ and 54.86, host feeding, superparasitism and parasitism rates, respectively; $df = 6,63$; $P < 0.0001$) (Fig. 5.2).

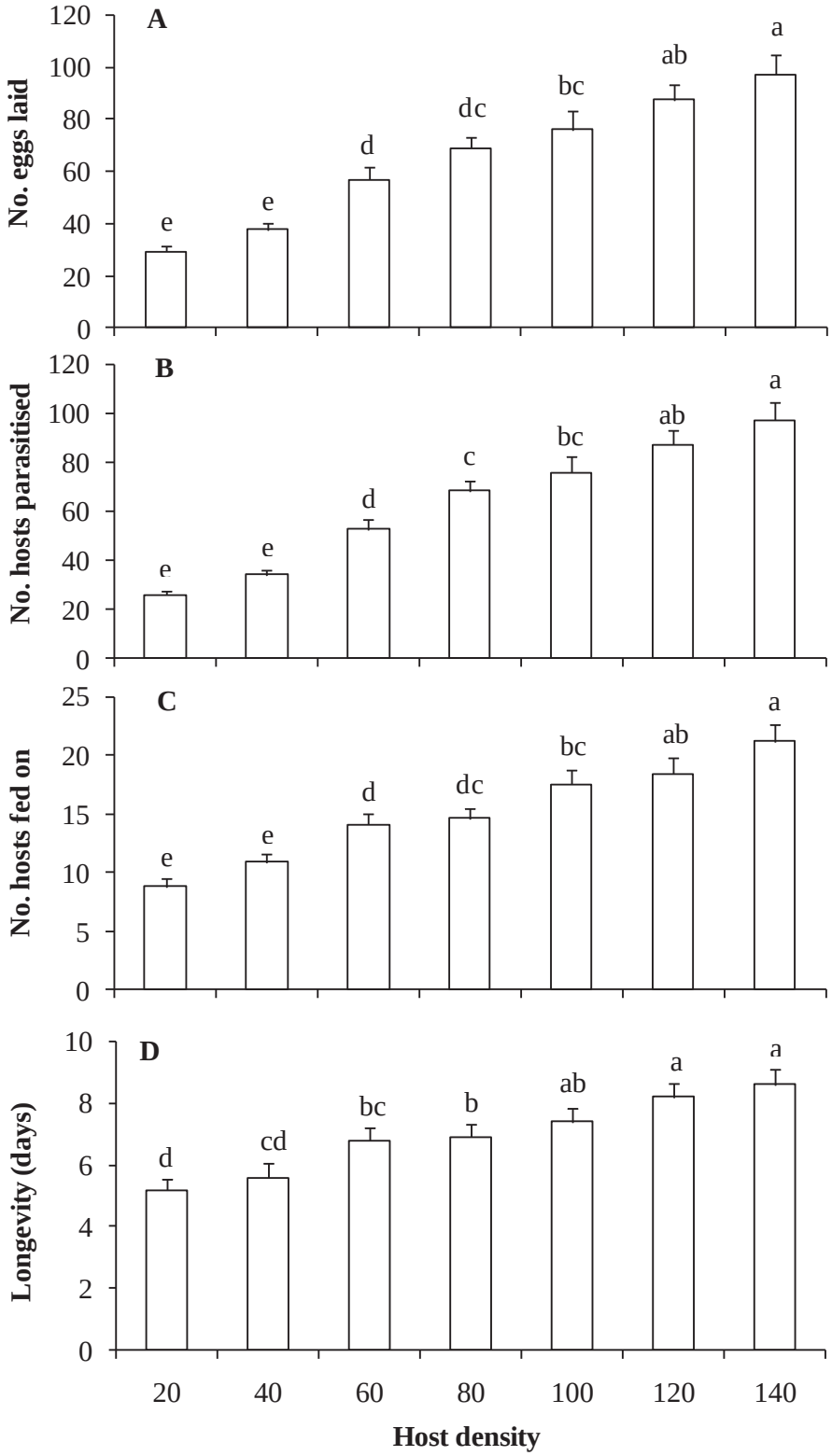

Figure 5.1 Effect of host density on the number of eggs laid (A), number of hosts parasitised (B), number of hosts fed (C), and longevity (D) in *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$).

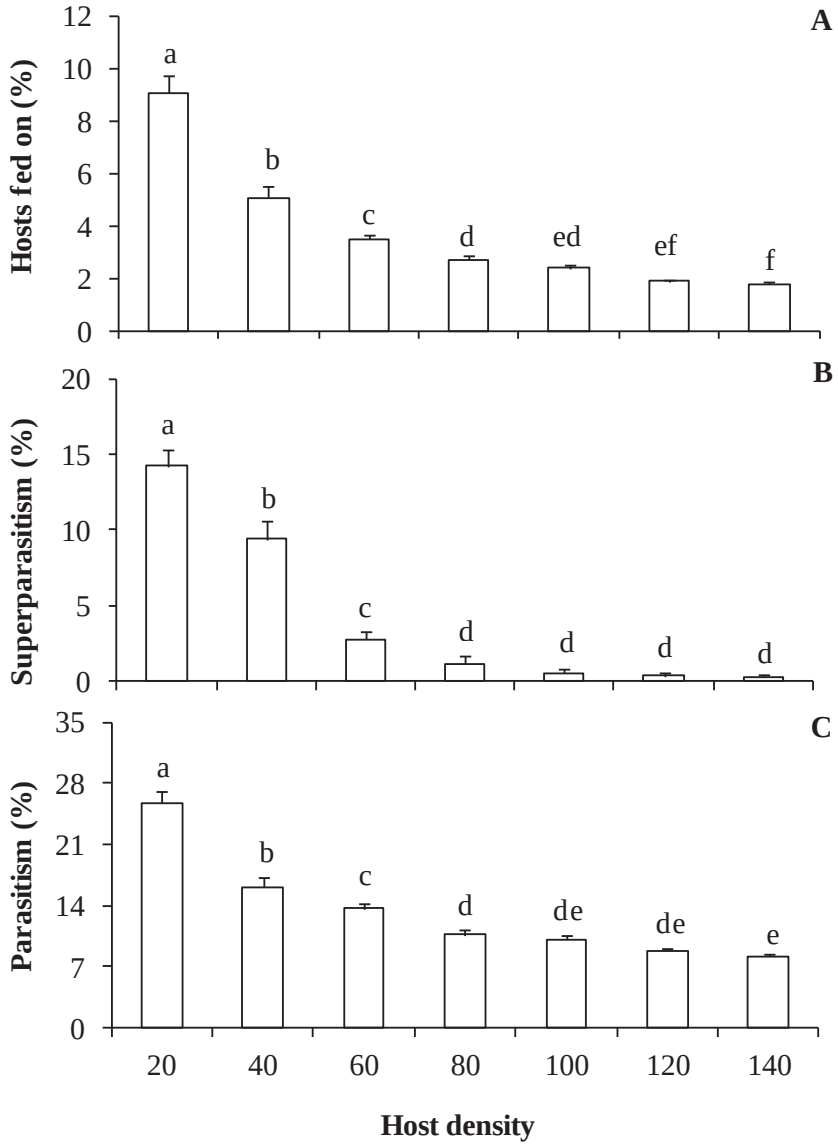

Figure 5.2 Effect of host density on host feeding (A), superparasitism (B) and parasitism rates (C) in *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$).

5.2.4 Discussion

Egg load, host stage and host availability influence the oviposition and host feeding behaviour of many parasitoid species (Rosenheim & Rosen 1992; Heimpel & Collier 1996; Hansen & Jensen 2002). In the present study fecundity, parasitism, host feeding and longevity significantly increased with the increase of host density in *E. warrae* (Fig. 5.1). These results suggest that at higher host densities the parasitoid

obtains more energy by host feeding for egg development and maturation as well as prolonged longevity. Sahragard et al. (1991) also report that in *Dicondylus indianus* Olmi (Hymenoptera: Dryinidinae) increased fecundity at higher host densities is a positive function of increased longevity. Furthermore, host haemolymph fed by adults may provide lipids and carbohydrates necessary for higher fecundity and greater longevity of adult parasitoids in *Eupelmus vuilletti* (Crowford) (Hymenoptera: Eupelmidae) (Giron et al. 2004).

However, the proportion of hosts fed on, parasitised and superparasitised significantly decreased with the increase of host density in *E. warrae* (Fig. 5.2). This pattern is consistent with a type II functional response (Holling 1959), which is also founded in *A. ervi* (He et al. 2006), *E. mundus* (Zandi-Sohani et al. 2008) and *Encarsia acaudaleyrodis* Hayat (Shishehbor & Zandi-Sohani 2011). These results suggest that *E. warrae* can adjust its feeding and oviposition strategy according to host availability.

The results imply that *E. warrae* can be a good candidate for the control of greenhouse whitefly because it significantly increases its feeding, fecundity and parasitism with the increase of host density. However, the highest parasitism and host feeding rates are found when the host density is 20 nymphs, suggesting that 20 *T. vaporariorum* nymphs per *E. warrae* adult is a good starting point for determining the optimal rearing density and release rate for augmentative releases.

5.3 Effect of Parasitoid and Host Density on Reproductive Success of Adult and Progeny Fitness in *E. warrae*

5.3.1 Introduction

Functional response has long been considered as a relationship between parasitoid attack rate and host density. However, more recently, parasitoid density (ratio dependence) has been included and considered as the refined form of functional response (Mills & Lacan 2004; Chong & Oetting 2006; Montoya et al. 2000). In the field parasitoids seldom occur as a single individual but often encounter other conspecifics foraging in the same patch (Chong & Oetting 2006). Although the per capita parasitism is lower when parasitoids are searching in a group as compared to a single parasitoid, they parasitise more hosts as a whole at a given host density. For example, *T. minutum* and *A. kamali* parasitise more hosts when foraging in groups (Sagarra et al. 2000; Mills & Lacan 2004). Moreover, superparasitism is also ubiquitous when there are a number of competitors foraging in the same patch (Montoya et al. 2000). Superparasitism may result in reduced survivorship, size, longevity and fecundity of emerging offspring (Potting et al. 1997; Jones et al. 1999).

In biological control programmes, parasitoids' success depends upon the knowledge of several factors, including the parasitoid-host interaction. However, no published information is available on the parasitoid-host interactions and their effects on the offspring fitness in *E. warrae*, making it difficult to develop strategies for effective mass-rearing and field release of this parasitoid. Thus, the objectives of this study were to investigate the interactions of parasitoid and host densities and their effects on the reproductive potential of adult *E. warrae* and its offspring. Information produced here would be helpful in mass rearing and recommending the release rates of *E. warrae* against *T. vaporariorum*.

5.3.2 Materials and methods

5.3.2.1 Effect of parasitoid and host density on host feeding, fecundity, parasitism and superparasitism in *E. warrae*

To determine how parasitoid and host density affected the reproductive potential

(fecundity, parasitism and superparasitism) and host feeding of *E. warrae*, experiments were conducted with parasitoid densities of 1, 3 and 5 females × three host densities of 40, 60 and 80 second instar nymphs (a total of 9 treatments): (1-3) 1 parasitoid × 40, 60 and 80 second instar nymphs, (4-6) 3 parasitoids × 40, 60 and 80 second instar nymphs, and (7-9) 5 parasitoids × 40, 60 and 80 second instar nymphs. Ten females (10 replicates) were used for each treatment. For each replicate, newly emerged (<12 h) parasitoids were released into a Petri dish with a fresh leaf infested by the test number of nymphs. The parasitoids were allowed to stay in the Petri dish for 24 hours, after which time they were removed. Host feeding was recorded by counting the number of fed hosts while the fecundity, parasitism and superparasitism were estimated by randomly turning over half of the nymphs. The remaining nymphs were reared in the Petri dishes to determine the juvenile developmental period and offspring reproductive fitness (see below) of *E. warrae* in each treatment.

5.3.2.2 Effect of parasitoid and host density on offspring fitness in *E. warrae*

To determine the effect of parasitoid and host density on reproductive fitness of the offspring, the egg load of emerging parasitoids was determined by dissecting 10 parasitoids from each of the above treatments in a drop of 70% alcohol on a slide under a stereomicroscope. One drop of 1% acetocarmine was added to the alcohol and allowed to stand for 3 to 5 minutes for staining. The chorion of mature eggs prevents the stain but immature eggs absorb the stain (Edward 1954). Then the number of mature eggs in the ovaries was counted under a compound microscope. Before dissection, body size (from the frons to the tip of the abdomen) (Jones et al. 1999) of emerging parasitoids was measured under the stereomicroscope.

To determine the longevity of the offspring, 10 newly emerged parasitoids from each treatment were taken randomly and tested in Petri dishes by supplying 100 second instar whitefly nymphs once a day until they died.

5.3.2.3 Statistical analyses

A central composite design (CCD), response surface (Box & Draper 1987), was applied to investigate the effect of parasitoid density and host density on host feeding and reproductive potential of *E. warrae*. After running the full regression models only

significant terms were kept in the final models. A log likelihood rate test (McCullagh & Nelder 1989) was then applied to determine whether parasitoid and host density had different effect on host feeding and reproductive potential of *E. warrae*.

5.3.4 Results

5.3.4.1 Effect of parasitoid and host density on host feeding, fecundity, parasitism and superparasitism in *E. warrae*

The CCD model indicates that both parasitoid and host density significantly positively affected the host feeding (CCD: $F = 176.26$; $df = 3,86$; $P < 0.0001$; $R^2 = 0.8601$), parasitism (CCD: $F = 65.71$; $df = 3,86$; $P < 0.0001$; $R^2 = 0.6962$), fecundity (CCD: $F = 86.88$; $df = 3,86$; $P < 0.0001$; $R^2 = 0.7520$), and adult emergence (CCD: $F = 43.91$; $df = 4,85$; $P < 0.0001$; $R^2 = 0.6738$) in *E. warrae* (Fig. 5.3). However, parasitoid density had significantly more effect on host feeding, parasitism, fecundity, and offspring emergence than did host density (Likelihood rate test: $\chi^2 = 5326.87$, 1094.10, 2578.97 and 574.95, for host feeding, parasitism, fecundity, and offspring emergence, respectively; $df = 2$; $P < 0.0001$).

The CCD model also shows that the increasing host and parasitoid density significantly affected the host feeding (CCD: $F = 151.72$; $df = 3,86$; $P < 0.0001$; $R^2 = 0.8410$) and superparasitism rates in *E. warrae* (CCD: $F = 531.82$; $df = 3,86$; $P < 0.0001$; $R^2 = 0.9488$) (Fig. 5.4). Increasing host density had significantly negative effect on host feeding ($\chi^2 = 24.18$; $P < 0.0001$) and superparasitism rates ($\chi^2 = 26.90$; $P < 0.0001$) while increasing parasitoid density had significantly positive effect on host feeding ($\chi^2 = 87.38$; $P < 0.0001$) and superparasitism rates ($\chi^2 = 290.74$; $P < 0.0001$) (Fig. 5.4). However, the likelihood rate test indicates that the parasitoid density had significantly more effect on host feeding and superparasitism rates than did the host density (Likelihood rate test: $\chi^2 = 27849.42$ and 10371.52, for host feeding and superparasitism rates, respectively; $df = 2$; $P < 0.0001$) (Fig. 5.4).

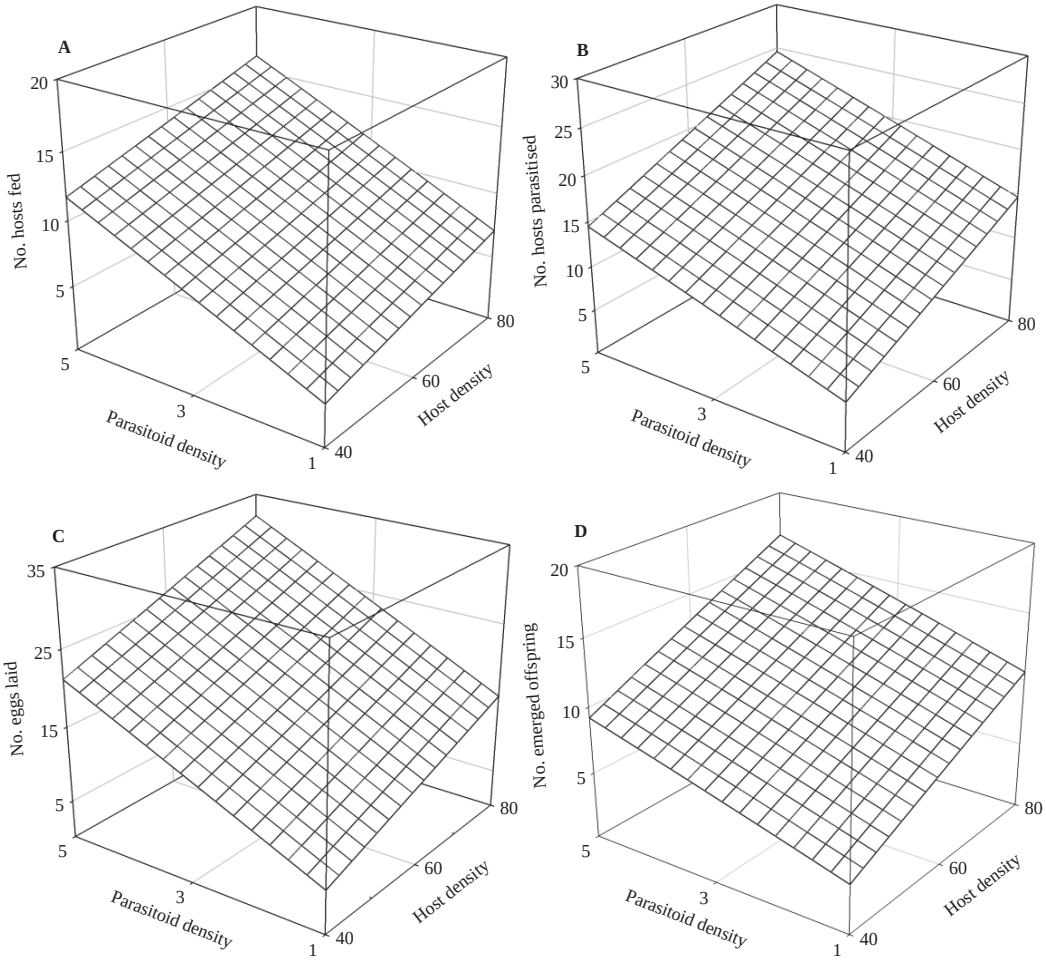

Figure 5.3 Effect of host and parasitoid density on (A) host feeding = exp (0.3238.9817 + 0.0109 HD + 0.6342 PD - 0.0623 PD^2); (B) parasitism = exp (0.8948 + 0.0166 HD + 0.3744 HD - 0.0339 PD^2); (C) fecundity = exp (0.9474 + 0.0140 HD + 0.5410 PD - 0.05403 PD^2); and (D) number of emerged offspring = exp (-1.4487 + 0.0795 HD - 0.0005 HD^2 + 0.5036 PD - 0576 PD^2). HD = host density, PD = parasitoid density.

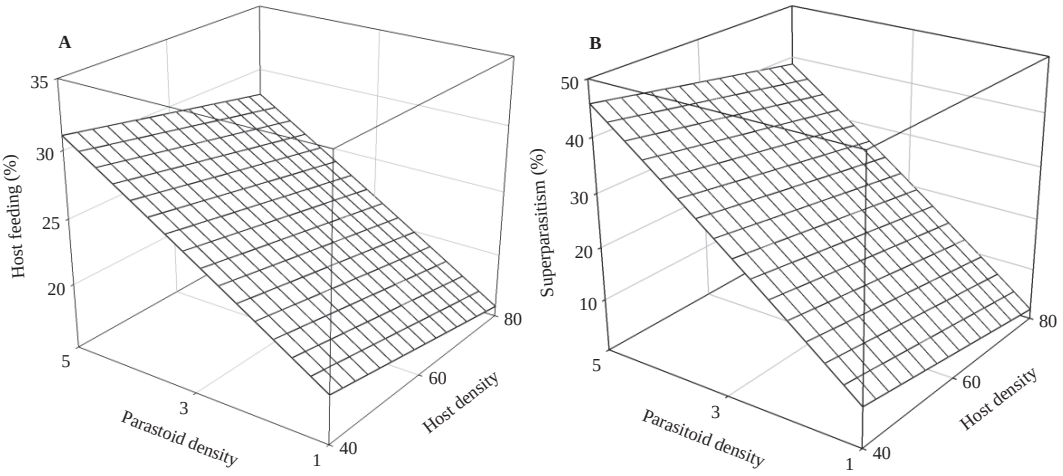

Figure 5.4 Effect of host and parasitoid density on (A) host feeding rate = exp (2.6937 - 0.0032 HD + 0.3361 PD - 0.0329 PD^2) and (B) superparasitism rate = exp (-3.1949 - 0.0068 HD + 3.7262 PD - 0.4557 PD^2). HD = host density, PD = parasitoid density.

5.3.4.2 Effect of parasitoid and host density on offspring fitness in *E. warrae*

My results show that the increasing host and parasitoid density significantly affected the developmental time (CCD: F = 18.10; df = 2,911; P < 0.0001; R^2 = 0.0380), body size (CCD: F = 19.21; df = 2,87; P < 0.0001; R^2 = 0.3051), egg load (CCD: F = 21.82; df = 2,87; P < 0.0001; R^2 = 0.3340) and longevity in *E warrae* offspring (CCD: F = 24.95; df = 3,86; P < 0.0001; R^2 = 0.4653) (Fig. 5.5).

The increasing host density had a significantly negative effect on the offspring developmental time (χ^2 = 5.32; P < 0.02) and a positive effect on the body size (χ^2 = 4.20; P < 0.05), egg load (χ^2 = 7.73; P < 0.01) and longevity of offspring adults (χ^2 = 14.33; P < 0.001). However, the increasing parasitoid density had a significantly positive effect on the offspring developmental time (χ^2 = 31.11; P < 0.0001) and a negative effect on the body size (χ^2 = 33.89; P < 0.0001), egg load (χ^2 = 35.44; P < 0.0001) and longevity of offspring adults (χ^2 = 15.26; P < 0.0001) (Fig. 5.5).

The likelihood rate test indicates that the parasitoid density had significantly more effect on the developmental time and body size, egg load and longevity of offspring adults (Likelihood rate test: χ^2 = 11656.75, -8542.40, 762.46 and1379.22, for the developmental time, body size, egg load and longevity, respectively; df = 2; P <

0.0001) (Fig. 5.5).

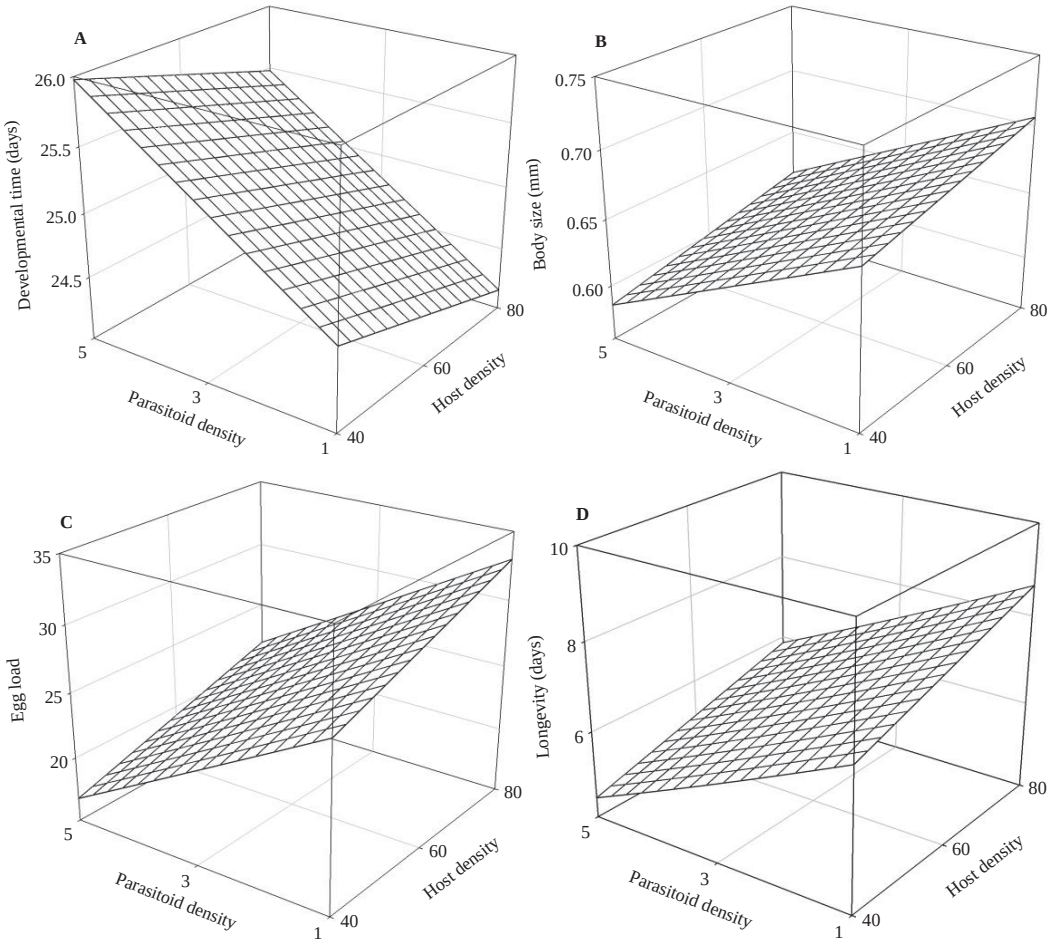

Figure 5.5 Effect of host and parasitoid density on (A) development time = exp (3.2116 - 0.0005 HD + 0.0132 PD); (B) body size = exp (-0.4072 + 0.0012 HD - 0.0348 PD); (C) egg load = exp (3.2162 + 0.0052 HD - 0.1128 PD), and (D) longevity of emerged offspring = exp (2.0439 + 0.0055 HD - 0.3036 PD + 0.0335 PD^2). HD = host density, PD = parasitoid density.

5.3.5 Discussion

In the present study, *E. warrae* parasitised and fed on more hosts in response to increasing parasitoid and host density, suggesting that this parasitoid can suppress whitefly population when the latter increases. It is possible that with the increase in parasitoid and host densities parasitoid-host encounter rate increases, which has a

positive effect on parasitisation and host feeding (Sahragard et al. 1991; Knipling 1992). Similar results were also recorded by Sagarra et al. (2000) in *A. kamali* on *Maconellicocus hirsutus* Green (Hemiptera: Pseudococcidae).

Although parasitoids of the genus *Eretmocerus* avoid superparasitism when provided with abundance of hosts (Headrick et al. 1995, 1996), they superparasitise when confined with limited number of hosts (Gerling 1966; McAuslane & Nguyen 1996). In the present study, superparasitism significantly increased with the increase in the parasitoid density and decreased with the increase in the host density. This suggests that superparasitism could be more common under the situations with a number of parasitoids foraging in the same patch or when the unparasitised hosts are depleted. It is also possible that to increase the probability of the survival of their offspring, parasitoids allocate more than one egg under the host (Visser et al. 1990). Solitary parasitoids may also make larger clutch sizes when a large number of individuals are competing for a limited number of hosts in a same patch (Visser et al. 1990; Crespo & Castelo 2009). For example, *A. kamali* increases its clutch size from 1.4 at 1 parasitoid per host to 2.4 at 20 parasitoids per host (Sagarra et al. 2000).

Among several biotic and abiotic factors that affect the development, size and longevity of emerging parasitoid progeny, nutritional adequacy is the most important (Jervis & Kidd 1986). The present study shows that the developmental time of progeny significantly increased with the increase in the parasitoid density and decreased with the increase in the host density. These results suggest that the increased superparasitism at these densities (Fig 5.4B) increases the competition among the developing larvae for the resources, resulting in their delayed development and growth. These results agree with the findings of Tunca and Kilincer (2009) who report that *Chelonus oculator* Panzer (Hymenoptera: Braconidae) requires a longer time to develop in superparasitised host, *Cadra cautella* (Walker) (Lepidoptera: Pyralidae). *Microctonus vittatae* Muesebeck (Hymenoptera: Braconidae) takes shorter time to complete development in singly parasitised hosts of *Phyllotreta crucifer* Goeze and *Phyllotreta striolata* F. (Coleoptera: Chrysomelidae) (Wylie 1983).

In hymenopteran parasitoids, body size has a positive correlation with egg load and longevity (He et al. 2005; Karut 2007). The current results also show that the progeny that emerged from the lower parasitoid density and higher host density had

significantly larger body size with higher egg load. They also lived significantly longer than the parasitoids that emerged from the higher parasitoid density and lower host density. These suggest that the larger parasitoid females hold greater quantity of resources, which enhances their egg production and prolongs their longevity. These results support the finding of Jones et al. (1999) on *E. mundus*.

The information obtained from this experiment has improved our knowledge on the interaction of *E. warrae* with its host *T. vaporariorum* at different parasitoid and host densities. The density of both parasitoids and their hosts should be taken into consideration before estimating release rates of *E. warrae* on whitefly in the greenhouses or the field. The optimal parasitoid-host ratio which can maximize the biological control efficiency of *E. warrae* on the whitefly appeared to be 5 parasitoids: 80 whitefly nymphs.

5.4 Effect of Food Supply on Reproductive Potential of *E. warrae*

5.4.1 Introduction

Adult females of many insects depend on carbohydrate-rich food as their main source of energy for longevity, fecundity and mobility (Jervis & Kidd 1986). In hymenopteran parasitoids, including *Eretmocerus* spp., longevity and fecundity may be influenced by a range of factors, such as temperature (Greenberg et al. 2000), host stage (Jones & Greenberg 1998), host density (Sahragard et al. 1991; Hanan et al. 2012), host plant species (Qiu et al. 2005) and presence of food (Leatemia et al. 1995; McAuslane & Nguyen 1996; Hardin et al. 2008). Among these factors, availability of food sources appears to be the most practical and economical means of promoting longevity (McDougall & Mills 1997). Previous studies on *Eretmocerus* demonstrated that sugar sources (e.g. saccharose and honey) and host haemolymph have a significant effect on longevity of *E. debachi* (Kuwana) and *E. mundus* (Mercet) (Sengonca et al. 1994; Ghahari et al. 2005). *E. eremicus* lives significantly longer when fed with carbohydrate diets (Hardin et al. 2008). However, whether host feeding and supply of food such as honey affect longevity and reproductive output of *E. warrae* is not clear.

Parasitoids can be classified as synovigenic (females continue to mature eggs during their adult lifetime) and pro-ovigenic (females complete oogenesis prior to eclosion) (Jervis & Kidd 1986). *E. warrae* carries some mature eggs at eclosion and continues to mature eggs during adult lifespan (Unpubl. data), suggesting that it is a pro-synovigenic species (Jervis & Kidd 1986). It is well known that nutrients obtained by host feeding are used to mature eggs and sugar sources are used for the maintenance of life (Heimpel & Collier 1996). Food supply may allow pro-ovigenic species to increase longevity (Thompson 1999) and synovigenic species to increase both longevity and fecundity (Heimpel & Collier 1996). Jervis & Kidd (1986) suggested that synovigenic parasitoids whose adults feed on host fluid still require sugars as their main source of energy during the adult stage. Therefore, understanding the effect of food supply for adults on parasitoid fitness is important for mass-rearing and field enhancement of parasitoids for biological control programmes.

Prior to the present study there was no published information on the effect of non-host food (honey or sugars) on longevity, fecundity and parasitism in *E. warrae*,

making it difficult to develop strategies for effective mass-rearing and field manipulation of this parasitoid. Therefore, the primary aim of this study was to determine the effect of food supply on the longevity, host feeding, fecundity and parasitism of *E. warrae.*

5.4.2 Materials and methods

5.4.2.1 Experiments

All experiments were carried out in plastic Petri dishes. To investigate whether and how food supply affected the host feeding, fecundity, parasitism and longevity of *E. warrae*, two experiments were set up. In the first experiment, the longevity of *E. warrae* was recorded without hosts with three treatments: (1) no food and no host, (2) water-fed, and (3) 10% honey solution and no host. Water and 10% honey were provided in a cotton wick inserted into the Petri dish through a 1 cm hole in the lid. For each treatment, a newly emerged parasitoid was released into a Petri dish and checked daily until she died. Then the longevity of parasitoids was recorded. There were 20 (no. of parasitoids) replicates for each treatment in this experiment.

In the second experiment, the host feeding, fecundity, parasitism and longevity of *E. warrae* were recorded with two treatments: (1) 40 second instar nymphs per day and 10% honey solution, and (2) 40 second instar nymphs per day and no honey. For each treatment, a parasitoid (< 12 h after emergence) was released into a Petri dish, allowed to stay for 24 h, and then moved into another Petri dish. This process was repeated until she died.

As *E. warrae* place their eggs between the venter of whitefly nymphs and leaf surface, all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope. The oviposition and host feeding patterns were determined by counting the numbers of eggs laid and hosts fed upon by the parasitoid. The longevity of adult parasitoids was also recorded. There were 10 replicates (no. of parasitoids) for each treatment.

5.4.2.2 Statistical analyses

A goodness-of-fit test was used to test the distribution of data before analysis.

All data were normally distributed and analysed by ANOVA. The percentage data were arcsine transformed before analysis. When significant differences in variables occurred, means were separated using a Tukey's test. The daily host feeding and fecundity with respect to parasitoid age were analysed using linear regression, and slopes of each category (no. of hosts fed upon or no. of eggs laid) were compared using analysis of covariance (ANCOVA).

5.4.3 Results

The parasitoids that only got access to honey lived significantly longer than those that were unfed (no hosts and no food) and water-fed ($F = 210.54$; $df = 2,57$; $P < 0.0001$) (Fig. 5.6). The parasitoids that got access to honey and host lived significantly longer than those that only fed on hosts ($F = 5.44$; $df = 1,18$; $P > 0.05$) (Table 5.1).

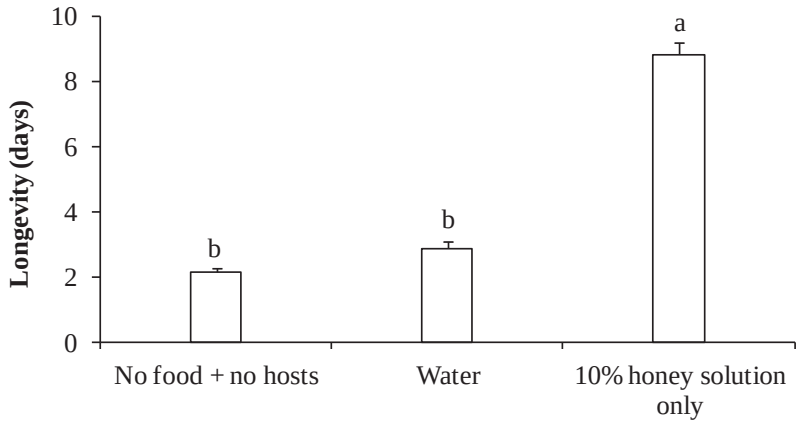

Figure 5.6 Effect of food supply on the longevity of *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$).

In the treatments 'hosts only' and 'honey + hosts', the number of hosts fed on and eggs laid significantly decreased with the parasitoid age ($F = 22.26$ and 81.76, for hosts only and honey + hosts, respectively; $df = 1,47$; $P < 0.0001$) (Fig. 5.7). However, the decrease in host feeding was significantly faster in the 'host only' treatment than in the 'honey + hosts' treatment ($F = 5.72$; $df = 1,95$; $P < 0.01$) (Fig. 5.7a) while the decline in the number of eggs laid with increasing parasitoid age was not significantly different between these two treatments ($F = 0.12$; $df = 1,95$; $P > 0.05$) (Fig. 5.7b).

When lifetime data were considered, honey supply significantly reduced the number of hosts fed on but increased the longevity in *E. warrae* (F = 6.10 and 5.44 for number of hosts fed and longevity, respectively; df = 1,18; P < 0.05) (Table 5.1). However, honey solution did not significantly increase *E. warrae* fecundity, parasitism or superparasitism rates (F = 0.38, 0.27 and 0.44 for fecundity, parasitism and superparasitism rates, respectively; df = 1,18; P > 0.05) (Table 5.1).

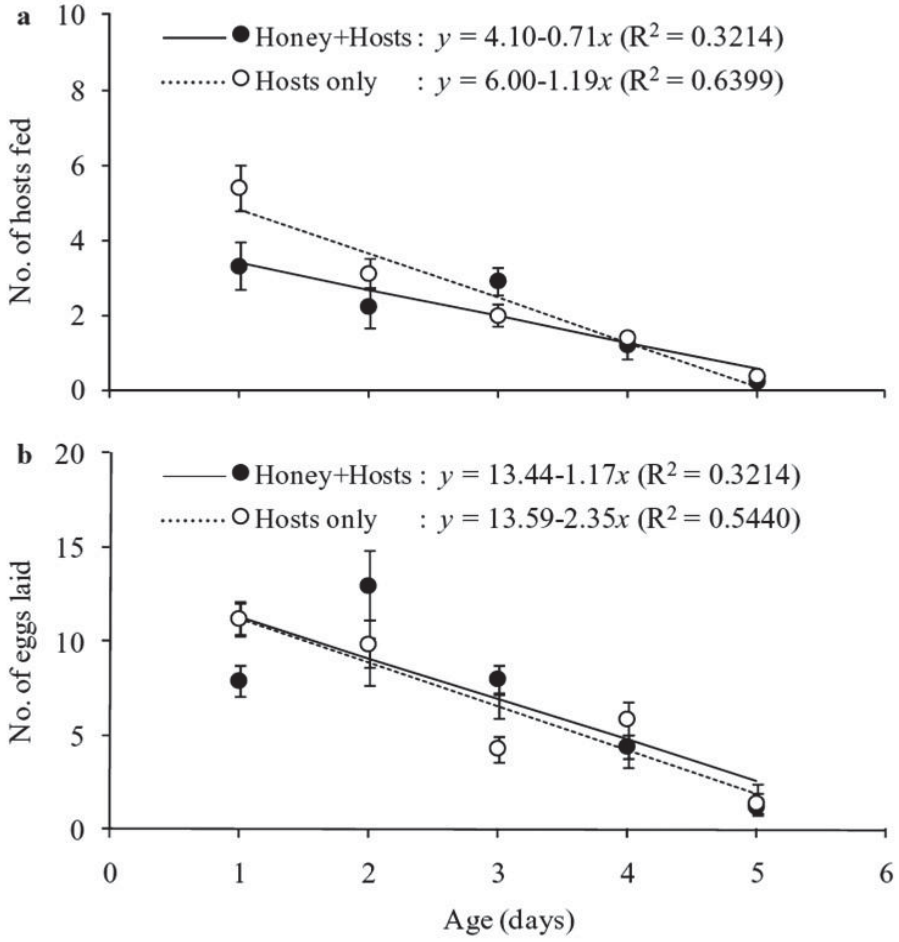

Figure 5.7 Daily number of hosts fed (a) and eggs laid (b) by *E. warrae* when provided with hosts only and honey + hosts. All data were pooled for regression but means (± SE) were presented.

Table 5.1 Effect of host and/or honey solution on lifetime host feeding and reproduction in *E. warrae*.

Treatment	No. hosts fed	No. eggs laid	Parasitism (%)	Superparasitism (%)	Longevity (days)
Host only	12.4±0.65a	32.4±2.51a	14.7±1.12a	11.4±2.5a	4.8±0.13b
10% honey + hosts	9.6±1.05b	34.7±2.77a	16.0±1.18a	10.3±1.5a	5.5±0.27a

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

5.4.4 Discussion

Host feeding by adult hymenopteran parasitoids contributes to pest control (Jervis & Kidd 1986). The present study indicates that honey solution significantly prolongs the longevity of this parasitoid (Fig. 5.6). These results are highly consistent with the results of Hardin et al. (2008) who demonstrated that *E. eremicus* lived significantly longer when fed with carbohydrate diets. In some parasitoids like *Trichogramma* spp., honey increases adult longevity 8-11 times compared to unfed or water-fed adults (McDougall & Mills 1997). My results also show that *E. warrae* adults lived significantly longer when provided with both hosts and honey than those provided with hosts only. These results imply that although adult female feeds on host fluid, she still requires sugars to prolong her longevity. Similarly, *T. minutum* adults that feed on honey as well as hosts live significantly longer than those that feed on hosts only (Bai & Smith 1993).

Heimpel and Collier (1996) reported that when presented with hosts directly after feeding on water or honey, *A. aonidiae* fed upon fewer hosts, implying that availability of honey or water limits the host feeding rate. My results also show that provision of honey solution significantly reduced host feeding by *E. warrae* (Table 5.1), suggesting that honey supply for this species can lower its biological control efficiency by host feeding. However, in the laboratory, provision of honey may reduce the rearing costs as parasitoids kill fewer hosts by feeding, which may result in more hosts available for oviposition. Furthermore, with the presence of hosts honey did not significantly increase *E. warrae* reproductive output (Table 5.1), suggesting that sugar

sources may not be necessary in mass-rearing programmes of this species for biological control. However, when hosts are rare or temporarily absent in a mass-rearing or biological control programme, the increase in longevity of *E. warrae* from 5 to 9 days due to provision of honey may enable the parasitoids to survive before they find hosts.

5.5 Effect of Host Stage on Reproductive Potential in *E. warrae*

5.5.1 Introduction

A parasitoid's fitness can be established by the amount of nutritional resource allocated by its host during the larval development (Ellers & Jervis 2003). Therefore, host stage is considered as an essential component for determining parasitoid reproductive fitness (Da Rocha et al. 2007).

Host stage and size can affect fitness of developing parasitoid in variety of ways, such as survival, development, body size and longevity (Jervis & Kidd 1986; McGregor 1997; Murdoch et al. 1997; Qui et al. 2004, 2007; Greenberg et al. 2008). The larger and nutritionally rich hosts increase the larval survival and growth of emerging offspring and are usually preferred by the parasitoids for oviposition (Godfray 1994; McGregor 1997; Murdoch et al. 1997; Hu et al. 2002; Harvey et al. 2004). For example, in *Anagyrus indicus* (Subba Rao) (Hymenoptera: Encyrtidae) more rapid development and higher survival occur when larger hosts are parasitised (Nechols & Kikuchi 1985). *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae) develops more quickly when prepupae or pupae are parasitised than when younger hosts are parasitised (Lauzière et al. 2001).

The reproductive potential of a parasitoid usually relates to its size at emergence (Hardey et al. 1992; Da Rocha et al. 2007). Both theoretical and empirical models support the fact that parasitoid body size correlates strongly with life history parameters such as egg load and longevity (Jervis & Copland 1996; Murdoch et al. 1997; Hu et al. 2002; Ellers & Jervis 2003; Harvey et al. 2004; Da Rocha et al. 2007). Larger female offspring have higher egg load, search the host more efficiently and live longer than smaller ones (He et al. 2005; Karut 2007; Kazmer & Luck 1995).

In host feeding parasitoid species, host feeding and oviposition preference is shaped by the stage and size of the host (Jervis & Kidd 1986). Theory of optimal host feeding strategies predicts that parasitoids usually prefer to feed on lower quality hosts, particularly on earlier host stages and oviposit on/under/in higher quality hosts or later host stages (Jervis & Kidd 1986; Chan & Godfray 1993; Heimpel & Collier 1996). For example, *C. stephanoderis* prefers to feed on eggs of *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) and lays eggs in later stages (Lauzière et al. 2001).

Prior to the present study there was no published information on the effect of host stage on parasitism, host feeding and longevity of adult *E. warrae*, and fitness of progeny, making it difficult to develop strategies for effective mass-rearing and field manipulation of this parasitoid. The objectives of this study were to investigate the effects of host stage on adult *E. warrae* parasitism and feeding rates and ultimately on longevity, and to examine whether host stage had significant effects on the fitness of emerging progeny.

5.5.2 Materials and methods

5.5.2.1 Effect of host stage on adult fitness

To determine whether and how host stage affected the reproductive fitness of *E. warrae* adults, we offered parasitoid adults whitefly nymphs of the same stage (1st, 2nd, 3rd, or 4th instar) every day and recorded their life time fitness. Ten females were used for each host stage. For each replicate, one newly emerged parasitoid (< 1 h old) was released into a Petri dish containing a fresh leaf infested with 100 nymphs of the same stage. The parasitoid was allowed to stay in the Petri dish for 24 hours, after which time she was removed and placed into another Petri dish containing the same number and stage of nymphs until she died. The adult fecundity was determined by randomly turning over 50 nymphs and counting the number of eggs laid in each Petri dish. The remaining nymphs were reared in the Petri dishes until adult emergence. Adult emergence was recorded daily to determine the juvenile developmental period and offspring reproductive fitness (see below) of *E. warrae* in each treatment. The adult longevity in each treatment was also recorded.

5.5.2.2 Effect of host stage on offspring fitness

To determine how host stage affected the reproductive fitness of the offspring, the egg load of emerging parasitoids was determined by dissecting 10 parasitoids from each of the above treatments in a drop of 70% alcohol on a slide under a stereomicroscope. One drop of 1% acetocarmine was added to the alcohol and allowed to stand for 3 to 5 minutes for staining. The chorion of mature eggs prevents the stain but immature eggs absorb the stain (Edward 1954). Then the number of mature eggs in the ovaries was counted under a compound microscope. Before dissection, body size

(from the frons to the tip of the abdomen) (Jones et al. 1999) of emerging parasitoids was measured under the stereomicroscope.

To determine the effect of host stage at parasitisation on the offspring longevity, I randomly took 10 parasitoids from each treatment and reared them individually in Petri dishes. I provided 100 2nd instar host nymphs for each dish once a day until the parasitoid died.

5.5.2.3 Statistical analyses

A goodness-of-fit test was used to test the data distribution. Data for developmental period of juveniles were not normally distributed even after transformation and thus analysed using the nonparametric Kruskal-Wallis test (KWT). Means were separated by Dunn's procedure for multiple comparisons. All other data were normally distributed and analysed by one-way ANOVA followed by Tukey's test. The percentage data were arcsine transformed before analysis but non-transformed means were presented here.

5.5.3 Results

5.5.3.1 Effect of host stage on adult fitness in *E. warrae*

The parasitoids fed on significantly more hosts ($F = 17.30$; $df = 3,36$; $P < 0.0001$), laid significantly more eggs ($F = 11.45$; $df = 3,36$; $P < 0.0001$) and lived significantly longer ($F = 3.72$; $df = 3,36$; $P < 0.05$) (Fig. 5.8) when they were offered the 2nd and 3rd instar nymphs as compared to younger ones.

The host feeding and parasitism rates were significantly higher in intermediate hosts instar nymphs than in the 1st and 4th instar nymphs ($F = 6.89$ and 8.78 for host feeding and parasitism rates, respectively; $df = 3,36$; $P < 0.001$) (Fig. 5.9).

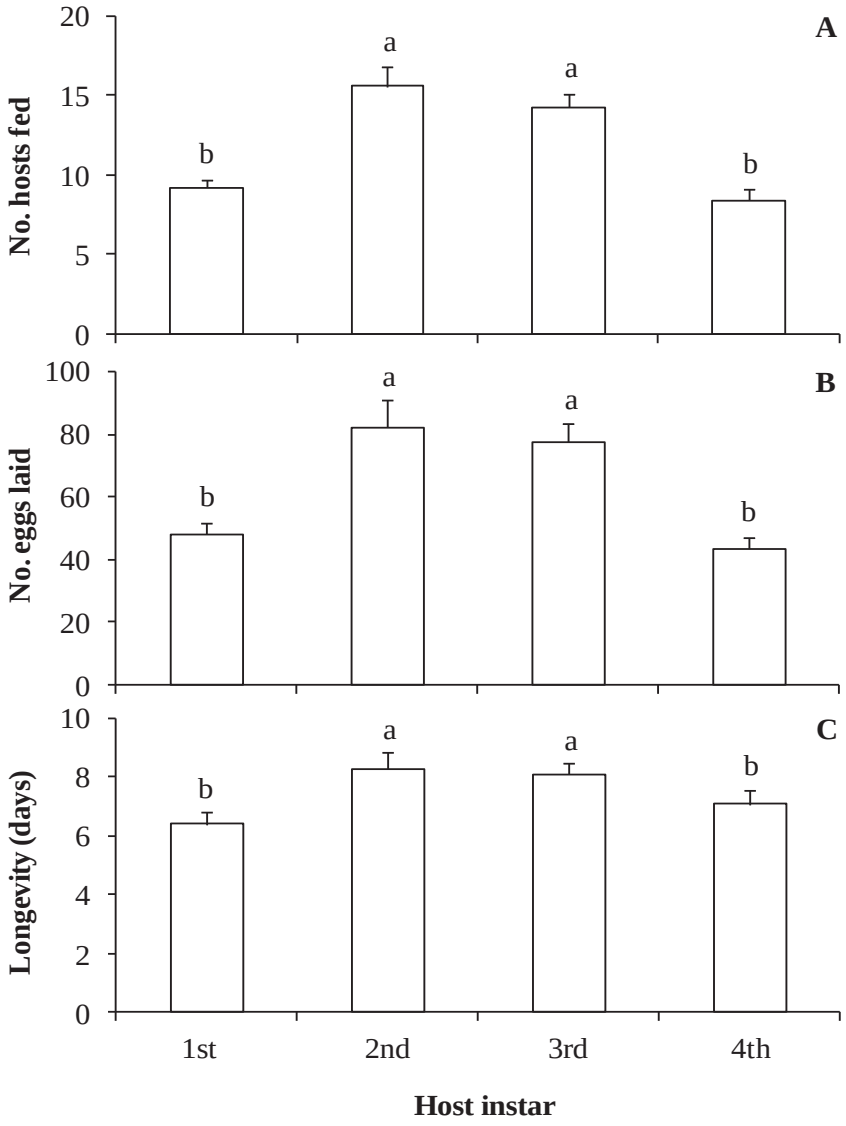

Figure 5.8 Effect of host stage on the number of hosts fed (A), number of eggs laid (B), and longevity (C) in *E. warrae*. Columns with the same letters are not significantly different ($P > 0.05$).

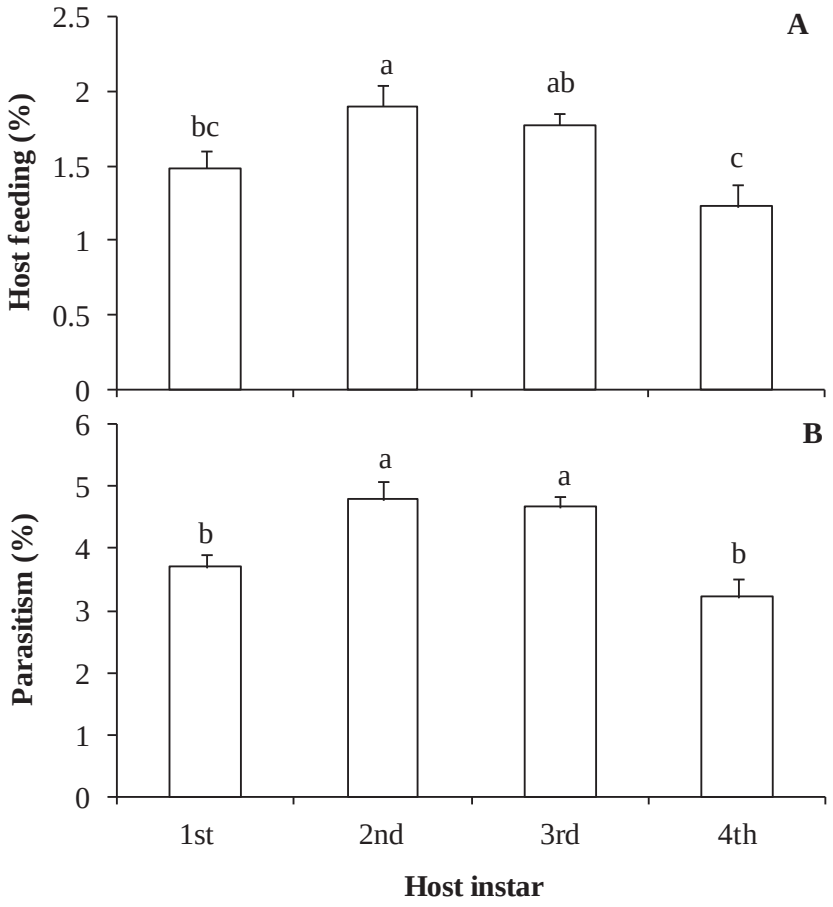

Figure 5.9 Effect of host stage on host feeding rate (A) and parasitism rate (B) in *E. warrae*. Columns with the same letters are not significantly different (P > 0.05).

5.5.3.2 Effect of host stage on offspring fitness in *E. warrae*

The results show that with the increase of the host stage (up to 4th instar) parasitised by the parasitoids, their offspring gained significantly more in terms of body size, egg load, juvenile developmental time and adult longevity (Table 5.2). When juvenile survival rate was taken into consideration, parasitising the 3rd instar nymphs by the parasitoids gave their offspring the highest overall fitness return (Table 5.2).

Table 5.2 Effect of host stage on parasitoid progeny fitness.

Host instar	Survival (%)	Developmental period (days)	Body length ($\times10^{-1}$mm)	Head width ($\times10^{-1}$mm)	Hind tibia length ($\times10^{-1}$mm)	Egg load (no)	Longevity (days)
1st	58.31±2.74b	25.54±0.25a	6.13±0.13c	2.41±0.04c	2.39±0.05c	17.50±2.28c	5.40±0.40c
2nd	72.55±2.37a	23.58±0.14b	6.95±0.10b	2.73±0.06b	2.64±0.04b	27.60±1.95b	7.50±0.64b
3rd	74.21±1.64a	21.82±0.11c	7.40±0.08a	2.98±0.04a	2.90±0.04a	37.10±3.04a	8.90±0.48ab
4th	56.13±2.36b	21.43±0.12c	7.50±0.06a	2.99±0.03a	2.93±0.03a	38.50±3.25a	9.20±0.49a
F(χ^2)	16.69	(284.29*)	41.50	38.06	41.09	13.20	11.58
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
df	3,36	3	3,36	3,36	3,36	3,36	3,36

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

* Kruskal-Wallis test.

5.5.4 Discussion

Theory of optimal host feeding strategies predicts that a parasitoid should feed and oviposit on different host stages, preferentially feeding on lower quality hosts and ovipositing on/under/in higher quality hosts (Kidd & Jervis 1991). However, my results show that *E. warrae* preferred the second and third instar nymphs for both oviposition and host feeding. These results suggest that the exoskeleton of the fourth instar nymphs may be hard for the parasitoids to make a wound for feeding (Kidd & Jervis 1991; Ardeh 2004) and the fluid in the first instar nymphs may be too little for parasitoids to sustain fecundity and longevity (Present study). Thus, food supply is not the sole reason for host selection but optimal trade-off between food supply and overall fitness matters.

My results indicate that *E. warrae* laid significantly more eggs and lived significantly longer on the second and third instar nymphs, suggesting that more host feeding on these instars (Fig. 5.8A) increases the fecundity and longevity of *E. warrae*. The present results also show that the *E. warrae* survival rate was significantly lower when the first and fourth instar nymphs were parasitised. The lower parasitoid survival under the first instar nymphs may be attributed to the higher mortality of the parasitoid first instar larvae under these instars (Section 3.5.3.1). The transformation of the fourth

instar nymphs into the pharate adult stage prior to the egg hatch may be responsible for the lower survival of the parasitoid under the fourth instar nymphs (Nechols & Tauber 1977). Therefore, it is concluded that the second and third instar host stages are the most suitable stages for the survival of *E. warrae* adults and their offspring.

In many parasitoids, host stage and size at parasitisation influence the growth and development of the parasitoid progeny (Jervis & Kidd 1986; McGregor 1997; Murdoch et al. 1997). In the present study, with the increase of host stage the developmental period of *E. warrae* significantly decreased. The faster development in the older instars may be attributed to the more resources available in these instars, supporting the rapid growth and development of the parasitoids (Mackauer & Sequeira 1993; Godfray 1994). Moreover, as reported earlier (Section 3.5.3.2) and in other *Eretmocerus* parasitoids (Gerling 1966; Gerling et al. 1990, 1998; Gelman et al. 2005), when parasitism occurs under the older instar nymphs, the parasitoid first instar larvae penetrate into the hosts sooner.

In solitary parasitoids, overall reproductive fitness is often positively correlated with the host size at parasitisation (Ellers & Jervis 2003; Harvey et al. 2004; Fand et al. 2011). With the increase of host stage the parasitoid offspring gained more fitness with larger body size, higher egg load and longer longevity. This is because the larger hosts are nutritionally rich with more haemolymph (Godfray 1994; Hu et al. 2002; Fand et al. 2011).

The findings of this study have implications for the laboratory mass rearing and field application of *E. warrae*, for example, the second and third instar nymphs appear to be the most suitable stages for the mass rearing programme. Therefore, in order to get maximum results, the parasitoids should be reared on the second and third instar nymphs in the laboratory and released in the field when these instars are available.

Chapter 6

General Discussion and Conclusion

6.1 Introduction

Prior to this study there was a lack of information on *E. warrae*, which provided me an opportunity to investigate its reproductive biology. In this thesis, I investigated the host feeding, oviposition and developmental strategy of *E. warrae*. Such knowledge is vital to better understanding biological control ecology of *E. warrae*.

In this chapter, I summarise and discuss my main findings on general biology, feeding and oviposition behaviour of *E. warrae* and their relevance for the development of biological control programmes.

6.2 General biology

The results demonstrate that *E. warrae* adults show circadian rhythmicity in emergence, feeding and oviposition (Section 3.3.3.1). Emergence occurs exclusively during the photophase and peaks during the early hours of the photophase, suggesting that the onset of light may act as a signal for adult emergence. However, *E. warrae* carries on host feeding and oviposition activities throughout 24 hours with the former peaking between 4 to 6 h and the latter between 10 to 14 h after lights on. The fact that host feeding occurs before oviposition peak suggests that host feeding may provide nutrients for egg maturation and promote egg production (Jervis & Kidd 1986; Giron et al. 2004; Burger et al. 2005). Information generated here could be useful for further studies on its reproductive and feeding behaviour and for optimal timing of pesticide application without massive removal of the parasitoid.

Eretmocerus warrae is very sensitive to temperature (Sections 3.4.3.1). Although higher temperature accelerates the development of parasitoids, larval survival and emergence rates are significantly lower at temperatures of 15 and 30°C. At lower temperatures, the lower survival can be attributed to the incomplete development of the embryo while the low emergence can be ascribed to the low degree of activity of the wasp inside the whitefly pupa, preventing its emergence (Chong et al. 2008). Conversely, at higher temperatures, the digestion and circulation rates become unstable,

causing the animal to die quickly (Chapman 1998). The increased daily feeding rates at higher temperatures (Section 3.4.3.1) can be attributed to the increased action of enzymes, leading to the increasing metabolism and ultimately feeding and growth rates (Howe 1967; Tomberlin et al. 2009).

The maximum fecundity, survival and emergence in *E. warrae* detected at 20 and 25°C (Section 3.4.3.1) indicate that the physiological processes such as respiration, digestion and circulation work optimally within this range of temperatures (Chapman 1998). Therefore, the biological control efficiency of *E. warrae* should be higher at the optimal temperatures from 20 to 25°C. Moreover, increased adult longevity at the lower temperatures can be useful in mass-rearing or biological control programmes, for example, when the hosts are rare or temporarily absent, the increase in longevity at lower temperatures may enable the parasitoids to survive before they find the hosts.

Eretmocerus warrae successfully parasitises whitefly nymphs up to the fifth stage of the fourth instar but does not lay eggs once the whitefly develops to the pharate adult stage (Section 3.5.3.4). When parasitism occurs under the third-fifth stages of the fourth instar nymphs, *E. warrae* does not complete its development because the egg that hatches under the pharate adult stages fails to penetrate the host body possibly due to the tough pharate adult structure (Gelman et al. 2005). Moreover, significantly higher mortality of the first instar larvae of *E. warrae* occurs under the first and fourth instar nymphs (Section 3.5.3.1). The high mortality under the first instar nymphs may be due to the delay in the penetration process which is restricted to the fourth instar nymphs. Under the fourth instar nymphs it may be attributed to the transformation of the whiteflies into the pharate adult stage, making penetration difficult (Gelman et al. 2005).

Koinobiont parasitoids arrest their hosts' development when the latter reach a specific stage (Beckage & Gelman 2004). No matter which host instar is parasitised, *E. warrae* arrests the whitefly development when the latter develops to the fifth stage of the fourth instar. The arrestment occurs significantly sooner if the later instar nymphs are parasitised (Section 3.5.3.3). This suggests that *E. warrae* does not affect the whitefly development until the latter reaches the fifth stage of the fourth instar.

6.3 Host feeding and oviposition behaviour

My study shows that in both choice and non-choice tests, *E. warrae* encounters the larger hosts more often than the smaller ones (Sections 4.2.3.2 & 4.2.3.3). This suggests that the parasitoids perceive and locate the larger hosts more easily due to their larger size and greater amount of odour (semiochemicals) released from their body (van Lenteren et al. 1976; van Roermund & van Lenteren 1995; Ardeh 2004).

The theory of optimal host feeding predicts that a parasitoid should feed on and oviposit on/under/in different host stages (Jervis & Kidd 1986; Kidd & Jervis 1991). However, contrary to the suggested theory, *E. warrae* feeds on and parasitises all four instar nymphs but prefers the second and third instar nymphs for both feeding and oviposition (Sections 4.2.3.2, 4.2.3.3 & 5.5.3.1). This may be attributed to that (1) the exoskeleton of the fourth instar nymphs is too hard to probe while the first instar nymphs contain less and lower quality fluid (Kidd & Jervis 1991; Ardeh 2004), and (2) feeding on and ovipositing under the second and third instar nymphs result in increased fecundity and survival of *E. warrae* adults and relatively higher fitness of their progeny (Section 5.5.3). This knowledge allows us to improve *E. warrae* laboratory mass rearing techniques.

Many parasitoid species are able to distinguish between the hosts parasitised by the same females and those by different females (reviewed by van Alphan & Visser 1990; Buckner & Jones 2005). My results indicate that *E. warrae* superparasitises significantly more hosts at the lower host density (10 hosts) than at the higher host density (15 hosts) (Section 4.3.4). The higher superparasitism of *E. warrae* at the lower host density can be advantageous to the parasitoid because it can increase the possibility of survival of offspring (reviewed by van Alphan & Visser 1990). The present study suggests that *E. warrae* is able to discriminate between the parasitised and unparasitised hosts, and that superparasitism is an adaptive strategy of *E. warrae* when unparasitised hosts are in short supply.

Food and host deprivation for a certain period of time affects the oviposition and feeding behaviour of many parasitoids (Legner & Gerling 1967; Hickman et al. 2001). My study shows that when *E. warrae* is initially deprived of food and hosts for 5 hours, host feeding significantly increases, which subsequently enhances its fecundity and longevity (Section 4.4.3.1). Thus, to maximise biological control efficiency of *E.*

warrae, parasitoids should be kept away from food and hosts for 5 hours before laboratory or field releasing.

In pro-ovigenic species egg resorption occurs if they are deprived of food and host for a certain period of time (Jervis & Kidd 1986). In the present study, no significant egg resorption occurs in *E. warrae* for up to two days of food and host deprivation, suggesting that *E. warrae* maintains its egg load for up to two days. However, in honey-fed parasitoids, egg resorption is detected after 5-7 days, indicating that *E warrae* keeps its egg load for up to 5-7 days if provided with honey (Section 4.4.3.2). These findings could be useful in mass rearing techniques, for example, when hosts are rare, *E. warrae* can survive on the honey solution for 5-7 days without egg depletion.

6.4 Reproductive fitness in *E. warrae* in relation to different factors

Several factors such as host and parasitoid density, host stage and availability of food influence the oviposition and host feeding behaviour of *E. warrae* (Chapter 5). My results indicate that the fecundity, parasitism, host feeding and longevity in *E. warrae* significantly increase with the increase of host density (Sections 5.2.3 & 5.3.4.1). This suggests that at higher host densities the parasitoid obtains more energy through host feeding, which increases its egg production and longevity. However, the proportion of hosts fed, parasitised and superparasitised significantly decreases with the increase of host density in *E. warrae*. This indicates that *E. warrae* can adjust its feeding and oviposition strategy according to the host availability and potentially suppress the high whitefly populations.

Although *E. warrae* avoids superparasitism when provided with abundance of hosts (Hanan et al. 2012), superparasitism occurs when the parasitoid density is high (Gerling 1966; McAuslane & Nguyen 1996). The increasing superparasitism at higher parasitoid densities (Section 5.3.4.1) suggests that superparasitism in *E. warrae* is more widespread under the situations with a high number of parasitoids foraging in the same patch or when the unparasitised hosts are depleted. However, *E. warrae* can be a good candidate for the control of greenhouse whitefly because it significantly increases its feeding, fecundity and parasitism with the increase of parasitoid and host densities. The results also show that at higher parasitoid density, the developmental time of *E. warrae*

progeny is significantly longer with smaller body size, lower egg load and shorter longevity (Section 5.3.4.2). This suggests that the increased superparasitism at higher parasitoid density increases the competition among the developing larvae for the resources, thus reducing the reproductive fitness of *E. warrae*. The optimal parasitoid-host ratio which can maximize the biological control efficiency of *E. warrae* on the whitefly appears to be 5 parasitoids : 80 whitefly nymphs.

As detected in many parasitoid species (McDougall & Mills 1997; Siekmann et al. 2001; Hardin et al. 2008), carbohydrate diets such as honey (alone or together with the hosts) significantly prolong the longevity of *E. warrae* (Section 5.4.3). These results indicate that although adult females feed on host fluids, they still require sugars to survive longer (Jervis & Kidd 1986). Furthermore, honey feeding significantly reduces the number of hosts fed on, implying that the availability of honey limits the host feeding rate of *E. warrae*, and thus reduces the laboratory rearing costs because *E. warrae* kills fewer hosts by feeding. This is important for mass-rearing of this species.

6.5 Conclusion and Future Directions

In this thesis, I have reported and discussed my main findings on the general biology, feeding and oviposition behaviour, and factors affecting the reproductive potential of adult *E. warrae*, and survival and fitness of offspring. This work has provided a much firmer basis of knowledge of this parasitoid that existed hitherto. Such knowledge is vital to appraising prospects for further investigation for the improvement of biological control strategies.

My findings highlighted the optimal release time and rate of *E. warrae*. Results on emergence, feeding and oviposition rhythms would help avoid pesticide application during the peak periods of these activities in the parasitoid. The highest parasitism and host feeding rates of *E. warrae* occurred at 20 nymphs per parasitoid, suggesting that 20 *T. vaporariorum* nymphs per *E. warrae* adult is the optimal rearing density and release rate for augmentative releases.

E. warrae can develop successfully at a wide range of temperatures with a significantly higher level of parasitisation and host feeding at 20 and 25°C, indicating that this temperature range is optimal for *E. warrae* oviposition and survival. Key findings on host preference behaviour for host feeding and oviposition imply that *E.*

warrae could be a good candidate for testing optimal feeding and oviposition theories.

However, the biological characteristics of *E. warrae* in the natural or greenhouse conditions are unknown. Further research could focus on assessing the biological control efficiency of this parasitoid under greenhouse conditions, for example,

- host stage preference for oviposition and host feeding;
- optimal release ratio of the parasitoid and its hosts;
- oviposition and parasitisation ability of the parasitoid at fluctuating temperatures, and
- efficacy of this parasitoid on different host plants.

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DIURNAL RHYTHMS OF EMERGENCE, HOST FEEDING AND OVIPOSITION OF *ERETMOCERUS WARRAE* (HYMENOPTERA: APHELINIDAE)

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ABSTRACT

Eretmocerus warrae is a parasitoid of greenhouse whitefly, *Trialeurodes vaporariorum*. It was first detected in New Zealand in 1997 during a survey of greenhouses in Auckland. In the laboratory at 22±1°C, 60±5% RH and 16:8 h light:dark, significantly higher adult emergence occurred after 2–3 h of light. No emergence was observed during the scotophase. Host feeding and oviposition occurred in both the photophase and scotophase. In the photophase, host feeding by *E. warrae* tended to be higher after 4–6 h of light than at other stages of photophase. In the scotophase, the number of hosts fed on by *E. warrae* was significantly higher 2 h before lights came on. The number of eggs laid was significantly higher 10–14 h into the photophase than at other stages. There tended to be higher oviposition in the first 2 h of darkness.
Keywords: *Eretmocerus warrae*, whitefly, emergence, host feeding, eggs laid.

INTRODUCTION

Whiteflies (Homoptera: Aleyrodidae) are well known highly polyphagous insect pests and feed on almost any terrestrial plant (van Lenteren et al. 1996). The most important species are the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), and sweet potato whitefly, *Bemisia tabaci* (Gennadius), which cause serious economic damage to agronomic, horticultural and ornamental crops throughout warm regions and glasshouses in temperate regions of the world (Byrne et al. 1990). *Trialeurodes vaporariorum* was first found in greenhouses in UK in 1856 (Van Lenteren et al. 1996). It causes billions of dollars of damage worldwide in crop losses each year (Henneberry et al. 1997; Chu & Henneberry 1998). It is well known that whitefly nymphs are sessile and susceptible to parasitism (Gerling 1990) and *T. vaporariorum* has been successfully managed in glasshouse systems with parasitoids (Vet et al. 1980).

Among the six species of *Eretmocerus* that have been reared from *T. vaporariorum* (Zolnerowich & Rose 2008), *E. warrae* (Nauman & Schmidt) (Hymenoptera: Aphelinidae) is a newly described thelytokous (no males) species (Workman et al. 2008). *Eretmocerus* sp. was observed to parasitise *T. vaporariorum* during a survey of greenhouses in Auckland, New Zealand, in 1997 (P.J. Workman, Plant & Food Research, pers. comm.). Ten years later, this species was identified as *E. warrae* using DNA sequencing (Workman et al. 2008). During the present study, adult wasps were sent to the Natural History Museum, London, for identification, and were confirmed as *E. warrae* (A. Polaszek, pers. comm.). However, little is known about the biology of this wasp.

Many behavioural, developmental and physiological events displayed by insects are controlled by endogenous circadian rhythms, which, in many cases, are modulated by external factors (Saunder 1982). The knowledge of a parasitoid's emergence, oviposition and feeding rhythms is fundamental for understanding the ecology and evolution of their reproductive strategies, which in turn contributes to the development and implementation

of biological control programs (He et al. 2004). Therefore, for better understanding of biological control ecology of *E. warrae*, experiments on the circadian patterns of emergence, oviposition and feeding were undertaken.

MATERIALS AND METHODS

Breeding colony and experimental conditions

The colonies of *T. vaporariorum* and *E. warrae* were initiated with parasitised and unparasitised pupae of the whitefly obtained from BioForce Limited, Auckland, New Zealand. 'Moneymaker' tomato plants were used for rearing whitefly. The colonies of *T. vaporariorum* and *E. warrae* were maintained and experiments were carried out at 22±1°C with 60±5% RH and 16:8 h light:dark, in the Entomology and IPM Laboratory, Massey University, Palmerston North, New Zealand. All parasitoids used for experiments emerged from pupae parasitised at the stage of 2nd and 3rd instar nymphs; and 2nd instar nymphs were used as hosts of parasitoids in all experiments.

Emergence

To observe the circadian emergence rhythm of *E. warrae,* two bioassay rooms were set up: a normal light regime in which photophase was set from 0800 h to 2400 h and a reverse-light regime in which scotophase was set from 1000 h to 1800 h. High-frequency, broad-spectrum biolux tubes (Osram, Germany) were used as light source. Observations in the scotophase were made under red photographic safe lamps (Phillips, Greensboro, NC).

To obtain parasitised whitefly nymphs, a tomato leaf infested with about 80-100 2nd instar nymphs were placed onto a Petri dish with a 0.5 cm layer of 1% agar solution for keeping the tomato leaf fresh. One newly emerged female parasitoid was released into the Petri dish for 24 h, and then moved each day into further Petri dishes containing the same number of whitefly nymphs until the parasitoid died. When nymphs had developed to pupal stage, they were collected and kept singly in glass vials (5 cm in height × 1.5 cm in diameter, with a 0.5 cm mesh covered hole in the lid) in the same bioassay room. Twenty female parasitoids were used in each room. Adult emergence was observed hourly in the entire photophase in the normal-light regime room, and in the entire scotophase in the reverse-light regime room.

Oviposition and feeding

To determine circadian oviposition and feeding rhythms of *E. warrae*, the light regimes in the two bioassay rooms were set up as above. One newly emerged parasitoid was released into an agar-based Petri dish (as above) containing a tomato leaf infested with 20 2nd instar whitefly nymphs. The female was allowed to oviposit for 2 h (first oviposition and feeding period), then moved into another agar based Petri dish containing the same number of nymphs (second oviposition and feeding period). This procedure was repeated until eight oviposition and feeding periods in the photophase and four oviposition and feeding periods in the scotophase were completed. Ten replicate females were tested in each light regime. As *E. warrae* place their eggs between the venter of whitefly nymphs and leaf surface (Qiu et al. 2007), all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope (Leica MZ12, German) after each oviposition period. The oviposition and host feeding patterns were determined by counting the number of eggs laid and host feeding by the parasitoid in each oviposition and host feeding period. Host feeding was recorded if the nymph body fluid was found to have escaped as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs (Vet et al. 1980; Viggiani 1984). The period between emergence and first oviposition was recorded as the pre-oviposition period.

Statistical analysis

Data on hourly emergence and number of eggs laid and hosts fed per period were not normally distributed even after transformation and thus were analysed using the non-parametric Kruskal-Wallis test (KWT), followed by Dunn's procedure for multiple comparisons (Zar 1999). ANOVA was used to examine the difference in the mean number of eggs laid and hosts fed per 2 h period between the photophase and scotophase.

RESULTS

Emergence

No emergence was observed in the scotophase. In the photophase, adult emergence was significantly higher between 2 and 3 h into the photophase and then significantly decreased (KWT:χ^2=80.00> $\chi^2_{5,0.05}$=11.07, P<0.0001) (Fig. 1). No adults emerged after 7 h into the photophase.

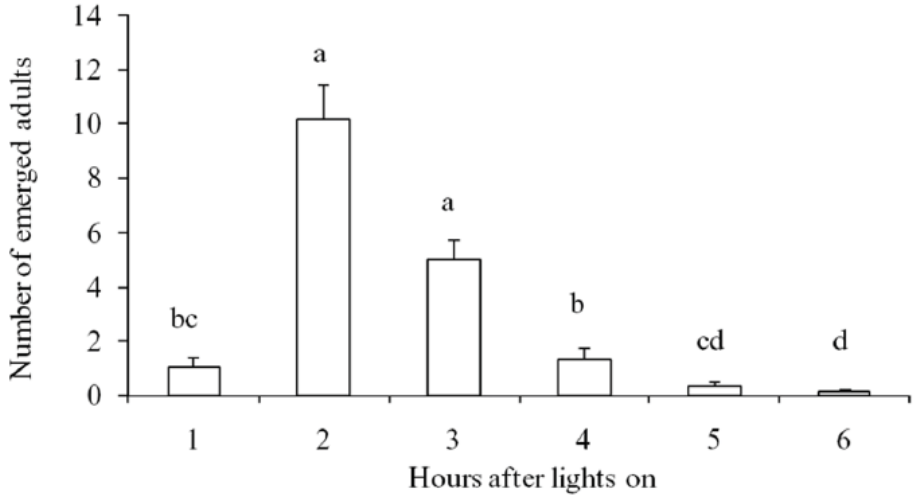

FIGURE 1: Emergence of *E. warrae* in the photophase. Bars with the same letters are not significantly different (P>0.05).

Oviposition and feeding

In the photophase, host feeding by *E. warrae* tended to be higher after 4–6 h of light than at other stages of photophase, but no significant difference was detected between feeding periods (KWT: χ^2=6.84<$\chi^2_{7,0.05}$=14.07, P>0.05) (Fig. 2). In the scotophase, the number of hosts fed by *E. warrae* was significantly greater after 6 h of the scotophase (KWT:χ^2=13.16>$\chi^2_{3,0.05}$=7.82, P<0.01) (Fig. 2).

Females laid significantly more eggs between 8 and 12 h after lights on than in other periods of the photophase (KWT:χ^2=19.30>$\chi^2_{7,0.05}$, P<0.01) (Fig. 2). In the scotophase, although higher oviposition was detected in the first 2 h after lights off, no significant difference was found between oviposition periods (KWT:χ^2=5.74<$\chi^2_{3,0.05}$=7.82, P>0.05) (Fig. 2). There was no difference in the mean number of eggs laid (0.75±0.23 and 0.50±0.11, respectively) and hosts fed (1.28±0.39 and 0.33±0.17, respectively) per period between the photophase and scotophase (P>0.05). The pre-oviposition period of *E. warrae* was 7.20±1.27 h.

DISCUSSION

Results of this study indicate that emergence occurred exclusively during the photophase, peaking during the first few hours of the photophase and then decreasing rapidly afterwards (Fig. 1). It is suggested that the onset of light may act as a signal for adult emergence. Fantinou et al. (1998) suggested that in *Telenomus busseolae* Gahan, a solitary egg parasitoid of various Lepidoptera, emergence during early photophase probably coincides with more favourable conditions for their survival, as in the morning field temperature is lower and humidity is higher than the rest of the day. Furthermore, *E. warrae* emergence early in the morning (Fig. 1) may facilitate maximum oviposition in the afternoon (Fig. 2).

The present results also show that *E. warrae* is active throughout a 24 hour period, suggesting that oviposition and host feeding by *E. warrae* is not controlled by

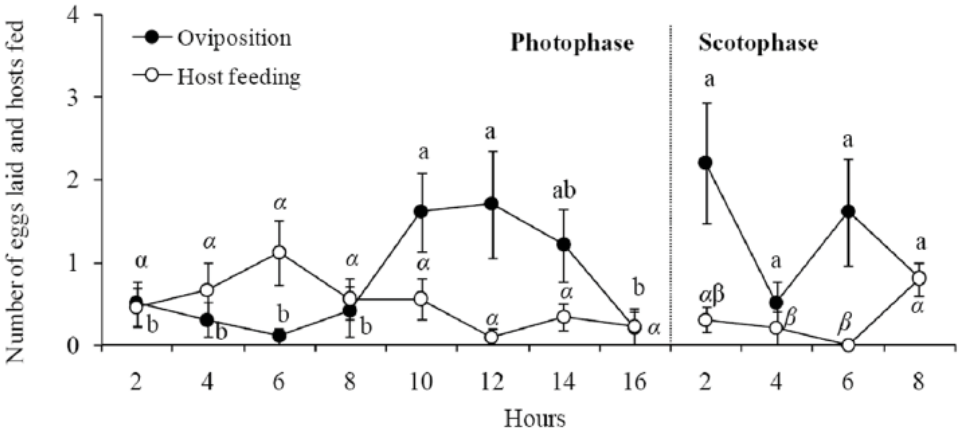

FIGURE 2: The number of eggs laid and hosts fed on by *E. warrae* throughout the photophase and scotophase. Means (±SE) followed by the same English letters within the oviposition line and the same Greek letters within host feeding line are not significantly different (P>0.05). Data from the photophase and scotophase were analysed separately.

endogenous oscillator or exogenous factor (i.e. the light), but rather the parasitoid may respond to cues from the host (Couch 1997). These properties may enable *E. warrae* to act successfully as an agent in the biological control of greenhouse whitefly.

Jervis & Kidd (1986) suggested that the primary role of host feeding is to secure nutrients necessary for egg maturation and studies have demonstrated that host feeding can promote parasitoid egg production (Giron et al. 2004; Burger et al. 2005). The main host feeding of E. warrae occurred before the oviposition peak in the photophase, suggesting that host feeding supplied nutrients for egg maturation.

The findings of this study have implications for laboratory mass rearing and field release of *E. warrae*. For example, pre-emerged *E. warrae* (i.e. beige pupal colour) should be placed in the greenhouse early in the morning so that with the light signal, parasitoids emerge and begin to feed upon whiteflies to promote oviposition.

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Effect of food supply on reproductive potential of *Eretmocerus warrae* (Hymenoptera: Aphelinidae)

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Abstract *Eretmocerus warrae* (Naumann & Schmit) is a thelytokous parasitoid of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). It was first detected in New Zealand in 1997 during a survey of greenhouses in Auckland. Under 22±1°C, 60±5% RH and 16:8 h light:dark, the effect of food supply on longevity, host feeding, fecundity and parasitism in *E. warrae* was investigated with four treatments: (1) no food and no host, (2) 10% honey solution and no host, (3) 40 2nd instar nymphs per day and no honey, and (4) 40 2nd instar nymphs per day and 10% honey solution. Results showed that parasitoids lived significantly longer when given honey but no host (8.8 days) than parasitoids given the other treatments (2.5~5.5 days) ($P<0.0001$). Honey supply significantly reduced host feeding ($P<0.05$). There was no significant difference ($P>0.05$) in lifetime fecundity (32.4~34.7 eggs), parasitism rate (14.7~16.0%) and superparasitism rate (10.3~11.4%) between parasitoids given hosts with or without access to honey.

Keywords *Eretmocerus warrae*, *Trialeurodes vaporariorum*, host feeding, fecundity, longevity.

INTRODUCTION

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), is one of the most important whitefly species, causing serious economic damage to crops throughout tropical and subtropical regions and in glasshouses in temperate regions of the world (Byrne et al. 1990). It was first found in greenhouses in UK in 1856 (Van Lenteren et al. 1996). The sessile nymphs of *T. vaporariorum* have been successfully managed in glasshouse systems with parasitoids (Vet et al. 1980; Gerling 1990). Among the six *Eretmocerus* species that have been reared from *T. vaporariorum* (Zolnerowich & Rose 2008), *E. warrae* (Nauman & Schmidt) is a newly described thelytokous (no males) species (de Barro et al. 2000). It was first found in greenhouses in Auckland, New Zealand, in 1997 (Workman et al. 2008). During the present study, adult wasps were sent to the Natural History Museum, London, for identification, and were confirmed as *E. warrae* (A. Polaszek, pers. comm.).

Adult females of many insects depend on carbohydrate-rich food as their main source of energy for longevity, fecundity and mobility (Jervis & Kidd 1986). In hymenopteran parasitoids, including *Eretmocerus* spp., longevity and in some cases fecundity may be influenced by a range of factors, such as temperature (Greenberg et al. 2000), host stage (Jones & Greenberg 1998), host density (A. Hanan, unpubl. data), host plant species (Qiu et al. 2005) and presence of food (Leatemia et al. 1995; McAuslane et al.

1996; Hardin et al. 2008). Among these factors, availability of food sources appears to be the most practical and economical means of promoting longevity (McDougall & Mills 1997). Previous studies on *Eretmocerus* demonstrated that sugar sources (e.g. saccharose and honey) and host hemolymph have a significant effect on longevity of *E. debachi* (Kuwana) and *E. mundus* (Merct) (Sengonca et al. 1994; Ghahari et al. 2005). It has also been demonstrated that *E. eremicus* lives significantly longer when fed with carbohydrate diets (Hardin et al. 2008). However, whether host feeding affects longevity and reproductive output of *E. warrae* is not clear.

Based on egg maturation, parasitoids can be classified as synovigenic (females continue to mature eggs during their adult lifetime) or pro-ovigenic (females complete oogenesis prior to eclosion) (Jervis & Kidd 1986). *Eretmocerus warrae* carries some mature eggs at eclosion and continues to mature eggs during adult lifespan (A. Hanan, unpubl. data), suggesting that it is a pro-synovigenic species (Jervis & Kidd 1986). It is well known that nutrients obtained by host feeding are used to mature eggs and sugar sources are used for the maintenance of life (Heimpel & Colier 1996). Food supply may allow pro-ovigenic species to increase longevity (Thompson 1999) and synovigenic species to increase both longevity and fecundity (Heimpel & Colier 1996). Jervis & Kidd (1986) suggested that synovigenic parasitoids whose adults feed on host fluid still require sugars as their main source of energy during the adult stage. Therefore, understanding the effect of food supply for adults on parasitoid fitness is important for mass-rearing and field enhancement of parasitoids for biological control programmes.

Prior to the present study there was no published information on the effect of non-host food (honey or sugars) on longevity, fecundity and parasitism in *E. warrae*, making it difficult to develop strategies for effective mass-rearing and field manipulation of this parasitoid. Therefore, the primary aim of this study was to determine the effect of food supply on the longevity, host feeding, fecundity and parasitism of *E. warrae*.

MATERIALS AND METHODS

Breeding colony and experimental conditions

The colonies of *T. vaporariorum* and *E. warrae* were initiated with parasitised and unparasitised pupae of *T. vaporariorum* obtained from BioForce Limited, Auckland, New Zealand. 'Moneymaker' tomato plants were used for rearing the colonies. The colonies of *T. vaporariorum* and *E. warrae* were maintained and all experiments were carried out at 22±1°C with 60±5% RH and 16:8 h light:dark, in the Entomology and IPM Laboratory, Massey University, Palmerston North, New Zealand. All parasitoids used for experiments emerged from *T. vaporariorum* pupae that were parasitised as 2nd or 3rd instar nymphs, and 2nd instar nymphs were used as hosts of parasitoids in this study.

Experiments

All experiments were carried out in plastic Petri dishes (5.5 cm diameter and 1.2 cm height). To investigate whether and how food supply affected host feeding, fecundity, parasitism and longevity of *E. warrae*, four treatments were set up: (1) no food and no host, (2) 10% honey solution and no host, (3) 40 2nd instar nymphs per day and no honey, and (4) 40 2nd instar nymphs per day and 10% honey solution. In treatments (2) and (4), 10% honey was provided in a cotton wick inserted into the Petri dish through a 1 cm hole in the lid. For each treatment, one parasitoid (<12 h since eclosion) was released into a Petri dish, allowed to stay for 24 h, and then moved into another Petri dish. This process was repeated until she died. As *E. warrae* place their eggs between the venter of whitefly nymphs and leaf surface (Hanan et al. 2009), all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope (Leica MZ12, German). The oviposition and host feeding patterns were determined by counting the numbers of eggs laid and hosts fed upon by the parasitoid. Host feeding was recorded if the nymph body fluid was found to have effused as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs (Vet et al. 1980; Viggiani 1984). The longevity of adult parasitoids was also recorded. There were 20 replicates (no. of parasitoids)

in treatments (1) and (2) and 10 replicates in treatments (3) and (4).

Statistical analysis

A goodness-of-fit test was used to test the distribution of data before analysis. All data were normally distributed and analysed by ANOVA. The percentage data were arcsine transformed before analysis. When significant differences in variables occurred, means were separated using a Tukey's studentised range (HSD) test. The responses of hosts fed upon and eggs laid per day to parasitoid age were analysed using linear regression, and slopes of each category (no. of hosts fed upon or no. of eggs laid) were compared using analysis of covariance (ANCOVA).

RESULTS

The parasitoids that only got access to honey lived significantly longer than those in other treatments, and those that fed on hosts only or both hosts and honey had significantly greater longevity than those without honey and hosts ($P<0.0001$) (Figure 1). However, the longevity was similar between parasitoids provided with hosts only and those with both hosts and honey ($P>0.05$) (Figure 1).

In the treatments 'hosts only' and 'honey + hosts', the number of hosts fed and eggs laid significantly decreased with parasitoid age ($P<0.0001$) (Figure 2). However, the decrease in host feeding was significantly faster in the 'host only' treatment than in the 'honey + hosts' treatment ($P<0.0001$) (Figure 2a), while the decline in the number of eggs laid with increasing parasitoid age was not significantly different between these two treatments ($P>0.05$) (Figure 2b).

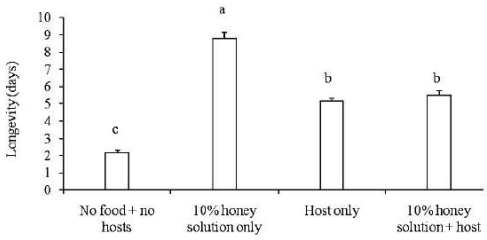

Figure 1 Effect of food and host supply on the longevity of *E. warrae*.

When lifetime data were considered, honey supply significantly reduced the number of hosts fed upon by the parasitoids ($P<0.05$) (Table 1). However, honey solution did not significantly increase *E. warrae* fecundity, parasitism or superparasitism rates ($P>0.05$) (Table 1).

DISCUSSION

Host feeding by hymenopteran parasitoids contributes to pest control (Jervis & Kidd 1986). The present study indicates that honey solution significantly prolongs the longevity of this parasitoid (Figure 1). These results are highly consistent with the results of Hardin et al. (2008) who demonstrated that *E. eremicus* lives significantly longer when fed with carbohydrate diets. In some parasitoids like *Trichogramma* spp. honey increases adult longevity 8–11 times compared to unfed or water-fed adults (McDougall & Mills 1997). However, when both hosts and honey are provided, *E. warrae* adults live significantly shorter than those provided with honey only. This phenomenon may be attributed to the costs associated with egg maturation and oviposition when hosts are present (Charnov & Stephens 1988; Mangel 1989). It is also possible that although parasitoids still feed on honey when hosts are present, they ingest less honey due to host feeding, resulting in shorter longevity.

Heimpel & Colier (1996) reported that when presented with hosts directly after feeding on water or honey, *Aphytis aonidiae* (Mercet) fed upon fewer hosts, implying that satiation from honey or water limits the host feeding rate. The present results also show that provision of honey solution significantly reduced host feeding by *E. warrae* (Table 1), suggesting that honey supply for this species can lower its biological control efficiency by host feeding. Furthermore, with the presence of hosts honey did not significantly increase *E. warrae* reproductive output (Table 1), suggesting that sugar sources may not be necessary in mass-rearing programmes of this species for biological control. However, when

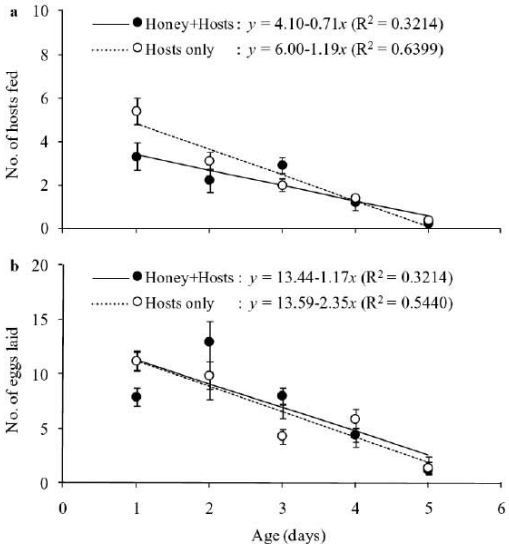

Figure 2 (a) Daily number of hosts fed and (b) eggs laid by *E. warrae* when provided with hosts only and honey + hosts. All data were pooled for regression but means (±SE) are presented.

hosts are rare or temporarily absent in a mass-rearing or biological control programme, the increase in longevity of *E. warrae* from 5 to 9 days due to provision of honey may enable the parasitoids to survive until they find hosts.

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Table 1 Effect of host and/or honey solution on lifetime host feeding and reproduction in *E. warrae*.

Treatments	No. hosts fed	No. eggs laid	Parasitism (%)	Super-parasitism (%)
Host only	12.4±0.5a	32.4±2.5a	14.7±1.1a	11.4±2.5a
10% honey + hosts	9.6±1.0b	34.7±2.8a	16.0±1.1a	10.3±1.5a

Mean (±SE) followed by the same letters in each column are not significantly different (P>0.05).

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Host feeding and oviposition strategy of *Eretmocerus warrae* (Aphelinidae: Hymenoptera) under different host densities

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Abstract *Eretmocerus warrae* Naumann & Schmidt is a thelytokous parasitoid of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). In conditions of 22±1°C, 60±5% RH and 16:8 h light:dark, the host feeding and oviposition strategy of *E. warrae* under seven host densities (20, 40, 60, 80, 100, 120 and 140 whitefly nymphs) was investigated. With an increase in host density the number of nymphs parasitised and fed upon by *E. warrae* significantly increased, but the percentage of nymphs parasitised, superparasitised and fed on significantly decreased ($P<0.0001$). The parasitoid longevity significantly increased with an increase in host density up to 100 nymphs ($P<0.0001$). These results suggest that *E. warrae* can adjust its feeding and oviposition strategy according to the host availability, and can potentially contribute to biological control of greenhouse whitefly.

Keywords *Eretmocerus warrae*, host density, fecundity, host feeding, parasitism, *Trialeurodes vaporariorum*.

INTRODUCTION

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), is an important pest, causing serious economic damage to crops throughout tropical and subtropical regions and in glasshouses (Byrne et al. 1990). The sessile nymphs of *T. vaporariorum* have been successfully managed in glasshouse systems with parasitoids (Vet et al. 1980; Gerling et al. 1990). Among the *Eretmocerus* species that have been reared from *T. vaporariorum* (Zolnerowich & Rose 2008), *E. warrae* Nauman & Schmidt is a newly described thelytokous (no males) species (de Barro et al. 2000). It was first found in greenhouses in Auckland, New Zealand, in 1997 (Workman et al. 2008). This species is a pro-synovigenic parasitoid because its ovaries contain a limited number of mature eggs and it continues to feed on hosts and mature eggs throughout its life (Hanan et al. 2010).

Parasitoid response to varying host density is an important parameter for measuring the parasitoid-host dynamics (Holling 1959). Understanding this in a parasitoid-host system can help evaluate the potential of a parasitoid for maintaining the host population (Murdoch & Briggs 1996). Hymenopteran parasitoids can increase their fecundity and alter the sex ratio of their progeny in response to host density (Waage

& Hassell 1982; Wang & Keller 2005). For example, *Aphidius ervi* Haliday, a parasitoid of pea aphid, parasitises a higher number of hosts and allocates a higher proportion of female progeny in response to higher host densities (He et al. 2006). However, in parasitoid species that use their hosts for both oviposition and feeding (Jervis & Kidd 1986; Heimpel & Collier 1996), the decision to oviposit or feed is manipulated by several factors depending upon the host and parasitoid itself (Lauzière et al. 1999). Egg load, maturation delay and life expectancy are parasitoid-related, while host quality and quantity are host-related factors (Sahragard et al. 1991; Rosenheim & Rosen 1992; Heimpel & Collier 1996; Videllet et al. 1997). Some studies suggest that host feeding is density-dependent and the proportion of hosts fed upon by a parasitoid decreases with increasing host density (Kidd & Jervis 1989). However, other studies demonstrate that host feeding is density-independent (Sahragard et al. 1991; van Lenteren et al. 1996) and oviposition is density-dependent where parasitoids respond positively to increasing host densities (Lauzière et al. 1999).

So far, little is known about the host feeding and oviposition strategy of *E. warrae* in response to the density of *T. vaporariorum*. The objective of this study was to investigate the influence of host density on fecundity, host feeding, parasitism, superparasitism and longevity of *E. warrae*. The information generated here would help optimise mass-rearing procedures and field release rates of *E. warrae* against whiteflies.

MATERIALS AND METHODS

Breeding colony and experimental conditions

The colonies of *T. vaporariorum* and *E. warrae* were initiated with parasitised and unparasitised pupae of *T. vaporariorum* obtained from BioForce Limited, Auckland, New Zealand. 'Moneymaker' tomato plants were used for rearing the colonies. *Trialeurodes vaporariorum* and *E. warrae* were maintained and all experiments carried out at 22±1°C with 60±5% RH and 16:8 h light:dark, in the laboratory. All parasitoids used in this study emerged from *T. vaporariorum* that were parasitised at the second or third instar.

Experiments

To determine whether and how host density affected host feeding, fecundity, parasitism and longevity of *E. warrae*, seven host densities were set up: 20, 40, 60, 80, 100, 120 and 140 second instar nymphs of *T. vaporariorum* per female *E. warrae*. Ten adult females (ten replicates) were used for each treatment. For each replicate, one female parasitoid (<12 h after adult emergence) was released into a Petri dish (5.5 cm in diameter × 1.3 cm in height) with a fresh leaf infested by a test number of nymphs, allowed to stay for 24 h, and then moved into another Petri dish containing the same number of nymphs. This process was repeated until she died. As *E. warrae* place their eggs between the venter of nymphs and leaf surface (Hanan et al. 2009; 2010), all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope (Leica MZ12, Germany). The daily and life time oviposition and host feeding patterns were determined by counting the numbers of eggs laid and hosts fed upon by the parasitoid. Host feeding was recorded if the nymph body fluid was found to have effused as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs (Hanan et al. 2010). The longevity of adult parasitoids was also recorded.

Statistical analysis

A goodness-of-fit test was used to test data normality. All data were normally distributed, thus analysed using ANOVA followed by Tukey's test. The percentage data were arcsine transformed prior to analysis but non-transformed means are presented.

RESULTS AND DISCUSSION

Egg load, host stage and host availability influence the oviposition and host feeding behaviour of many parasitoid species (Rosenheim & Rosen 1992; Heimpel & Collier 1996; Videllet et al. 1997; Hansen & Jensen 2002). The present study shows that with the increase in host density *E. warrae* laid more eggs, lived longer, and parasitised and fed on more whiteflies ($P<0.0001$) (Figure 1). These results suggest that at higher host densities

parasitoids obtain energy by feeding on hosts, which enables greater egg development and maturation as well as prolonged longevity. Sahragard et al. (1991) also report that in *Dicondylus indianus* Olmi (Hymenoptera: Dryindinae) increased fecundity at higher host densities is a positive function of increased longevity. Furthermore, host haemolymph fed on by adult parasitoids may provide lipids and carbohydrates necessary for higher fecundity and enable greater longevity of adult parasitoids as reported for *Eupelmus vuilletti* (Crawford) (Hymenoptera: Eupelmidae) (Giron et al. 2004).

In the present experiment with *E. warrae*, the proportion of hosts fed on, parasitised and superparasitised was significantly higher in lower host densities of 20 and 40 nymphs than in higher host densities of 60-140 nymphs ($P<0.0001$) (Figure 2). This pattern is consistent with a type II functional response (Holling 1959), which is also found for *A. ervi* (He et al. 2006), *E. mundus* Mercet (Zandi-Sohani et al. 2008) and *Encarsia acaudaleyrodis* Hayat (Shishehbor & Zandi-Sohani 2011). These results suggest that

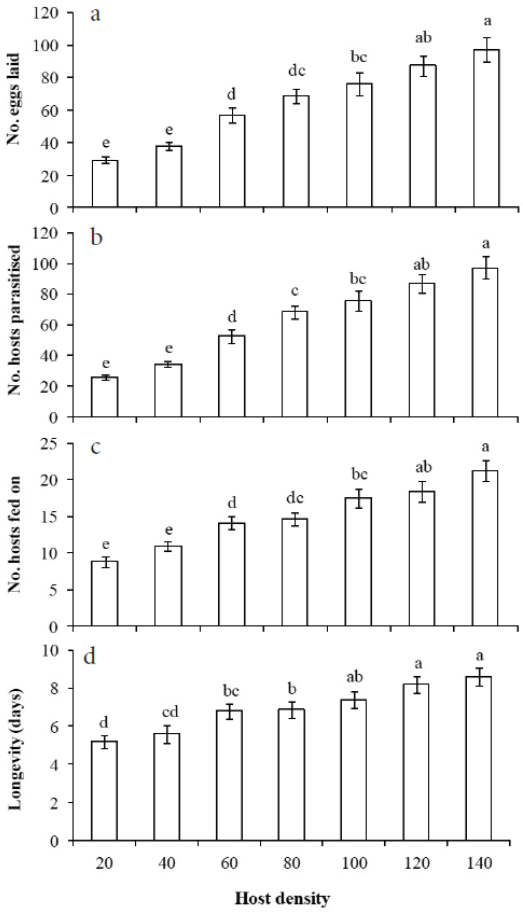

Figure 1 Mean (± SEM) values for (a) number of eggs laid, (b) hosts parasitised, (c) hosts fed on and (d) longevity as an adult, in *E. warrae* exposed to different host densities. Columns with the same letters are not significantly different ($P>0.05$).

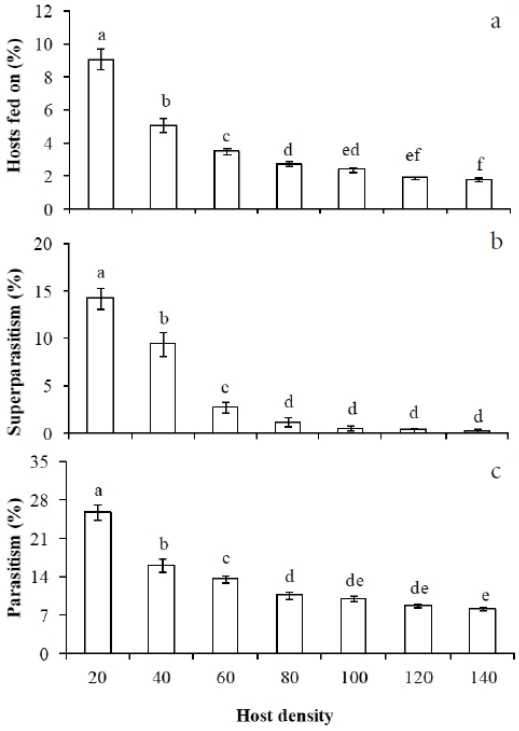

Figure 2 Mean (±SEM) percentage of hosts (a) fed on, (b) superparasitised and (c) parasitised, by *E. warrae* exposed to different host densities. Columns with the same letters are not significantly different ($P>0.05$).

E. warrae can adjust its feeding and oviposition strategy according to host availability.

The present results imply that *E. warrae* can contribute to the biological control of greenhouse whitefly because it significantly increases its fecundity and the number of whiteflies parasitised and fed on with an increase in host density. However, the highest parasitism and host feeding rates are found when the host density is 20 nymphs, suggesting that 20 *T. vaporariorum* nymphs per *E. warrae* adult is a good starting point for determining the optimal rearing density and release rate for augmentative biological releases.

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