Reproductive behaviour and fitness trade-offs in the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

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Abstract

Parasitoids are fascinating insects that lay eggs in or on the body of their hosts where parasitic immatures grow and develop by exploiting the fixed resources available in a host. This study investigated host-parasitoid interaction between the cabbage aphid *Brevicoryne brassicae* and its parasitoid *Diaeretiella rapae.* The research explored the reproductive decisions made by *D. rapae*, and how these decisions affect its fitness and pest suppression ability.

The haplodiploid nature of reproduction in *D. rapae* imposes strongly contrasting outcomes of mating and oviposition decisions that directly affect population sex ratio. This study found that parasitoid fitness is the integral outcome of lifetime mating and oviposition behaviours. Poor host-parasitoid synchronisation was found in an uncontrolled/open system in spring in New Zealand; a low female/male ratio and a significant number of erroneous male-male mating pairs were detected in this *D. rapae* population. Adult emergence occurred only during the light period, with males emerging before females (protandry). Light triggered both mating and oviposition in *D. rapae*. Female *D. rapae* preferred to mate before oviposition, which allowed them to produce female-biased offspring. Females were found to allocate more time for choosing their mates whereas males were more active during mating and selected their mates quickly. Females mated once (monandrous), while males mated multiple times (polygamous) and became sperm depleted after their third mating. The monandrous and polygamous nature of *D. rapae* changed the female-biased sex ratio to a highly male-biased operational sex ratio, resulting in mating interference. Several factors including age, body size, mating status and previous mating experience affected mate selection behaviour in males and females. Female *D. rapae* emerged with developed eggs and did not require additional food to mature their eggs (autogenous), however, it took about two days for all their eggs to mature (weakly synovigenic). The nutrients acquired during the larval stage (by feeding on host resources) and during adult stage (by feeding on 10% honey solution) both affected individual fitness. Parasitoids lived longer after feeding on honey solution and this effect was more pronounced in females than in males. Female *D. rapae* fed on honey also carried their eggs longer without resorbing them. Females preferred to oviposit in larger hosts than in smaller ones, despite stronger defensive behaviour of the larger hosts. Females also preferred the larger hosts for ovipositing fertilised eggs that resulted in larger female offspring; the females that emerged from larger hosts lived longer and produced more offspring than those emegered from smaller hosts. Female oviposited multiple eggs per host (superparasitism) after repeatedly attacking their hosts. This resulted in two to eight parasitoid larvae developing in a host, but only one adult emerged from each (solitary parasitoid). Female *D. rapae* produced more female offspring when hosts were limited, and the number of males only increased when host density was higher. Females oviposited more unfertilised eggs when competing with conspecifics, which allowed them to conserve their fertilised eggs for future oviposition.

Thus, the study suggests that strong intrasexual competition and intersexual selection exist during mating and oviposition in *D. rapae*. This study provides comprehensive information on interactions between cabbage aphid and *D. rapae* which can be used to develop effective biological control programmes for cabbage aphid and other aphid species using *D. rapae* or other parasitoids. Release of honey-fed, mated and 1-day old females in early morning and on sunny days would be most effective and result in quicker suppression of aphid populations. Raising females in low competition situations with large size hosts (5-7 day old) could help in producing efficient and female-biased broods in insectaries.

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Preface

A thesis is presented on reproductive behaviour and fitness trade-offs in aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae). The thesis is comprised of four main parts – General Introduction, experimental chapters (Chapters 1-13), General Discussion and Conclusions, and an Appendix (Appendices 1-3). The experimental work was carried out at Massey University, Palmerston North, New Zealand.

I am the author of each section and chapter, and the first author on published papers from this research. The co-authors of the published papers are my PhD supervisors. I designed the initial experiments, executed the experiments, analysed the results and discussed findings of each chapter. The supervisors gave their inputs in finalising the experiments, helping with statistical analyses, providing comments on the results, and reviewing the drafts in terms of language and clarity.

Various aspects of reproductive, mating and oviposition behaviour of *D. rapae* are presented in **Chapters 1-13**. Each Chapter is presented as a standalone paper with its own Introduction, Methods, Results, Discussion and References, and as a result, there is some repetition between the Chapters. The numbering of figures and tables restarts at the beginning of each Chapter.

The thesis begins with a **General Introduction,** which covers the background information and literature relevant to the species used in the research, as well as theories related to reproductive fitness and rationale for this research. **Chapter 1** investigates parasitism and mating strategies of *D. rapae* in a wild population (uncontrolled conditions), identifying some problems in oviposition and mating in *D. rapae*, for example, erroneous male-male mating. All other studies in this thesis were carried out in the laboratory at controlled temperature, humidity and light period. **Chapter 2** looks at the emergence pattern and diurnal variations in mating and oviposition activities of *D. rapae*. Sexual receptivity, courtship and mating behaviour of emerged adults are reported in **Chapter 3**. Sexual selection in *D. rapae* is studied in **Chapter 4**. The effect of multiple matings on sperm transfer and on the fitness of males and females is investigated in **Chapter 5.**

I looked into general biology and the importance of adult food availability for longevity and reproductive potential of *D. rapae* in **Chapter 6**. Since *D. rapae* is a haplodiploid species, a newly emerged female has a valid choice between ovipositing unmated or after mating. I investigated the fitness consequences of this choice in

Chapter 7. Further, I examined the effects of age, and mating and oviposition delay on overall fitness of *D. rapae* in **Chapter 8**.

The general host searching, handling and oviposition behaviours of *D. rapae* are described in **Chapter 9**. **Chapters 10** and **11** investigate the preference–performance hypothesis in host selection, and the effect of host selection on reproductive fitness**.** Although *D. rapae* is a solitary parasitoid and only one adult emerges per host, the females can lay more than one egg per host (superparasitism). **Chapter 12** examines the consequences of superparasitism for fitness, and checks experimentally whether or not female *D. rapae* can discriminate between unparasitised and already parasitised hosts. The last **Chapter 13** deals with reproductive strategies of *D. rapae* females when they are competing for hosts and when more than one conspecific female are foraging together.

The findings from all the chapters are discussed in **General Discussion and Conclusions** in a broader context of reproductive fitness, biological control and evolution. The **Appendix 1** includes phylogenetic work on *D. rapae*. Some additional information on superparasitism that could not be included in published paper (**Chapter 12**) is reported in **Appendix 2**. Abstracts of the full papers published in journals, or abstracts published in conference proceedings which arose from this research are given in **Appendix 3**.

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General Introduction

Insect pests in Agriculture

Insect pests cause significant damage to agricultural and horticultural crops. Economic losses due to insect pests exceed US$ 500 billion per annum worldwide. Despite plant protection measures, approximately 42% of the total crop yield is lost. Without plant protection measures we could lose as much as 70% of total yield (Koul et al. 2004). Among various methods of pest management, use of pesticides is the most frequent control method worldwide. However, this control measure often fails or lasts for a short period only. The escalating cost of pesticides puts an extra burden on the growers, and makes pesticides less affordable to poor farmers. Moreover, several additional problems are associated with the use of pesticides, such as pesticide residue in food, environmental pollution, and, increasingly, pesticide resistance in many pest insects (Palumbi 2001).

Biological control provides an alternative to pesticide use in pest management. Biological control is not only effective in regulating pest populations, but is also considered as a sustainable, eco-friendly pest management tool which can improve ecological systems by minimising pesticide use.

Biological control using parasitoids

Biological control is the utilization of living organisms (bio-agents) such as predators, parasitoids, or pathogens for management of insect pests (Debach & Schlinger 1964), and is common in natural ecosystems (Waage 1986). The bio-agents keep the pest (host) population low, while maintaining their own self-sustaining population. Predators consume all stages of hosts, often from a wide range of species, including non-pest species. They are generally larger than their hosts, and they require several host individuals in order to complete their life cycle.

In contrast to predators, parasitoids are host stage- and species-specific. They are usually smaller than their hosts and require a single host to complete their life cycle (Alphen & Visser 1990). The immature stage is the only parasitic stage in parasitoids, when they feed upon the host’s body tissue or body fluids, eventually killing the host (Quicke 1997). There are about 70,000 described species of parasitoids, which constitute about 10% of all insect species. Nonetheless, it is estimated that altogether 800,000 described and undescribed species of parasitoids exist in the world (Godfray 1994). Most parasitoids (about 80%) belong to the order Hymenoptera, while about 15% are Diptera and the rest (5%) are from the orders Coleoptera, Neuroptera and Lepidoptera (Gaston 1991).

Biological control has a long history. Successful control of cottony cushion scale *Icerya purchasi* Maskell in California through use of the beetle *Rodolia cardinalis* Mulsant imported from Australia in 1888 paved the way for biological control in insect pest management (Caltagirone 1981). Biological control using parasitoids can be approached in three ways: (1) by importing host-specific parasitoids from the region of origin of the invasive pest, also called classical biological control (Gerson et al. 1975; Caltagirone 1981; Vargas et al. 2007) (2) by augmentative (periodic) release of laboratory-produced parasitoids in the field or in a glasshouse (Van Lenteren & Bueno 2003; Collier & Van Steenwyk 2004; Crowder 2007) and (3) by conserving the imported or native parasitoid through provisioning of limiting resources such as food resources and/or habitat modification (Tooker & Hanks 2000; Hopper 2003; Tylianakis et al. 2007).

In New Zealand several parasitoid species have been deliberately introduced for the management of insect pests, and some were accidently introduced. The present study investigated an aphid parasitoid *Diaeretiella rapae* which has been used in biological control of aphids in a number of countries including USA, Canada and Australia (Read et al. 1970; Carver & Stary 1974; Bernal & Gonzalez 1993). *D. rapae* was accidently introduced, and later established in New Zealand, but has received little attention in relation to its use in biological control. Wide genetic variability occurs in this parasitoid (Baker et al. 2003). Studies have also found that indigenous North American populations of *D. rapae* do not attack the invading Russian wheat aphid *Diuraphis noxia* Morvilko as effectively as re-introduced Old World parasitoid populations (Lee et al. 2005). This strongly supports the importance of understanding reproductive behaviour of local populations of *D. rapae* for its use for aphid suppression in New Zealand. Since the biology and behaviour of a parasitoid species depend on its geographical location, the study of local New Zealand populations of *D. rapae* is important for its use in pest management.

Classification and biology of *Diaeretiella rapae*

Diaeretiella rapae was first described in 1855 by Curtis as *Aphidius rapae,* and mentioned in the "Book of the garden" written by Charles McIntosh of England (1853). This species was previously known as *Diaeretus rapae* Curtis until 1960, when Stray (1961) revised its name as *Diaeretiella rapae* (McIntosh). It is the only species in the genus *Diaeretiella*. This parasitoid has also been described under various other names, later synonymised (Table 1).

Table 1: Synonymy of *Diaeretiella rapae*

Synonyms of *D. rapae*	Author	Year
Aphidius rapae	Curtis	1860
Diaeretus chenopodii	Forster	1877
Misaphidus halticae	Rondani	1877
Trioxys piceus	Cresson	1879
Lipolexis chenopodiaphidis	Ashmead	1889
Diaeretus ferruginipes	Ashmead	1890
Aphidius brassicae	Marshall	1896
Diaeretus californicus	Baker	1909
Lysiphlebus crawfordi	Rohwer	1909
Diaeretus nipponensis	Viereck	1911
Diaeretus obsoletus	Kurdjumov	1913
Diaeretus napus	Quilis	1931
Diaeretus croaticus	Quilis	1934
Diaeretus plesiorapae	Blanchard	1940
Diaeretus aphidum	Mukerji & Chatterjee	1950
Diaeretiella rapae	McIntosh	1960

The taxonomic details of *D. rapae* have been described by several authors (Smith 1944; Stary 1960; Hafez 1961; Mackauer 1961; Stary 1961; Kavallieratos et al. 2010; Akhtar et al. 2011). The general taxonomic classification of *D. rapae* is summarised in Table 2.

Table 2: Taxonomic position of *Diaeretiella rapae*

Kingdom: Animalia
  Phylum: Arthropoda
    Class: Insecta
      Subclass: Pterygota
        Order: Hymenoptera
          Suborder: Apocrita
            Superfamily: Ichneumonoidea
              Family: Aphidiidae
                Subfamily: Aphidiinae
                  Tribe: Aphidiini
                    Subtribe: Aphidiina
                      Genus: *Diaeretiella*
                        Species: *rapae*

Origin, distribution and host range of *Diaeretiella rapae*

Diaeretiella rapae is of Western Palaearctic origin (Carver & Stary 1974a), and has been established in various countries including India (Kundu et al. 1966; Sethumadhavan & Dharmadhikari 1969), China (Liu 1989b), Japan (Takada 1975), France (Bonnemai 1970), Germany (Madel & Kilger 1981), Nigeria (Akinlosotu 1974), Kenya (Bahana & Karuhize 1986), Canada (Read et al. 1970), United States (Bernal & Gonzalez 1993) and Australia (Carver & Stary 1974). In New Zealand it has been accidently introduced, and established, during the early twentieth century (Carver & Stary 1974).

Diaeretiella rapae parasitises more than 60 aphid species, among which five to six species are common hosts that attack crops of agricultural and horticultural importance (Mackauer & Stary 1967; Pike et al. 1999). *D. rapae* predominantly parasitises the cabbage aphid (George 1957) and is the only parasitoid reported from cabbage aphid. The parasitoid has played a significant role in suppressing populations of Mustard aphid *Lipaphis erysimi* Kalt in oilseed rape and mustard in Africa and India (Bahana & Karuhize 1986; Devi et al. 1999), and has been deliberately imported to the United States for biological control of Russian wheat aphid *Diuraphis noxia* (Bernal & Gonzalez 1993) and to Australia for control of cabbage aphid *Brevicoryne brassicae* L. (Carver & Stary 1974). It has been reported in New Zealand from various aphid species, predominantly from cabbage aphid (Walker & Cameron 1981) and from green peach aphid *Myzus persicae* Sulzer (Irvin et al. 2006).

Cabbage aphid as a preferred host

The cabbage aphid *Brevicoryne brassicae* (L) (Homoptera: Aphididae) is dull green to grey in colour and covered with a waxy powder on the entire body. Average adult size is about 2 mm in length and 1 mm in width. Both wingless (apterous) and winged (alate) forms are prevalent in natural populations (Fig. 1). The aphid undergoes incomplete metamorphosis and has four nymphal instars (Hughes 1963). The immature stages and adults are very similar in appearance. The aphid is parthenogenic and each adult produces 20-30 nymphs in her life time (Ulusoy & Olmez-Bayhan 2006). Nymph takes 7 days to become adult and begin producing offspring. The nymph carries the immature young in its body during its development. The life cycle duration varies from 25 to 35 days.

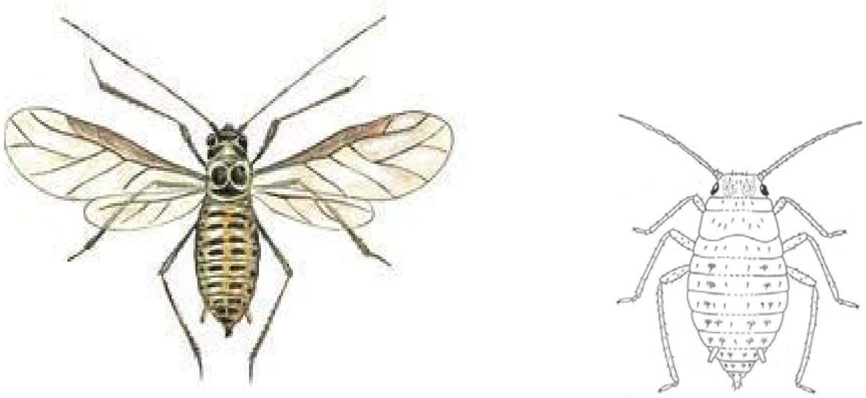

Figure 1: Alate (winged) and apterous (wingless) forms of the cabbage aphid *Brevicoryne brassicae*. (Source- http://mazinger.sisib.uchile.cl/repositorio/lb/ciencias_agronomicas/arayaj01/p2/c11/3.html)

Aphids are important cosmopolitan pests, and are responsible for substantial economic loss to agricultural and horticultural industries in New Zealand (Wellings et al. 1989; Teulon & Stufkens 2002). The cabbage aphid is a worldwide pest of Cruciferous plants family including cabbage, cauliflower, Brussels sprouts, rape, swede, turnips, broccoli, kale, mustard and some weed species (Ayal 1987) (Fig. 2).

In New Zealand, cabbage aphid is considered a severe agricultural pest (Costello & Altieri 1995). The aphid attacks plants of all ages, but prefers young plants. The aphids cause direct and indirect damage to the host plants by prolonged sucking of the phloem sap (Vos et al. 2007). This causes yellowing and curling of the leaves, and may cause wilting and stunting of the whole plant. The cabbage aphid is a vector of about twenty different plant viruses, including cauliflower mosaic and turnip mosaic viruses (Ellis et al. 1998). Vegetable crops infested with aphids are rejected by the market (causing economic loss to the grower), and feed-crops that are heavily infested with the aphid may also be unpalatable to livestock.

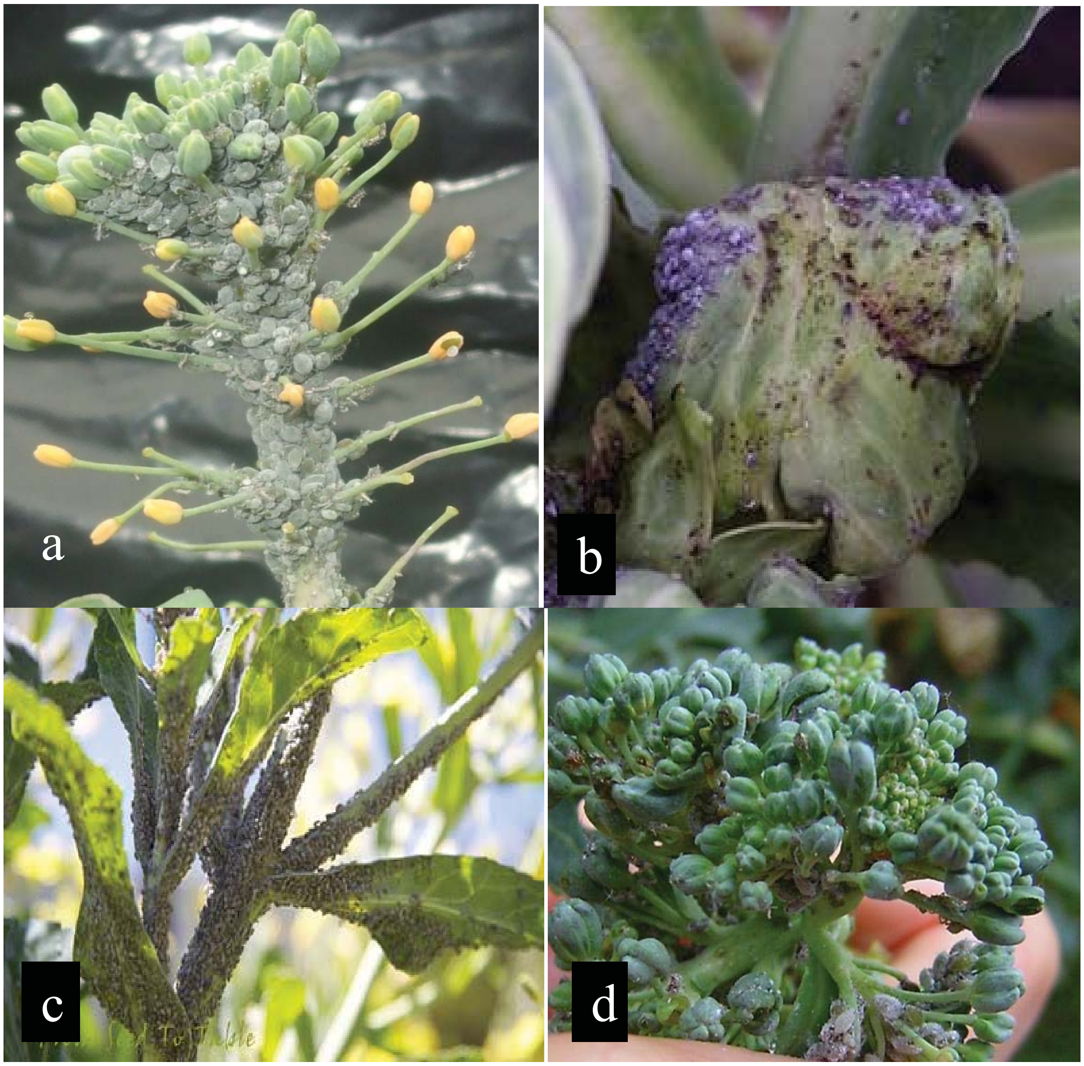

Figure 2: Cabbage aphid *Brevicoryne brassicae* infestation on crucifer plants: (a) Mustard, (b) Cabbage, (c) Brussel sprouts, and (d) Broccoli.

Several pesticides have been used to control the cabbage aphid. The waxy covering on the body of the aphid and the waxy nature of the crop mean that spraying adjustment and addition of surfactants are needed during pesticide spray. Moreover, the cabbage aphid has been reported to develop resistance against pesticides (Cameron & Fletcher 2005). Biological control of cabbage aphid has been done through predators such as syrphids (Sharma & Bhalla 1988), lady-bird beetles (Snyder et al. 2006) and lacewings (Messina et al. 1997; Islam & Chapman 2001), and the parasitoid *D. rapae* (Vidal 2007). In New Zealand, pesticides are widely used for control of these aphids, although there is now a trend toward alternative options of Integrated Pest Management (IPM) and biological control methods (Manktelow et al. 2005).

Life stages and biology of *Diaeretiella rapae*

The life cycle of a parasitoid can be divided into two stages: the immature stage and the adult stage (van Driesche & Bellows 1996). *D. rapae* is a holometabolic organism that undergoes complete metamorphosis, thus has distinct egg, larval, pupal and adult stages (Fig. 3). It lays one or more eggs inside the aphid (host) body and a single adult emerges from the mummified aphid. *D. rapae* overwinters in the larval stage. The different stages of *D. rapae* are described below.

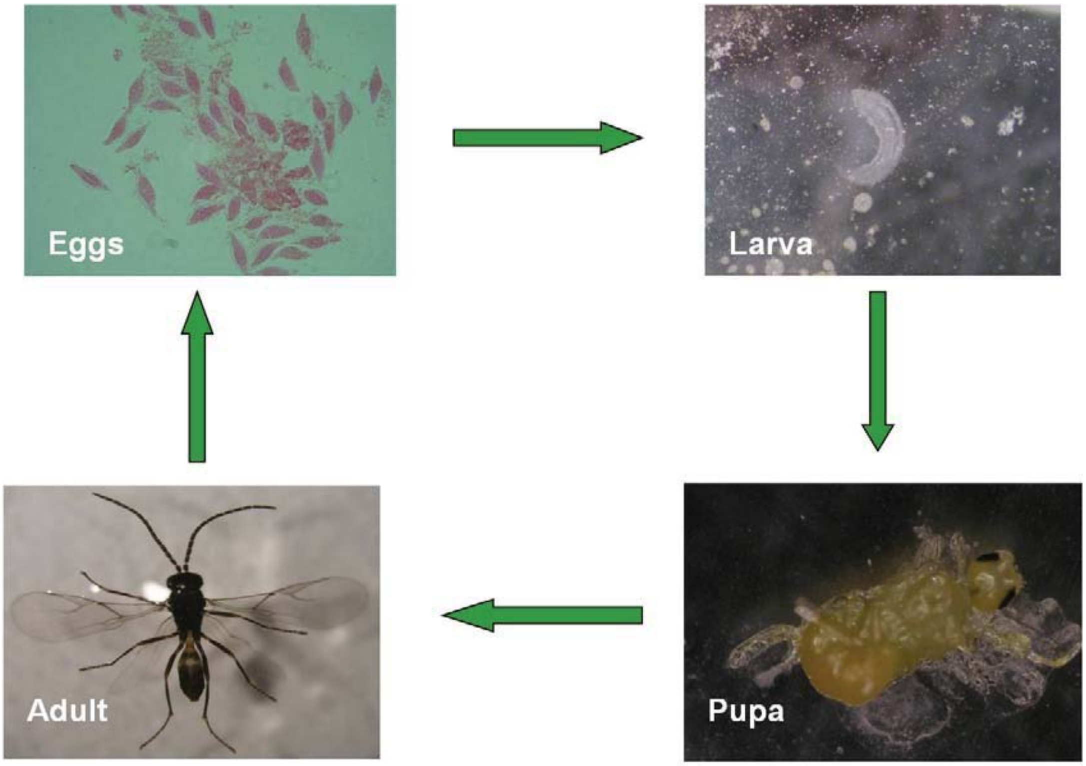

Figure 3: Life stages of *Diaeretiella rapae*: egg, larva, pupa and adult.

Egg

The female parasitoid has a pair of ovaries in which eggs (oocytes) develop. Eggs of *D. rapae* are ovoid in shape, with tapering ends, measuring 0.27 x 0.35 mm (Fig. 4). Hymenopteran parasitoids possess specialised ovipositors, which are used for stinging the host and for oviposition. During oviposition, the egg, as it passes through the female ovipositor, is squeezed and elongated; following deposition in host haemocoel it then absorbs host haemolymph, and increases in size (Jervis 2005). The egg stage lasts about 48 h before hatching.

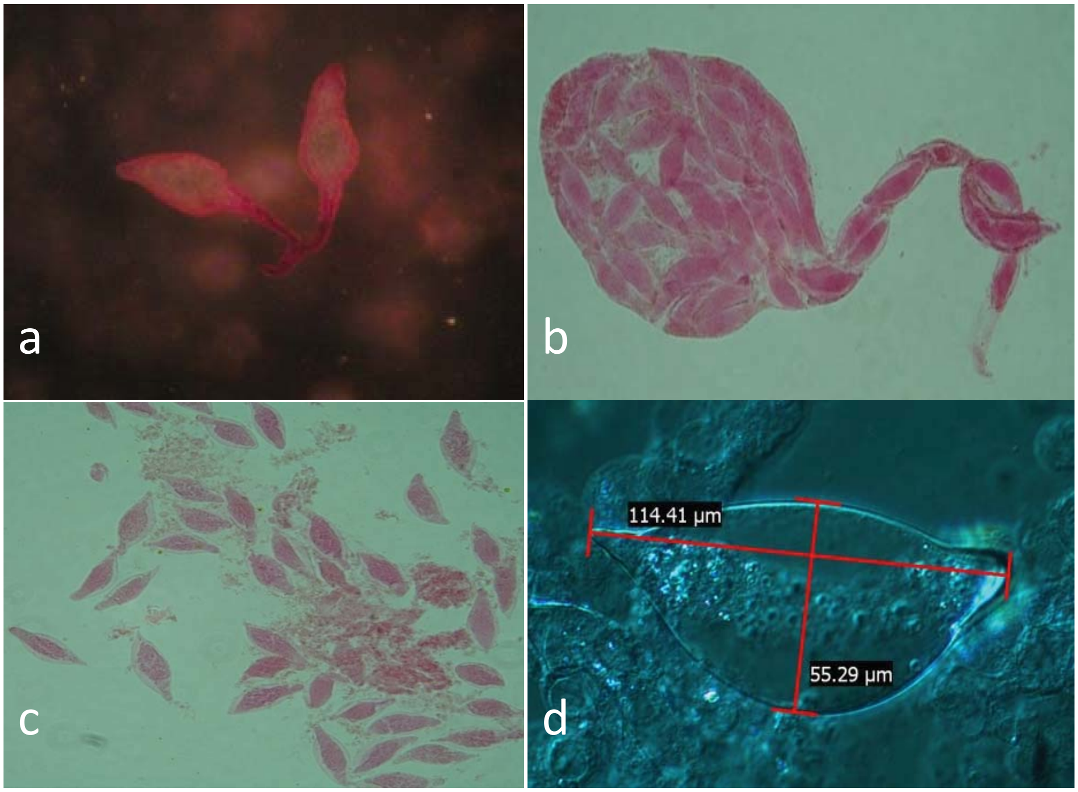

Figure 4: A pair of ovaries (a), ovary with mature eggs (b), eggs dissected from ovary (c) and measurement of a single egg (d) of *Diaeretiella rapae.*

Larva

The only parasitic stage in *D. rapae* is the larval stage. Larvae of *D. rapae* undergo four moults during their development (Spencer 1926). First and fourth instar larvae are mandibulates, while the second and third instar are hymenopteriform, that is lacking mandibles (Couchman & King 1977) (Fig. 5).

Figure 5: *Diaeretiella rapae* larvae: (a) 3-4 days after oviposition, larva with mandibles, (b) 4-5 days after oviposition, larva without mandibles.

After hatching, first instar larvae of *D. rapae* are attracted to the host embryos, migrate towards them, and use mandibles to break them apart (Broussal 1966). The presence of salivary glands in the first instar larvae enables them to produce catalytic secretions, which induce biochemical degradation of host tissue (Stary 1970) before ingestion. The thicker cuticle of the first and fourth instar larvae, compared to second and third instars (Couchman & King 1977), protects them from self-digestion. The second and third instar larvae lack salivary glands, lie in the host haemocoel, and feed on liquid or semi-liquid food using sucking movements created by the muscular pharynx. The fourth instar larvae are again active and consume the remaining host tissues such as the gut, musculature and nervous system, killing the host in the process (Couchman & King 1977).

Temperature affects the development and the life span of *D. rapae* and its host cabbage aphid. The development time decreases steadily with increased temperature from 10 to 25^{o}C, but an increase to 30^{o}C prolongs the development (Akinlosotu 1974). The maximum duration of development of the aphid and its parasitoid is 25 and 35 days, respectively, at 10^{o}C, and the minimum is 6 and 10 days, respectively, at 25^{o}C (Akinlosotu 1974). The threshold of development for aphids has been estimated to be 3.2^{o}C, while that of parasitoid varied from 1.7 to 3.6^{o}C depending on the developmental stage of the parasitised hosts. Aphid mortality was lowest (25%) at 20^{o}C, which also appears to be the optimum temperature for reproduction of the aphid and parasitic activity by *D. rapae* (Akinlosotu 1974). The development threshold and thermal constant for the period from egg to adult of *D. rapae* has been estimated to be 8.76°C and 139.51°C degree days, respectively (Liu 1989)

Pupa

The parasitoid larva pupates inside the host body (Fig. 3). Before pupation, the final instar larva makes a small hole on the ventral side of the host, which then fixes the aphid to the substrate by a secretion (Couchman & King 1977). The final instar larva spins a cocoon for protecting itself from any external injuries. When the aphid host dies, its body changes to a hardened exoskeleton called a mummy (Godfray 1994).

Adult

Under laboratory conditions, egg-to-adult development ranges from 9 to 15 days. Once the development inside the mummy is completed, the adult makes a small circular hole in the dorsal part of the abdomen of the mummified aphid to eclose (Fig. 6).

Figure 6: Parasitised aphid mummies fixed on the leaves, a circular orifice made by an emerging *D. rapae* adult on the posterior side of an aphid mummy (arrow).

The newly emerged adult expands its wings and grooms itself before walking. Adults are free-living, feeding on nectar or honey dew. The female has a protruding ovipositor (Fig. 7).

The number of adults emerging from a host varies with parasitoid species. Species in which more than one adult emerges per host are called gregarious parasitoids, while species in which only one adult emerges per host, irrespective of the number of eggs oviposited in the host, are called solitary parasitoids (Godfray 1994; Hackermann et al. 2007). *D. rapae* is a solitary parasitoid, only one adult emerges from host (Stary 1988), and it also falls into the category of koinobiont, where the parasitoid allows its host to feed and develop (Ayal 1987; Stary 1988), and sometimes to reproduce (Zhang & Hassan 2003) even after it has been parasitised.

Figure 7: A female and a male *D. rapae*. The female has an ovipositor protruding outside the abdomen (circle), and male has longer antennae with more antennal segments (arrow).

The longevity of the parasitoid adult stage (Blackburn 1991) affects the fitness of a parasitoid (Roitberg et al. 2001). Males with high longevities have more time to mate with more females and females living longer can also lay more eggs during their life time (Sandlan 1979; Godfray 1994). There are several biotic factors that could affect the longevity: food supply, body size of the adult, mating and oviposition. Climatic factors, including temperature, wind and rain can also affect the mortality/longevity of parasitoids. The longevity of *D. rapae* has been recorded in various studies. Adult females live for 10–15 days and males live for 7–10 days (Hafez 1961; Simpson et al. 1975; Reed et al. 1992). Akinlosotu (1974) found that adult *D. rapae* lived for a maximum of 12.9 days at 10^{o}C and a minimum of 1.8 days at 30^{o}C.

The total number of eggs laid by a female in her lifetime is referred to as fecundity, which is correlated with her reproductive potential (Ellers et al. 1998; Mills & Kuhlmann 2000). Fecundity is used as a measure for determining the fitness of a parasitoid (Ellers et al. 1998; Roitberg et al. 2001). Parasitoids having higher fecundity are able to produce more offspring in the next generation. Some female parasitoids emerge with a full complement of developed eggs (pro-ovigenic females) and others emerge with partially-developed eggs (synovigenic) (Flanders 1950). Jervis et al. (2001) developed an ovigeny index for parasitoid species. The index is based on the number of mature eggs and the total number of eggs (oocytes) present in the ovary of a female at the time of emergence. Proovigenic species have an index 1 while synovigenic species have index 0. Females whose indices lie between 1 and 0 are defined as weakly synovigenic. Time is an important constraint for synovigenic species, as they can produce more eggs if they live longer (Ellers et al. 2000b). However, the number of eggs in a female is an important constraint for pro-ovigenic females, which cannot produce more eggs after emergence (Heimpel et al. 1994; Casas et al. 2000).

The number of offspring that a female produces (fertility) is less than, or equal to the fecundity. Biotic factors such as host-parasitoid density, host size (host quality), and the size and mating status of the female influence the fertility of a female parasitoid (Honek 1993). Polyandrous females generally live longer and produce more offspring than monandrous females (Ridley 1988). Several factors, such as temperature, photoperiod and size of the adult female are known to influence the fecundity of a parasitoid (Force & Messenger 1964; van Steenis 1993).

Reproductive behaviour in parasitoids

Mating and oviposition are the two components of reproduction in insects. Most insects reproduce sexually and produce diploid males and females. In some parasitoids, reproduction does not require mating (thelytoky), while in others mating is required for producing female offspring (arrhenotoky). In arrhenotokous haplodiploidy, fertilised (diploid) eggs develop into female offspring and unfertilised (haploid) eggs develop into males (Crozier 1977; Jervis 2005). The unmated females can lay only haploid eggs and so produce only male offspring, while the mated females can store sperm in their spermatheca and thus can lay either fertilised or unfertilised eggs (Wilkes 1965; Dijken & Waage 1987; Godfray 1994). *D. rapae* is an arrhenotokous haplodiploid species.

The male and female parasitoids make important decisions during mating and oviposition, which affect the future generation (Hemerik et al. 2002). The behaviour of the parasitoids varies considerably, both within species and between species (Blackburn 1991; Godfray 1994; Jervis et al. 2003). Little information is available on reproductive behaviour of *D. rapae.*

Mating system and mating behaviour

After emergence, male and female parasitoids search for mates. Males and females display various courting behaviour before and during copulation to maximise their chances of mating, and after copulation to ward off other males. Alexander et al. (1997) described the general mating behaviour in parasitoids which includes pair formation, courtship, copulation and events after copulation. The behavioural display depends on the sexual receptiveness of the male and female. After emergence, an arrhenotokous female should mate to store sperm in order to produce females and maximise her lifetime fitness (Darrouzet et al. 2007). Male fitness is generally associated with competitive abilities, as both sperm and seminal fluids produced by males are sufficient for one or several reproductive activities (van den Assem et al. 1989; Reinhardt 2001). Males maximise their reproductive success by mating multiple times (Godfray 1994).

Darwin (1871) proposed natural selection and sexual selection as the central theme for his theory of "struggle for existence and survival of the fittest". For insects, the theory extends until oviposition, and for parasitoids it includes the female finding a suitable host for oviposition. Sexual selection occurs through intersexual selection, where males and females compete for mating (Panhuis et al. 2001). During sexual selection, males battle for mates in intrasexual competition, while females have intersexual choice during mating (Trivers 1972). Both intersexual and intrasexual competition become more intense when the number of females and males in the population is uneven, which is very common in haplodiploids. The main reason for the sexual selection is the variation in quality, quantity and availability of mates in space and time, which directly affects the reproductive fitness of an organism.

The reproductive system of females includes a pair of ovaries, oviduct, spermatheca, venom gland and Dufour's gland and an ovipositor (Fig. 8). Eggs develop in the ovaries and pass through oviducts to the ovipositor.

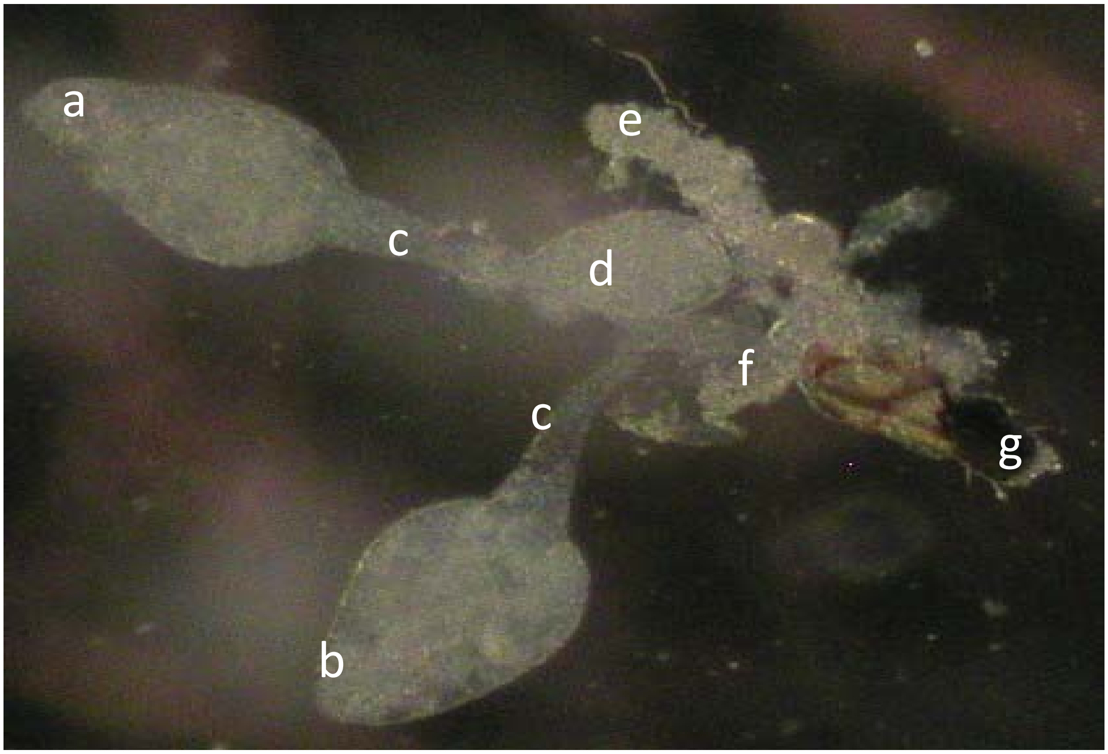

Figure 8: Dissected reproductive system in the parasitoid *Diaeretiella rapae*, ovaries (a and b), Oviduct (c), venom gland (d), Spermatheca (e), Dufour's gland (f) and ovipositor (g).

For producing the fertilized eggs, the sperm from spermatheca are released into the oviduct and eggs are fertilised before pass on to ovipositor. Therefore, fertilization may occur just before the eggs are laid. The general details of the reproductive system in parasitoids are described by Quicke (1997). The male reproductive system includes a pair of testes, vas deferens, accessory gland and aedeagus. The sperm released from testes passes through the vas deferens and the seminal vesicle and then mixes with accessory gland fluid before finally reaching the ejaculatory duct for ejaculation (Quicke 1997) (Fig. 9).

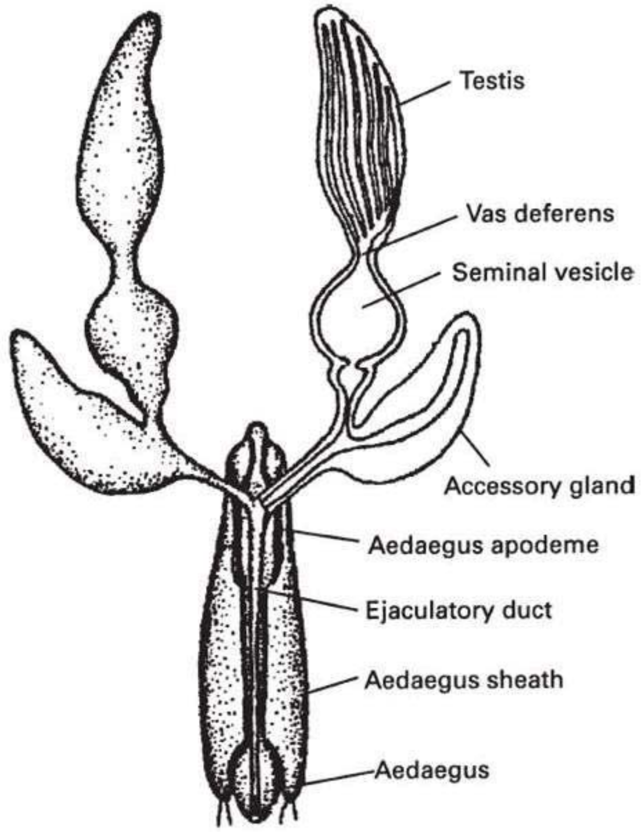

Figure 9: Male reproductive system in a hymenopteran parasitoid. This is a modified version of that from Sanger and King (1971).

Mating behaviour in *D. rapae* has not been studied so far. Askari and Alishah (1979) and Vaughn et al. (1996) suggested that mating in *D. rapae* occurs through the mediation of a sex pheromone.

Foraging and oviposition behaviour

Foraging behaviour of parasitoids is complex, and includes host searching, handling and oviposition. It varies both within and between species (Lewis et al. 1990). Selection of hosts by parasitoids is done in a sequence of steps. Vinson (1985) divided host selection into five steps: host habitat location, host location, host acceptance, host suitability, and host regulation. Parasitoids use visual, chemical, tactile and olfactory cues to locate and select their hosts (Reeve 1987; Harris & Miller 1988). Time budgeting for parasitoids is very important during foraging, and they have to balance their time between host-searching and host-handling for fitness gain (Hudak et al. 2003). Upon encounter with their host, parasitoids decide on the suitability of the host for oviposition, and offspring sex allocation (Godfray 1994). These decisions depend on several factors, including host age or size, host density, parasitoid age, previous foraging experience, competition among parasitoids and other factors (Godfray 1994; Jervis 2005).

Read et al. (1970) reported that *D. rapae* initially locates its hosts in response to odour emanating from the aphid's host plant, followed by visual searching. The volatile odour allylisothiocynate (AITC) which is released from damaged cruciferous plant tissues helps the parasitoid in locating hosts (Cole 1980). Vaughn et al. (1996) also found that only female *D. rapae* responded to AITC. However, female *D. rapae* have also been reported to parasitise aphids attacking plants that do not release AITC upon injury, such as wheat. Earlier studies have reported that *D. rapae* is not very efficient in searching, but has a high oviposition rate (Takada 1975).

Sex allocation and sex ratio

Allocation of male and female offspring is one of the important reproductive decisions a female parasitoid makes in her life; understanding this behaviour is one of the biggest challenges to understanding of evolutionary development in parasitoids (Charnov 1982; Hardy 2002). Fisher (1930) has shown that natural selection favours the equal production of male and female offspring, and the proportion of males and females is equal in a randomly mating population. However, parasitoid sex ratios are usually divergent from equality and generally female-biased (Godfray 1994; Reece et al. 2004; Shuker et al. 2006). From an evolutionary point of view, female-biased broods reduce competition among sons by providing them with more mates (if males mate multiple times) (Taylor 1981; Godfray 1994). Further, inbreeding in haplodiploids may lead to the mother being more related to its sons and daughters (Herre 1985). The sex allocation decision and distortion of sex ratio can affect the population dynamics and fate of the species (Godfray 1994; Ode & Hardy 2008). The production of females is always favoured in biological control programmes, because it is the females that are directly responsible for suppression of the pest population (Heimpel & Lundgren 2000). Therefore, the efficient mass production of parasitoids in augmentation biological control programme can be optimised by understanding sex allocation strategies in the target parasitod (Ode & Hardy 2008). There are various factors that affect sex allocation in parasitoids, including host quality, host density, competition with conspecifics, and the female's life history (Santolamazza-Carbone & Cordero Rivera 2003). There are two main theories that explain the sex allocation pattern in parasitoids, the "Local Mate Competition" theory (Hamilton 1967) and the "Conditional Sex Allocation" theory (Charnov et al. 1981).

Hamilton (1967) was the first to show that the optimal sex ratio of the offspring produced by a mother (called foundress) depends upon the number of mothers present on a habitat patch. When there is one female parasitoid on a patch, she may favour a female-biased sex ratio. By doing this, the female reduces the competition among her sons, so that they do not have to compete with their brothers for mating with their sisters (sib-mating). If the number of fertilised eggs laid by the mother is higher than the number of females in the population, the population increases and eventually the fitness of the parasitoid is increased. As the foundress density increases, the sex ratio becomes less female-biased. The theory explaining the female-biased sex ratio in the presence of one mother on a patch is widely known as Local Mate Competition (LMC). LMC theory has been extremely successful in explaining sex ratio variation within and among species, as well as explaining the facultative adjustment of offspring sex ratios in response to variable LMC (Charnov 1982a; Hardy 2002; West et al. 2002).

In Charnov's (1979) Conditional Sex Allocation theory, females usually oviposit fertilised eggs (produce females) in the good quality hosts. These hosts may be of "good quality" because they are large in size or unparasitised. The haplodiploidy of parasitoids allows them to choose hosts for allocating female and male offspring, and several parasitoid species prefer to allocate female offspring to larger hosts (Charnov et al. 1981; King 1993; Godfray 1994). However, there are two physiological constraints when females may not be able to produce female offspring: if the mother is not mated, or if the mother has depleted her store of sperm to fertilise all its eggs (Godfray & Waage 1990). The size of a parasitoid is usually determined by the size of host, with a positive relationship existing between them (Godfray 1994), and the size of parasitoid has been found to be positively related to its fitness (Charnov 1979;

Charnov 1982; Cloutier et al. 2000). However, Sequeira and Mackauer (1992) found that the relationship between the host size and emerged parasitoid is not linear across all host sizes. The parasitoids also change their offspring sex ratio in response to encountering parasitised and unparasitised hosts (Holmes 1972). It is important to test these sex allocation hypotheses in relation to *D. rapae*.

Environmental factors also affect the parasitism strategies of parasitoids. One of the important factors that affects sex ratios in parasitoids is temperature (King 1987). The sex ratio (female/male) of *D. rapae* in the Netherlands was found to be female-biased 3:1, but the ratio goes down in winter and early spring, when the parasitoid emergence is low (Hafez 1961). The sex ratio of *D. rapae* also varies with temperature. The ratios of females and males emerged at three temperatures, 10.0, 21.1 and 26.7^{o}C were 1.7, 2.1 and 1.2, respectively (Bernal & González 1997). Due to the lack of studies, there is no information on sex ratios of New Zealand populations of *D. rapae*.

Factors affecting reproductive fitness in parasitoids

Reproductive fitness in an organism is achieved by maximising offspring production. Understanding fitness consequences of reproductive behaviour in parasitoids provides useful insight into host-parasitoid interaction. The interaction directly affects host-parasitoid population dynamics (Godfray 1994; Fellowes et al. 2005) and could be utilised in designing and implementing biological control programmes of the host. Reproductive behaviour further provides information on ecological and evolutionary changes in host-parasitoid interactions (Bonduriansky et al. 2008). Reproduction in a parasitoid is directly linked to its host-suppression ability, because oviposition and production of resultant offspring occur at the cost of the host mortality. Thus, parasitoids are under strong pressure to utilise resources efficiently (van Alphen et al. 2003). Parasitoids with greater reproductive potential will be more efficient in suppressing the host population. In biological control, success in suppressing the pest population using parasitoids depends on behavioural decisions made by parasitoid females during parasitism (Godfray 1994; Mills & Wajnberg 2008). Therefore, reproductive fitness of parasitoids is the central theme in pest management through biological control.

Reproductive strategies of parasitoids depend on biotic factors such as host age, host body size, host availability, foundress competition, mating, and abiotic factors like temperature, photoperiod and humidity (Godfray 1994). The abiotic factors may not be effectively manipulated in the field, but can be easily adjusted in glasshouse conditions. However, biotic factors can be manipulated for increasing the efficiency of a parasitoid in biological control, if there is a clear understanding of biology and behaviour of the parasitoid (Heimpel & Casas 2008). Thus, it is important to understand the biotic factors that affect the behaviour and biology of the parasitoid. Behavioural ecologists have worked on theoretical predictions of biocontrol output in terms of biotic and abiotic factors, but not much work has been done in terms of optimising their output. Hence, understanding reproductive behaviour could be applied to manipulate the reproductive strategies of parasitoids in pest management.

The important factors that could affect the fitness of a parasitoid are briefly discussed below, and presented in more detail in the respective chapters of the thesis.

Host quality

The amount of resources available to a parasitoid in its host defines the quality of the host (Mackauer 1996). Growth and development of larvae depends on the availability of food to the immature larvae. Food requirements of males and females could vary among host species (Abe et al. 2003) or host ages/size (Godfray 1994). Immature parasitoids display various host-usage patterns, some prefer host tissue, and others host haemolymph, which affects their development process (Harvey et al. 2000).

Resources available in a host depend on several parameters, including resources available at the time of oviposition, the host's growth potential, and the time until the death of the host after parasitism, in the case of koinobiont parasitoids. Host quality in general varies with size and age of the host. Higher quality host increases the survival chance of the parasitoid. Adults emerging from good quality hosts could be more efficient in suppressing their host population (Lampson et al. 1996; Mills & Kuhlmann 2000; Teder & Tammaru 2002). Females foraging for hosts should prefer those of particular size/age for oviposition and sex allocation to maximise reproductive gain (Stephens & Krebs 1986). The fecundity of female parasitoids increases when the host patch is dominated by large size hosts (Hardy et al. 1992; Harvey et al. 2000). However, Corrigan & Lashomb (1990) and Mills & Kuhlmann (2000) found that the relationship between host body size and fecundity of female parasitoid is not uniform. Physical and physiological defences of hosts could also affect the host selection strategies of female parasitoids (Hagvar & Hofsvang 1991; Godfray 1994).

Repeated oviposition, referred to as superparasitism, is common in parasitoids (Van Alphen & Visser 1990). In the early stage, the parasitoid larvae compete for resources as well as defend themselves from attack by other co-developing larvae (Bai & Mackauer 1992). When multiple adults emerge from a host (in gregarious parasitoids) the resources are distributed among the individuals developing together. The emergence of only one adult per host (in solitary parasitoids) despite repeated oviposition and initial development of several larvae in a host, is due to cannibalistic behaviour of the larvae (Fig. 10).

Figure 10: Examples of up to 8 *Diaeretiella rapae* larvae developing per host in superparasitised *Brevicoryne brassicae* hosts.

The ability of a female parasitoid to detect whether the host has already been parasitised by herself or by another female is known as “host discrimination” (Salt 1961). This ability could help females to identify the quality of a host which could help them to avoid wasting egg by superparasitising. When competing with supernumerary larvae, during superparasitism, larvae of *D. rapae* attack and kill their competitors with strong mouthparts (Tremblay 1972; Couchman & King 1977) and feed upon them after biodegradation. Lester & Holtzer (2002) identified the possibility of superparasitism in *D. rapae*, other than that, not much information is published on the superparasitism in this species.

Host density

During foraging, female parasitoids encounter hosts in different patches. A patch may be considered as good if it contains large number of hosts in a patch. The density of the host can affect oviposition strategy, including the number of ovipositions, also called functional response, and sex allocation, and ultimately the fitness of the parasitoid. In general, higher host density usually has a positive correlation with fecundity, as the parasitoid spends less time and energy searching for hosts (Mackauer 1983). Life history theory predicts that both reproduction and longevity incur energy costs, and trade-offs exist between the two components (Roff & Fairbairn 2007).

Parasitoid density

Competition plays an important role in the fitness of an organism. When more than one female exploits a patch, females manipulate their oviposition and sex ratio (Hamilton 1967) to achieve maximum fitness. A high level of competition for a limited number of resources could also cause interference with reproductive strategies. Interference between the parasitoids decreases the fecundity of individual parasitoids (Evans 1976).

Parasitoid body size

The effect of male and female body size on fitness has been studied in several parasitoid species (Thornhill & Alcock 1983; Cloutier et al. 2000; Sagarra et al. 2001). The large body size of a parasitoid could affect the oviposition and mating process. Body size of females can affect their oviposition efficiency, as well as their ability to withstand stresses under extreme conditions, such as inadequate food availability (Colinet et al. 2007). Similarly, body size of a male can affect its mating opportunity and success rate, as it affects the sperm stock in the male and the amount of sperm transferred during mating. In a male-male competition, larger males usually have a greater ability to compete for mates (van den Assem et al. 1989; Kazmer & Luck 1995).

Age of parasitoid, and mating or oviposition delay

Age is an important factor that affects the physiology of most insects. Some insects emerge mature and reproduce immediately, while others require some time gap between emergence and maturation or reproduction. Parasitoids are time-limited and also egg-limited if they are proovigenic or weakly-synovigenic. When females emerge mature, even a short delay in their oviposition could reduce their reproductive success. Females can reabsorb their eggs (Richard & Casas 2009; Guo et al. 2011) and this reabsorption could affect their reproductive potential. When mating is delayed in female parasitoids, they may oviposit more unfertilised eggs than required in the population, and bring imbalance to the sex ratio in the next generation (King 1962). However, this effect was not found in *Trichogramma* sp. whose oviposition was delayed for a short period of time (Fleury & Bouletreau 1993). Similarly, mating delay in males could affect sperm viability and could induce sperm degeneration. Therefore, a delay in oviposition, mating, or both, could affect the fitness of male and female parasitoids. Very little information is available on mating or oviposition delay in *D. rapae* or any other parasitoid.

Mating history

Mating and sperm transfer during mating affect sex allocation in female parasitoids (Bissoondath & Wiklund 1996; Marcotte et al. 2007). The frequency of mating of males and females depends on several factors, including the mating history of males and the ratio of females and males in the population. Ridley (1993) studied 40 species of Hymenoptera, and found that most males mated multiple times but about 80% of females mated only once. When a female mates multiple times, she can simultaneously store sperm from several males in its spermatheca and choose a good quality sperm to fertilise her eggs (Schowalter 2006). However, when a female mates once in her life time, then she must choose a good quality male for mating. When a male mates multiple times, he may manipulate the quantity of sperm transferred during mating to maximise his fitness (King & Fischer 2010). Males may suffer sperm depletion after multiple matings (King 2000). Hardly any information is available on the mating system of *D. rapae*, and a detailed study on mating behaviour is required.

Research aim and objectives

The aim of this research was to understand the host-parasitoid interaction in cabbage aphid and its parasitoid *D. rapae*, and how mating and oviposition behaviour affects the reproductive fitness of this species. Although several studies have investigated parasitism in overseas populations of *D. rapae*, very little information is available on their reproductive behaviour. Most studies have focused on general parasitism and are limited to general biology of *D. rapae,* but not in relation to ecological aspects of this interaction. Reproduction in *D. rapae* or any other parasitoid is directly related to their pest suppression ability. A holistic knowledge of mating and oviposition behaviour and life history of *D. rapae* can be helpful in developing future strategies in biological control using *D. rapae* or in integrating biological control in current aphid management in New Zealand. A knowledge gap exists between parasitism efficiency and reproductive biology and behaviour of this parasitoid. Thus, the focus of this study was to generate information on reproductive ecology that would fill the knowledge gaps and this information could be utilised in implementing biological control of cabbage aphid or other aphid using *D. rapae* or other parasitoids. Furthermore, information currently available on *D. rapae* is from the studies of various overseas populations and it is becoming apparent that while these populations belong to the same named species, they also have distinct phenotypic and potentially genotypic characteristics. Generating detailed information on the New Zealand population will be valuable in biological control programmes in New Zealand, and provide important insights into the evolutionary trajectories of different lineages.

The present study, therefore, investigated reproductive behaviour and factors affecting reproductive fitness of *D. rapae* with respect to the previously discussed factors. The objectives of this research were:

1. To investigate the reproductive strategies of *D. rapae* in an open/uncontrolled system. Parasitism and mating strategies of a wild population of *D. rapae* were examined during spring (Chapter 1). The study assessed changes in parasitism rate and sex ratio over time, and mating and pairing of this parasitoid. This study formed the basis for further investigation of mating and oviposition behaviour of lab-reared *D. rapae* in New Zealand.

2. To understand emergence, mating and oviposition patterns in terms of diel variation in *D. rapae*. This study was of a preliminary type, and investigated effects of light on mating and oviposition in *D. rapae* (Chapter 2), which could be helpful in aphid management using parasitoids in glasshouse or during mass rearing of *D. rapae* for biological control.

3. To investigate sexual receptivity, courtship and mating behaviour of *D. rapae* (Chapter 3). Sexual selection in *D. rapae* was investigated in terms of age and adult body size (Chapter 4). The study further looked into multiple mating and its effects on reproductive fitness of male and female *D. rapae* (Chapter 5).

4. To investigate general biology - egg maturation, fecundity and longevity and how food supply affects these biological parameters (Chapter 6). The "mating versus oviposition" decision and the consequences of this decision on fitness were examined in *D. rapae* (Chapter 7). The study further investigated the effects of mating and oviposition delays on overall reproductive output of *D. rapae* (Chapter 8).

5. To investigate host searching and host preference behaviour of female *D. rapae* (Chapter 9). The study further examined the consequences of host selection strategies on fitness gain of *D. rapae* (Chapter 10 and 11). Oviposition strategies of *D. rapae* were further examined in terms of host discrimination and superparasitism (Chapter 12 and Appendix 2). Finally, parasitism strategies of *D. rapae* were studied in relation host density and conspecific competitions for hosts (Chapter 13).

Results of the component studies were integrated in the discussion section to reveal the factors that directly affect the reproductive fitness of *D. rapae* in different biotic conditions. Based on the results of the studies and existing information on the reproductive ecology of *D. rapae*, I will provide recommendations for use of *D. rapae* in biological control.

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Chapter One

Reproductive strategies of *Diaeretiella rapae* (Hymenoptera: Aphidiinae) during fluctuating temperatures of spring season in New Zealand

Abstract

Reproductive activities of naturally occurring population of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiinae) were studied during spring months in New Zealand. The cabbage seedlings were highly infested with the green peach aphids (272±25 individuals/plant) with about 30% on average parasitised by *D. rapae*. Rate of parasitism was positively correlated with the aphid density (R^2=0.64). Adult emergence from aphid mummies was 90%, with a higher frequency of females than males. Increase in the proportion of female was found between early and late spring (1.1 vs. 1.8). Mating in *D. rapae* occurred throughout the day. Nearly half of the mating pairs collected were male-male pairs, which were especially prevalent during morning and evening while more male-female mating pairs were found around midday. Male-male mounting was probably because of low numbers of virgin females in morning and evening populations. About 80% females were mounted by smaller-sized males. Male-male mounting is discussed in correlation to operational sex ratio of *D. rapae* population.

Keywords: mating, sex ratio, male-male mounting, ambient environment, *Diaeretiella rapae*

This chapter studied the reproductive strategies of a wild population of *Diaeretiella rapae*. The host-aphid in this study is *Myzus persicae* and this study was done in uncontrolled (natural) environmental conditions. All other studies in this thesis were done under controlled conditions (temperature, humidity and photoperiod), with the cabbage aphid *Brevicoryne brassicae* as a host for ***D. rapae.***

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Introduction

Aphids are significant and cosmopolitan agricultural and horticultural pests. In New Zealand, aphids cause substantial economic loss to the agricultural industry and it is predicted that global climate change will further aggravate the aphid problem (Gerard et al. 2010). The common method for controlling aphids is application of chemical pesticides, but the indiscriminate nature of these agents causes various problems, including evolution of insecticide resistance in aphids (Clark & Yamaguchi 2002), and toxicity to the beneficial natural enemies (Desneux et al. 2007). In New Zealand, pesticides are widely used, but there is a trend toward alternative options of Integrated Pest Management (IPM) and biological control methods (Manktelow et al. 2005).

Diaeretiella rapae (McIntosh) (Hymenoptera: Aphidiinae) is a solitary endoparasitoid of more than 60 different aphid species worldwide (Pike et al. 1999). The wasp originates from the Western Palaearctic, but is now distributed throughout the world, and was accidentally introduced to New Zealand where it has established (Carver & Stary 1974). Among the common hosts of *D. rapae* in New Zealand and other countries are cabbage aphid, green peach aphid and lettuce aphid (Mackauer & Stary 1967; Carver & Stary 1974; Kavallieratos et al. 2001; Kavallieratos et al. 2005). In the USA, *D. rapae* has been used in biological control of Russian wheat aphid (Bernal & Gonzalez 1993). In Australia it was deliberately introduced for controlling cabbage aphid (Carver & Stary 1974). Little attention has been given to *D. rapae* as a potential biological control agent of aphids in New Zealand, probably because *D. rapae* is not well studied here.

Unlike predators, the pest suppression ability of a parasitoid is directly linked to its reproductive strategy. Mating and oviposition behaviours are the two main aspects that affect the reproductive performance of parasitoids. Importantly, the reproductive potential of parasitoids is environmentally sensitive and it changes with climatic and geographical location (Jervis et al. 2008). Climatic conditions, including temperature and day length, strongly influence biological traits such as host searching, oviposition, sex allocation, immature development, adult emergence and mating, which further affects the host-parasitoid system in nature (Legrand et al. 2004). Rakhshani et al. (2008) showed the effect of altitude (and, therefore, temperature) on aphid parasitoids. For example *D. rapae* and *Aphidius rhopalosiphi*

De Stefani (Hymenoptera: Braconidae: Aphidiinae) parasitized cereal aphids at high altitudes, while *Ephedrus persicae* Froffat (Hymenoptera: Braconidae: Aphidiinae) and *Ephedrus plagiator* Nees (Hymenoptera: Braconidae: Aphidiinae) preferred lower altitude.

Studying reproductive behaviour of a local population will improve understanding of the reproductive strategies of *D. rapae*, which is essential for testing its suitability and efficiency in biological control programmes at a local level (Lewis et al. 1990). The current study investigated parasitism and mating activities of *D. rapae* in a wild New Zealand population in ambient conditions during the fluctuating weather of the spring season (September through December). The study sought to (1) assess parasitism patterns of *D. rapae* (2) determine sex ratio changes of *D. rapae* over time and in relation to parasitism rate and (3) reveal the scale of sexual dimorphism and its effect on mating attempts in *D. rapae* adults.

Materials and methods

Parasitism and mating activities of *D. rapae* were studied in a 25 m^2 shade-house in an open/ambient environment from September to December 2009 in Palmerston North, New Zealand. Weather conditions in New Zealand during the spring are typically variable and we recorded temperature and humidity during the study period (Table 1). The area contained approximately 544 potted cabbage plants (cv. ‘Summer globe’) aged 2 to 4 months, which were regularly irrigated with an automatic watering system (Temporizador T 14 e, Gardena AG, Germany). The temperature, humidity and day length conditions were ambient. Green peach aphid *Myzus persicae* and its parasitoid *D. rapae* colonised the area naturally, and were left undisturbed for 3 months prior to taking observations. The parasitoid was identified according to Powell (1982) and corroborated by comparison of mtDNA sequences with published data (Baker et al. 2003).

Table1: Mean average weekly temperature and humidity during the eleven week study period, Palmerston North, New Zealand. Source: The National Climate Database, NIWA, Wellington.

Week No.	**Week of**	**Mean Temp ^{o}C**	**Relative humidity (%)**
1	21-Sep	8.8	80.6
2	28-Sep	11.9	85.4
3	5-Oct	7.5	78.4
4	12-Oct	12.1	84.6
5	19-Oct	8.6	78.7
6	26-Oct	9.7	78.2
7	2-Nov	12.3	80.1
8	9-Nov	10.3	77.2
9	16-Nov	13.5	80.2
10	23-Nov	13.8	81.9
11	30-Nov	14.7	82.8

Parasitism rate, sex ratio and size dimorphism in D. rapae

Aphid infestation and parasitism were recorded at weekly intervals for an eleven week period starting from 21 September 2009. Each week, two infested plants were chosen at random, and the number of parasitised and unparasitised aphids on upper and lower leaves of the plants was recorded. The parasitized aphids (mummies) were transferred into individual 2ml microfuge tubes using a fine paint brush. The mummies were regularly monitored and the adults were sexed using a stereomicroscope (Olympus SZX12) as they emerged. The size of the males and females was measured for estimating the body size dimorphism in male and female *D. rapae*. The hind tibia length was measured as proxy for body size, as our earlier studies found them to be highly correlated (R^2=83.56, P<0.001) (R. Kant, unpublished data). The adults were killed by freezing at -20°C and then stored in 70% ethanol until measured. The hind tibia was dissected from the thorax of the insect on a glass slide in saline, and measured using a calibrated eyepiece micrometer.

Mating pairs of Diaeretiella rapae

To understand the relationship between the body size of mating male and female *D. rapae*, all pairs observed in the area during the one hour search were collected using a hand-held aspirator made with 10 ml glass vial. Mating activities of the parasitoids were observed, and mating pairs of *D. rapae* were collected once every three days at three times of the day – 7-8 am (morning), 12-1 pm (midday) and 5-6 pm (evening). The body size and sex of the pairs attempting to copulate were determined.

Statistical analyses

A goodness-of-fit test was performed to assess the normality of data before analyses and when required, non-normal data were transformed prior to analysis. Body sizes of males and females, and the sex ratio in the first and last five weeks were subjected to analysis of variance (ANOVA). Regression analysis was applied to examine the relationship between aphid density and parasitism rate, the trend in the sex ratio of parasitoids during the ten week study period, and the relationship between body sizes of male and female mates. A Chi-square test was used to determine the difference in the number of male-female and male-male matings observed at different times of the day. Analyses were carried out using SAS Enterprise Guide 4.2. Significance level set to the analyses was $\alpha=0.05$.

Results

Parasitism rate, sex ratio and size dimorphism in Diaeretiella rapae

The cabbage seedlings were heavily infested with aphids and parasitoids. The mean numbers (±SE) of live aphids and parasitised mummies were 272±25 and 71±7 per plant, respectively. Most of aphids (95%) and aphid mummies (91%) were recovered from undersides of cabbage leaves. The parasitism rate fluctuated between 23% and 39% during the study period (Figure 1) and no specific trend in parasitism rate during the eleven weeks study period was found (P=0.53).

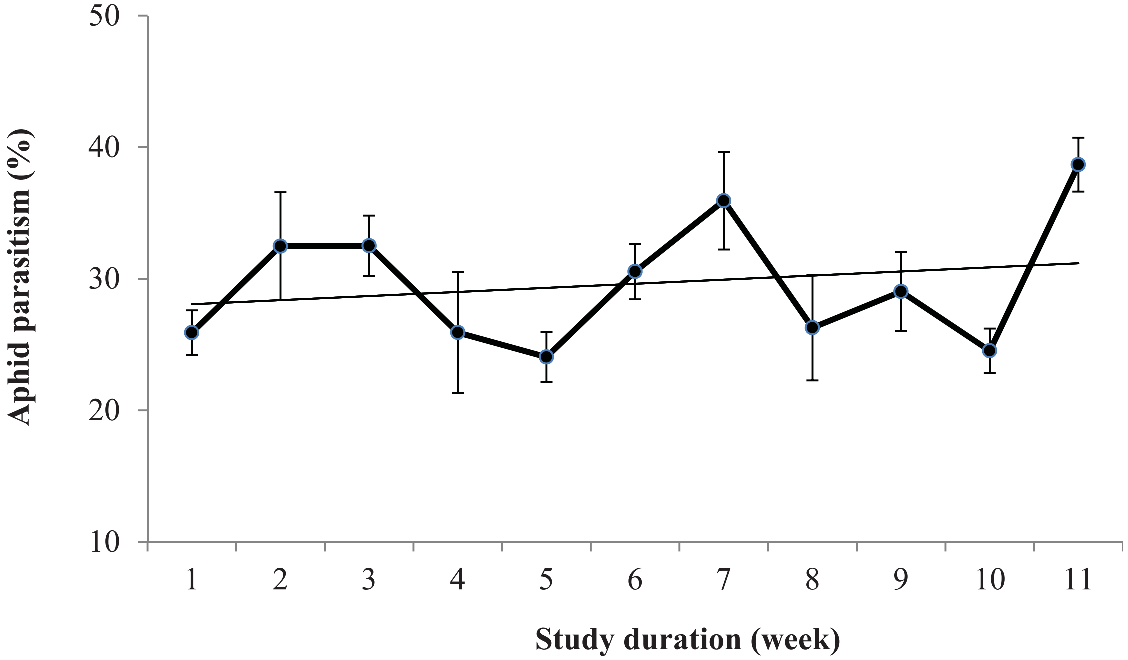

Figure 1: The mean (±SE) percentage parasitism of *Brevicoryne brassicae* nymphs parasitised by *Diaeretiella rapae* during eleven week period (3rd week of September to 1st week of December 2009).

The mean number (± SE) of aphids parasitised during the first five-weeks (94±10) was less than the number of aphids parasitised during the second five-week period (131±18) (P=0.023). The number of mummies recovered from infested plants was directly correlated with the density of the aphids on the plant (P<0.001) (Fig. 2).

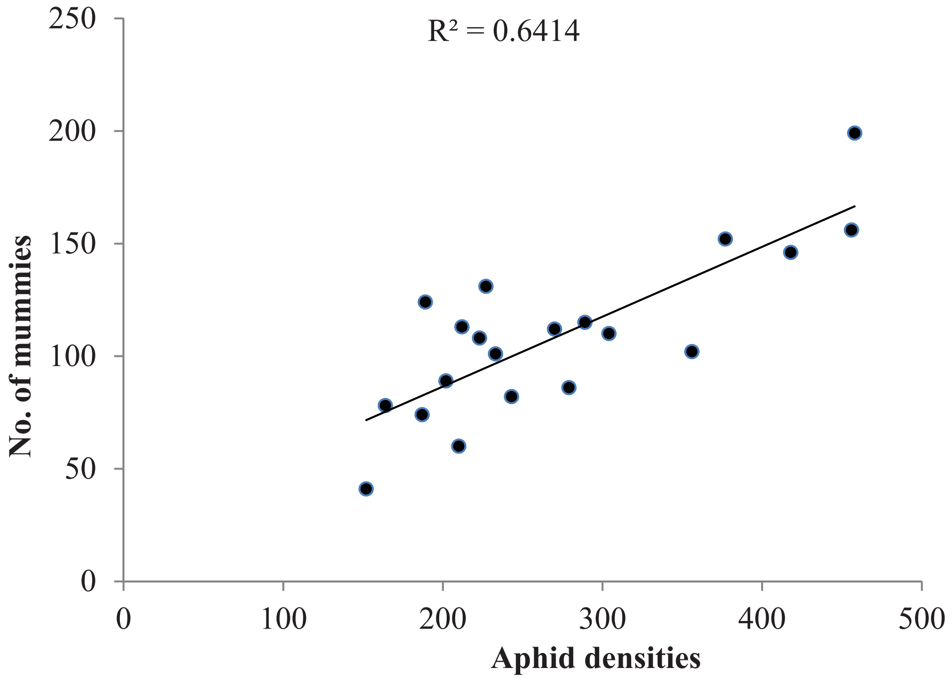

Figure 2: Relationship between aphid density and number of aphid mummies recovered per plant (parasitism rate).

The rate of parasitoid emergence from mummies was very high (92±6%), with the number of emerging females greater than the number of males during the entire study period. The proportion of female offspring increased from the beginning to the end of the study period (Fig. 3).

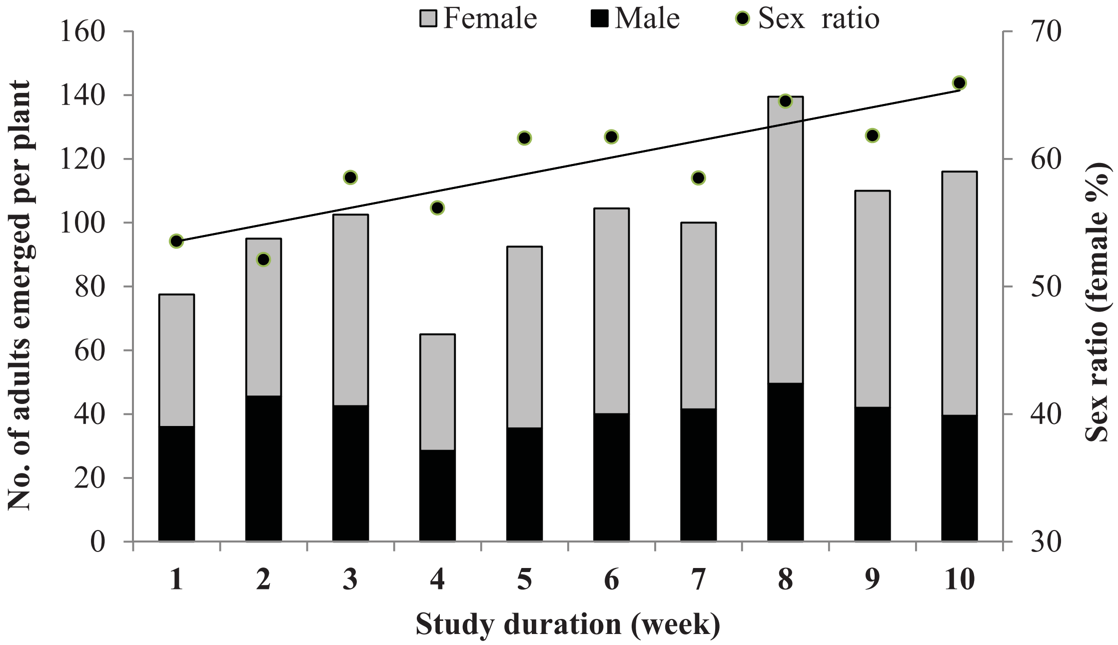

Figure 3: Mean number and the sex ratio (female %) of emerging *Diaeretiella rapae* (per plant) during a ten week period, beginning from 21st September 2009.

The mean female/male ratio of emerging parasitoids was significantly higher during the second five-week of the study (1.6±0.1 five-week average) compared to the first five-week period (1.2±0.1) ($P<0.05$).

The body size of male and female *D. rapae* ranged widely where hind tibia length varies between 433 and 601 µm in males and 408 and 622 µm in females. The hind tibia length of males was significantly smaller than that of the females ($P<0.001$) (Fig.4).

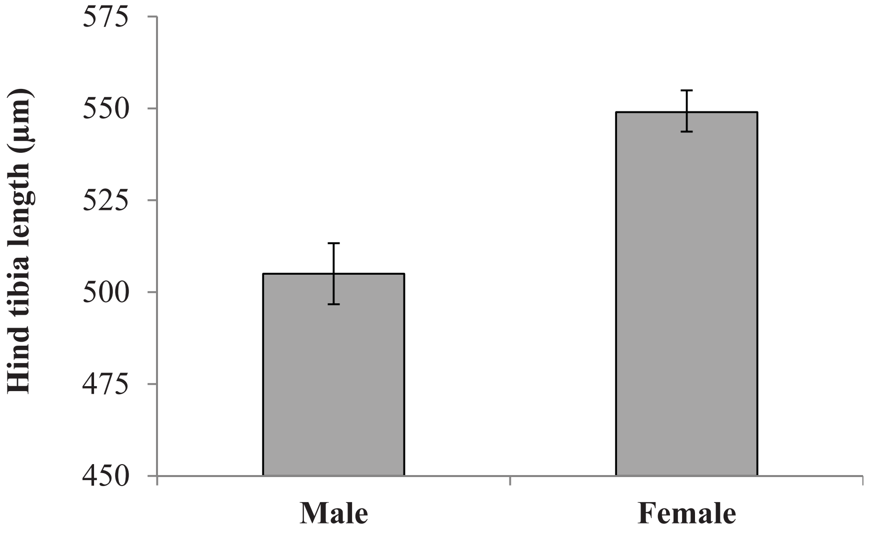

Figure 4: The mean (±SE) body size (as hind tibia length) of male and female *Diaeretiella rapae* emerged from parasitised aphids (mummies).

Mating pairs of Diaeretiella rapae

Overall, 49% of the mating pairs comprised a male mounted on a female and 45% were male mounted on another male. There were a few instances of female-female pairs (1%) and female-male-male mounting (5%).

When the mating pairs were partitioned by the time of day, it was found that male-female mating was more prevalent during midday compared to morning and evening ($\chi 2 = 8.89$; $P<0.05$). About half of the mating pairs were male-female during morning (48%) and evening (46%) (Table 2).

Table 2: Total number of mating pairs of *Diaeretiella rapae* captured in the morning (7-8 am), at midday (12-1 pm) and in the evening (5-6 pm). Within each column, the same letter indicates no significant difference (P>0.05).

Mating pairs	Morning	Midday	Evening
Male x Male	26 a	20 a	48 a
Female x Male	24 a	39 b	41 a
Total mating pairs	50	59	89

Most females (79%) in male-female mating pairs were found to be mounted by males with body size smaller than themselves, with males on average 16% smaller than their female partners (Fig. 5) ($P<0.005$). There was no significant difference in body size of males in male-male pairs ($F_{1,40}=0.14$, $P=0.711$)

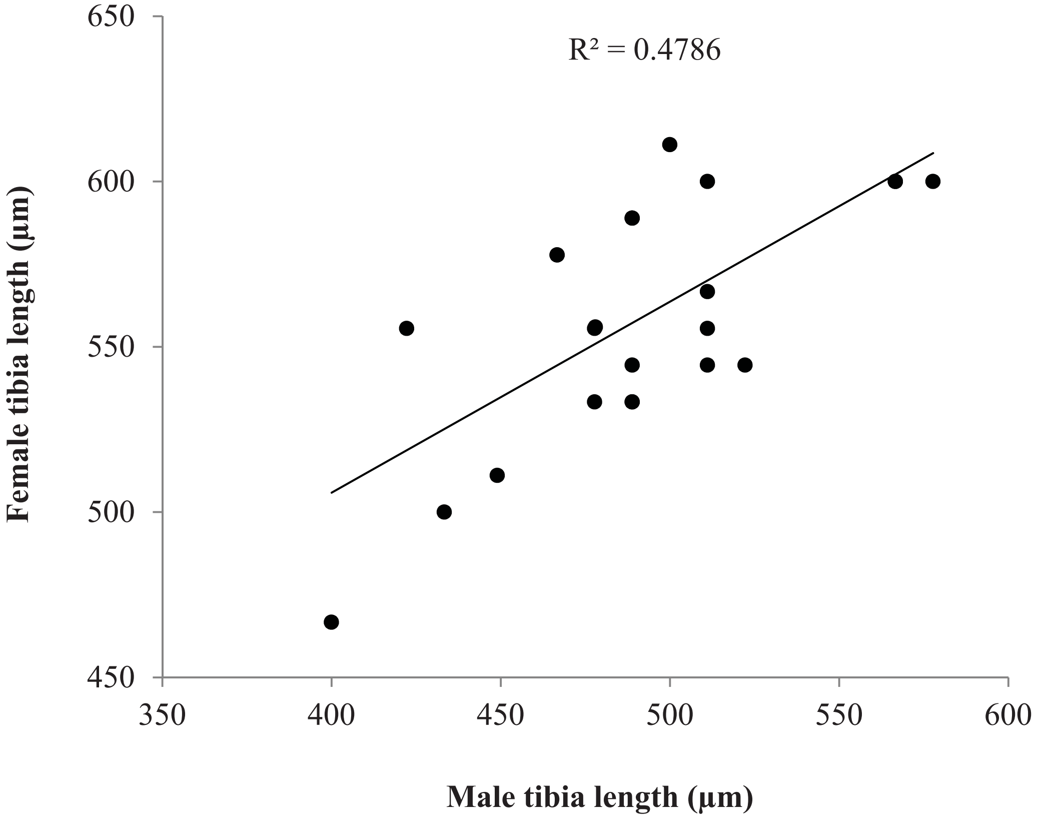

Figure 5: Relationship between body sizes (as hind tibia length) of male and female in *Diaeretiella rapae* mating pairs.

Discussion

The low rate of parasitism during the first five weeks of the experiment indicates low reproductive output of *D. rapae* during lower temperatures of spring. Temperature threshold and thermal constants for parasitoid activities are usually higher than those for their hosts (Campbell et al. 1974). After winter, the aphid population can grow quickly during the early season due to their low temperature threshold, whereas *D. rapae* take longer to develop at low temperatures (Shukla et al. 2008a), resulting in poor synchronisation between parasitoid and aphid populations (Liu 1989a). Kavallieratos et al. (2002) found that, for this reason Aphidiinae parasitoids could not suppress the aphids infesting citrus during spring and early summer. Zhang and Hassan (2003b), and Fathipour et al. (2006) suggested that the low parasitoid/host ratio during the spring season adversely affects the efficiency of *D. rapae* in suppressing the aphid population. In our study, a slight increase in the *D. rapae* parasitism rate of *D. rapae* was observed towards the end of spring, suggesting an increase in parasitoid/host ratio. This can be explained by increase of the mean air temperature in the late spring. According to Hayakawa et al. (1990) high temperatures (>30 ^{o}C) increased the fecundity and daily oviposition of *D. rapae* parasitising *Brachycorynella asparagi* Morvilko (Hemiptera: Aphididae) on asparagus.

In haplodiploid parasitoids, a female-biased sex ratio can facilitate rapid population increase (Godfray 1994). Although males are essential for the production of female offspring in *D. rapae*, their number does not need to be on a par with females, because males can mate up to six times, while females are monandrous (Kant 2009b). In this study, the proportion of females increased over time while that of males remained constant. The most likely explanation for this is scarcity of males for mating during emergence of early generations. Unmated females produce only male offspring. If a female remains unmated or mating is delayed, the parasitoid population in the subsequent generation will be even more male-biased, which affects the parasitoid ability to suppress the host population. Thus, an increase in female/male sex ratio is a primary requirement for *D. rapae* to provide better aphid suppression. The pattern of change in sex ratio found in this study suggests that there are higher parasitoid/aphid ratios in late spring and thus, better suppression of the aphid population is more likely to be achieved in summer.

Large numbers of males in the population could negatively influence the mating system. Among the pairs of *D. rapae* attempting copulation, only 49% were male-female pairs. The high number of erroneous male-male attempted copulations could not be explained by males "misinterpreting" the sex of larger individuals as females, as no size difference was found in male-male pairs. We think that male-male copulatory attempts may represent the intrasexual aggressive behaviour of males competing for mates, which has been found in other hymenopteran species (Al-Wahaibi et al. 2005).

A higher percentage of male-female mating around the midday indicates that more virgin females were available in the population during the midday, compared to morning and evening. Diurnal emergence patterns of *D. rapae* showed that males emerge prior to females, and the majority of males emerge during the first few hours of the morning (Kant & Sandanayaka 2009). Moreover, the males surviving from previous days further add to the male numbers in the morning population. Thus, morning populations may have an overabundance of males and be female-limited. The majority of females emerge by the middle of the day (Kant & Sandanayaka 2009), and by late afternoon/evening most of the newly emerged virgin females would have been mated, which leads to another shortage of females observed in the evening.

In an open system, where hundreds of males and females of different sizes are available simultaneously, a female may show its preference for a particular size of male for mating. Although males are aggressive during mating, the mating success largely depends on a female; they mate only once during their entire life so must choose a good quality male to maximise reproductive fitness. The quality of males can be inferred from their body size (Godfray 1994a) which differs between males and females in *D. rapae* (Kant et al. 2011c). Our results suggest better mating success or mating compatibility in *D. rapae* when males are slightly smaller than females.

During the relative unstable spring weather conditions of September to November in New Zealand, the aphid populations increase rapidly due to parthenogentic reproduction. However, we have found that periods of colder weather in New Zealand slow the generation time of *D. rapae* and thus, contributes to a low parasitism rate early in the season when the aphid population is already high. *Diaeretiella rapae* cannot use parthenogeny in response as this generates males, which have no parasitic capacity. Excess numbers of male wasps reduce parasitism rate and also mating rate and thus drive low female/male ratios in early spring. For biological control to be effective, pre-release of *D. rapae* for suppression of early population-explosion of the aphid host is required (Godfray 1994a). Therefore, careful consideration should be given to the measurement and management of sex ratio in wild and captive reared populations. These results highlight the need for more detailed study on mating and oviposition behaviour of *D. rapae* in New Zealand to optimise their performance in aphid suppression.

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Chapter Two

Diel asynchrony in reproductive behaviour of *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae)

Abstract

Diaeretiella rapae is an important parasitoid of cabbage aphid. Diel variation in emergence, mating and oviposition of *D. rapae* was studied in the laboratory to understand the biology and behaviour of the parasitoid. The emergence of the parasitoid was recorded hourly in two bioassay rooms set up at 22 ± 2°C with 16 h photoperiod from 0800-2400 hours or from 1800-1000 hours. Greatest emergence was found during the early photophase. The parasitoids that emerged during the scotophase did not mate until the following photophase. Unmated females that emerged during the scotophase had a lower incidence of host attack and oviposition during the dark. However, the parasitoids became active and had a greater incidence of mating and oviposition when they were brought into the light even during the scotophase. This research suggests that light triggers parasitoid activity and that the parasitoids lose their reproductive fitness if they emerge in the scotophase.

Keywords: *Diaeretiella rapae, Brevicoryne brassicae*, diel variation, emergence, mating, oviposition.

This chapter compared emergence, mating and oviposition of *Diaeretiella rapae* during photophase and scotophase.

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Introduction

Diaeretiella rapae (M'Intosh) (Hymenoptera: Aphidiidae) is an important parasitoid of cabbage aphid (*Brevicoryne brassicae* (L) (Homoptera: Aphididae)) (George 1957), which causes severe damage to most cruciferous crops (Hughes 1963). Beside parasitising aphids of cruciferous plants, *D. rapae* also attacks aphids that infest other plants, such as Russian wheat aphid *Diurpahis noxia*. *Diaeretiella rapae* is a solitary endoparasitoid (Ayal 1987) that lays one or more eggs in its host but only one egg develops into an adult (Godfray 1994). The diel pattern of parasitoid activity has not been documented.

Reproductive activities, such as mating and oviposition, in insects vary greatly during day and night. Some insects are active in light while others are active in dark, and their activities are very rhythmic (Saunders 1982). Behaviour of the parasitoids changes during day and night as well as during different times of photophase and/or scotophase (Saunders 1982; England 1995). Some parasitoids are active during early photophase (England 1995; Nakamura 1997) while others are active in late photophase (Allen 1998). To understand the reproductive strategies of parasitoids, it is important to know their emergence, mating and oviposition patterns (He et al. 2004). Parasitoids may also synchronise the timing of their emergence in order to increase their chances of mating and oviposition, which will ultimately maximise reproductive fitness.

In the current study, the diel pattern of emergence, mating and oviposition was investigated in *D. rapae*.

Materials and methods

Insect colonies

Colonies of cabbage aphid and its parasitoid *D. rapae* were established from a commercial cauliflower field near Palmerston North. Insects were reared in the laboratory on cabbage seedlings ('Autumn Pride') in plexiglass cages (30×30×30 cm). The colonies were maintained at 22 ± 2°C, 60-70% RH and 16 h photoperiod. Parasitoids used in the experiments emerged from 4-5 day old parasitised aphids.

Parasitoid emergence

Two controlled temperature rooms (22 ± 2°C) were set up at 16 h photoperiod to observe the emergence of the parasitoids during day and night. In room one, the photoperiod started at 0800 hours and ended at 2400 hours. In room two, the photoperiod started at 1800 hours and ended at 1000 hours. Five pairs of male and female parasitoids were released into an above mentioned cage that contained cabbage seedlings infested with about 100 aphids aged 4-5 days. Five sets of cages were used in each room. The parasitoids were allowed to parasitise aphids for 24 h and then transferred to another cage with fresh aphids. The process was followed until the death of the parasitoids. The aphids were allowed to feed and develop on the plants for 10 days. The mummified aphids were individually collected into 2-ml transparent Eppendorf tubes. The emergence of the parasitoids was recorded hourly during the light period in the room one and during the dark period in room two. The percentage emergence of males and females was estimated on the basis of total numbers of males and females that emerged during the entire period. The emerged parasitoids were used for the mating and oviposition experiments.

Mating behaviour

Upon emergence, the parasitoids were kept individually in a glass vial (2.5 cm in diameter and 7.5 cm in height), which contained a cotton wick soaked in a 10% honey solution, for at least 24 h before being paired for mating. The parasitoids were paired (n=67) in another glass vial during different times (early, middle and late) of photophase and scotophase without honey solution. The mating behaviour of parasitoids was observed and recorded for 1 h. Mating behaviour of parasitoid pairs (n=48) in scotophase was observed in the presence of red light. The pre-mating period (time between the pairing and mating) and the copulation time (duration between start and termination of mating) were recorded. The parasitoids that did not mate during the 1 h observation period were considered unsuccessful. The parasitoids that mated within the 1 h observation period were used for the oviposition experiment. Parasitoids that had been unsuccessfully paired during photophase were discarded. However, parasitoids that had been unsuccessfully paired during the scotophase were immediately brought into light and were observed for mating behaviour for another 1 h.

Oviposition behaviour

Oviposition behaviour of *D. rapae* was tested in scotophase and photophase. Thirty aphids (4-5 days old) were transferred to a cabbage seedling in a transparent plastic container (16 cm in diameter and 24 cm in height). A mated female was released into the container at the beginning of scotophase and then the female was removed from the container at the end of scotophase. The same female was released into another plastic container with a fresh set of 30 aphids at the beginning of photophase and her oviposition behaviour was observed during the photophase. Thirteen mated females were tested in this experiment. The aphids were allowed to feed and develop on the plants for 10 days and the number of mummies per container was recorded.

In order to test the effect of light on oviposition behaviour, a mated female *D. rapae* was released into a Petri dish (9 cm in diameter and 1.5 cm in height) with ten 5-day-old aphids during the middle of scotophase and her oviposition behaviour was observed in the presence of red light for 30 min. At the end of this 30-min period the same female was immediately released into a Petri dish with a similar set of aphids in light and the observations on oviposition behaviour were continued for another 30 min. Oviposition was considered successful even if the female oviposited only one host during the 30-min period. The oviposition behaviours of unmated females (n =23) were also tested using the same procedure.

Statistical analyses

A goodness-of-fit test was used to test the distribution of the data. ANOVA was used for analysing pre-mating duration and copulation time of different mating treatments, and for analysing parasitism during photophase and scotophase. The means were separated using a Tukey's studentised range (HSD) test. Analysis of regression (AOR) was used to determine the slope for hourly decline in male and female emergence. A chi-square test was used to determine the difference in parasitoid emergence during photophase and scotophase. Mating and oviposition success of parasitoids during photophase and scotophase was estimated by Mann-Whitney U Test (MWT). All analyses were done at the P=0.05 level of significance.

Results

Parasitoid emergence

A total of 293 adults emerged during hourly observations and more than 95% of them emerged during photophase. The number of adults that emerged during the 16 h photophase (3.5 adults per hour) was significantly higher than those emerged during the 8 h scotophase (0.25 adults per hour) ($P<0.0001$). The diel pattern of their emergence is shown in Figure 1.

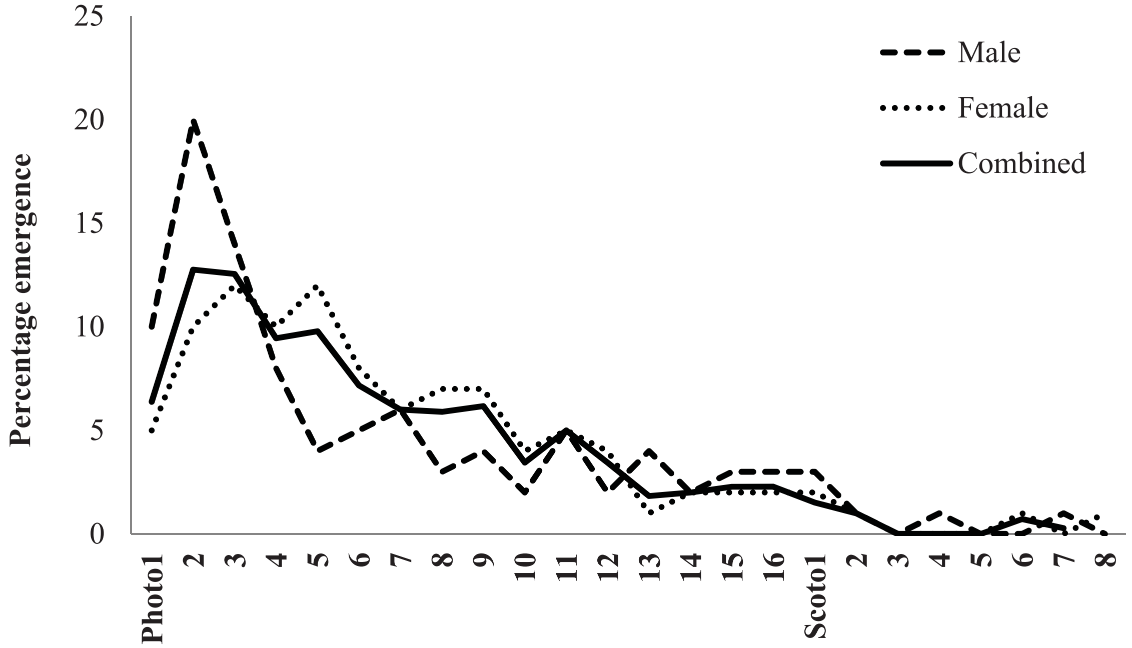

Figure 1: Percentage emergence of male and female *Diaeretiella rapae* during photophase and scotophase.

The emergence pattern was similar in males and females, with emergence peaking during the first 1-2 h of photoperiod. The emergence of males declined quickly after the second hour of photoperiod, while female emergence declined gradually over the photoperiod. The equations for the decline in male and female emergence over time (for 5 hours) were: $y = -4x + 22.2$ ($R^2 = 0.88$) and $y = -0.857x + 12.66$ ($R^2 = 0.47$), respectively, where y=percentage male or female emergence and x=time (hours) after the start of photoperiod.

Mating behaviour

The parasitoids paired during the photophase were highly successful in mating compared to those paired in scotophase (P<0.0001) (Fig. 2). Mating success in *D. rapae* was not significantly affected by the time of the photophase at which they were paired (P>0.05) (Fig. 2). However, the parasitoids were more successful in mating during early scotophase than the middle and the late scotophase (Fig. 2).

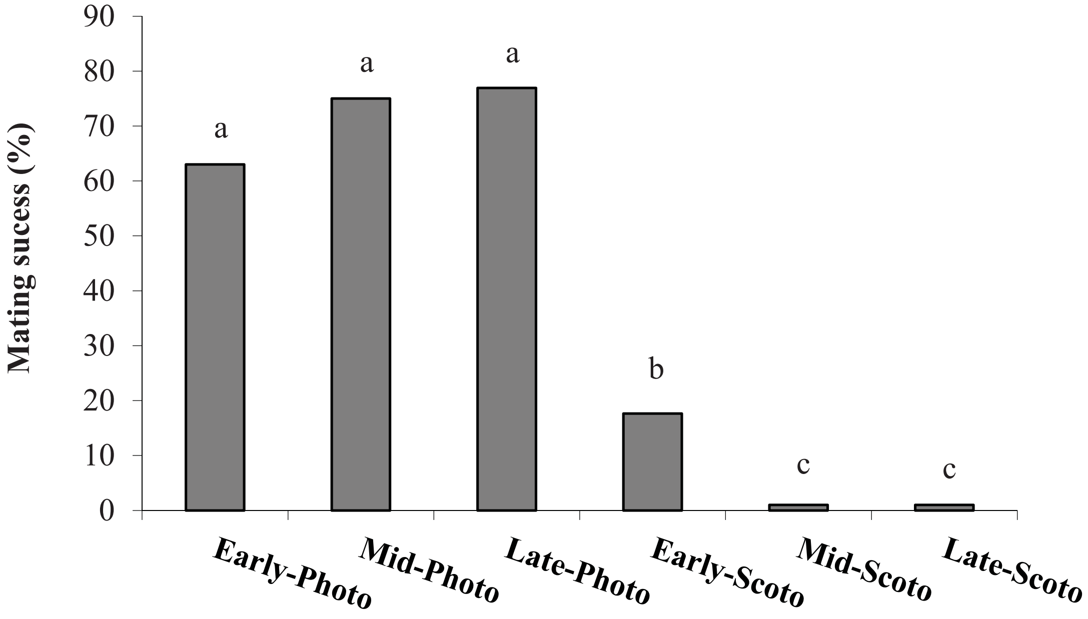

Figure 2: Mating success (% of pairs that mated within 60 min) in *Diaeretiella rapae* during different times of photophase and scotophase. Bars with the same letter are not significantly different (P>0.05).

The pre-mating periods of the parasitoid pairs that mated during different times of the photoperiod were not significantly different (P>0.05) (Table 1). A similar result was also found for their copulation periods (P>0.05) (Table 1).

Table 1: Mean (±SE) pre-mating period (min) and copulation time (s) of the parasitoids that emerged during different time of photophase.

Time of mating	Pre-mating time	Copulation time
Early photophase	15 ± 4	58 ± 4
Middle photophase	21 ± 6	61 ± 7
Late photophase	10 ± 5	60 ± 3
ANOVA	not significant	not significant

It was observed that light had a significant effect on the mating behaviour of *D. rapae*. Success in mating was not only higher in photophase but the pairs that were unsuccessful in mating in scotophase also mated when they were brought into light ($P<0.0001$). The highest mating success (69%) was observed in the pairs that were brought into light after being unsuccessful in mating during the late scotophase. However, the mating success was not significantly different when in early (49%) and middle (38%) scotophase parasitoids were brought in light ($P<0.0001$).

The parasitoids that were unsuccessful in mating during the late scotophase took a significantly longer time (53±3 min) to mate when they were brought into light, when compared to the parasitoids of early (12±3 min) and middle (24±9 min) scotophase ($P<0.001$). However, the copulation time of early (58±1 s), middle (56±3 s) and late (59±2 s) scotophase parasitoid pairs who mated when brought into light, were not significantly different ($P>0.05$).

Oviposition behaviour

Oviposition ability of females in scotophase and photophase was different. The mean number of aphids parasitised by a female was significantly higher in photophase (16±1.5) than in scotophase (1.5±0.5; $P<0.0001$). Females oviposited 96% of their total eggs during photophase.

Oviposition was quite low during scotophase, but was triggered ($P<0.0001$) when the females moved into the light, for both mated and unmated females (Fig. 3).There was no difference in the number of females ovipositing between mated and unmated females either during scotophase or after they were brought into the light ($P>0.05$) (Fig. 3).

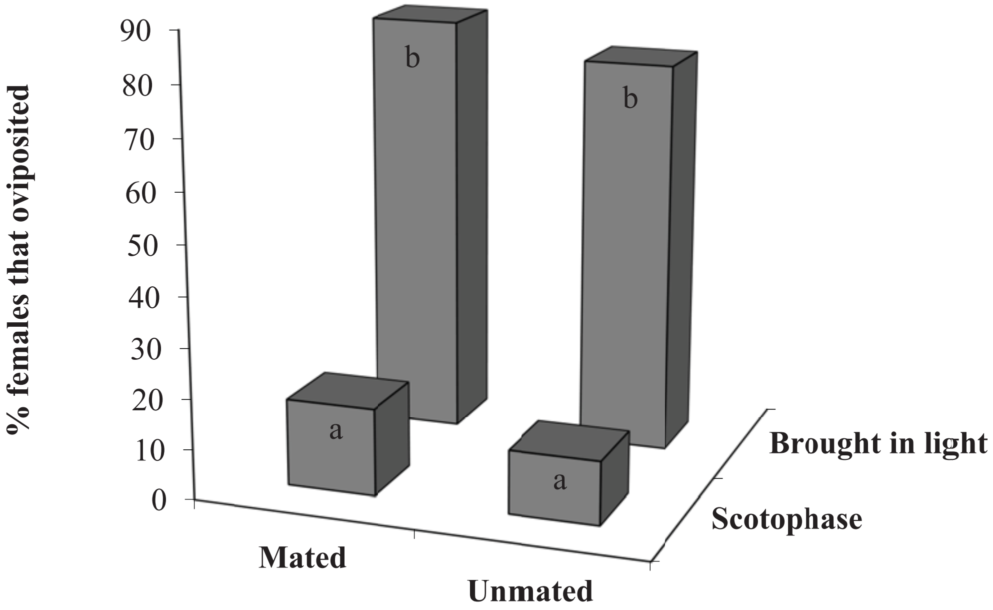

Figure 3: The percentage of mated and unmated *Diaeretiella rapae* females that oviposited at least one host (within 30 min) during scotophase or when brought from scotophase into light. Bars with the same letter are not significantly different (P>0.05).

Discussion

Reproductive behaviours like mating and oviposition are greatly influenced by light in many insects. Emergence of *D. rapae* was triggered by light and prevented by darkness. Emergence of parasitoids at the start of photophase has also been observed in other species, such as *Bathyplectes curculionis, Exorista japonica* and *Aphidius ervi* (England 1995; Nakamura 1997). The emergence of *D. rapae* during early photophase gives them more time and opportunity for finding suitable mates and hosts. *Eriborus terebrans,* a parasitoid of European corn borer, was found to be more active in the morning than in the afternoon and activity almost stopped in the dark (Dyer & Landis 1997). In most parasitic hymenopterans, males emerge earlier than females (Doutt 1964) and wait near the female pupae for females to emerge (Werren 1980) or leave the emergence site and mate when they find females (Myint & Walter 1990). Similarly, most *D. rapae* males also emerged earlier than females. Males get a better chance of encountering and mating with virgin females by emerging earlier than females (Nadel & Luck 1985b). Those males that emerge later than females may get poorer quality females for mating and the mating frequency may also be reduced due to a lack of virgin females.

The emergence of males and females in the dark was negligible when compared to that in the light. By emerging in the dark, parasitoids would have to wait until the following photophase for mating and oviposition. This could be a mechanism used by parasitoids to maximise their reproductive fitness. On the other hand, emergence during photophase could merely be a result of evolutionary processes, since mating and oviposition do not occur in the dark. It has been suggested that parasitoids might also utilise scotophase as a resting period, which is also termed “tucking” (Vogt & Nechols 1991).

In general, mating in hymenopterans is synchronised with day and night changes (Nadel & Luck 1985b). Mating in *D. rapae* occurred only during photophase. It is thought that males may not be attracted to females in the absence of light as females gradually stop releasing pheromone at the start of scotophase (McNeil & Brodeur 1995). Lack of visual cues in scotophase could also affect the courting capacity of males. However, a few *D. rapae* individuals had mated during early scotophase, which could be explained by the possibility that some females continued producing pheromone after the end of photophase. The mating success of *D. rapae* was not affected by the time of day. It is therefore suggested that *D. rapae* females continued to release their sex pheromones throughout the light period. However, other work has found that the mating activity of *A. ervi* was affected by the time of photophase (McClure et al. 2007a). The parasitoid pairs that were unsuccessful in mating during scotophase did mate when they were brought into light, although they took almost 1 h to mate after the pairing. It could therefore be suggested that the effective release of pheromone by the females did not start immediately after the start of photophase.

In hymenopterans, visual cues play an important role in host searching and oviposition (Michaud & Mackauer 1994). The low parasitism rate of *D. rapae* during scotophase and high parasitism rate during photophase would suggest that these females may also require light to select their host. It has been proposed that a female increases her reproductive fitness by laying the majority of her eggs in good quality hosts (Kant et al. 2008a). Similar diel oviposition behaviour was observed by Armstrong et al. (1996) in the parasitoid *Microctonus aethiopoides* and by Mityakina et al. (1993) in the egg parasitoid *Edovum puttleri*. However, in the case of *M. hyperodae* more parasitism occurred in dark (Armstrong et al. 1996).

It can be concluded that light plays an important role in instigating reproductive behaviour of *D. rapae*. The parasitoid synchronises its emergence with photoperiod to maximise opportunities for mating and oviposition.

Acknowledgments

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Chapter Three

Sexual receptivity and mating behaviour of *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae)

Abstract

Sexual receptivity and mating behaviour of the cabbage aphid parasitoid *Diaeretiella rapae* were studied under laboratory conditions. When male and female *D. rapae* were paired for mating, males actively courted for mating, while females showed "coy" behaviour. Males were sexually active immediately after emergence, whereas females took longer to become receptive. Virgin males quickly detected the presence of females resulting in significant increase in male activity. Males encountered females within 5 min of pairing, and 90% of the males displayed courtship behaviour by fanning their wings and chasing the female for mating. Before mating, males approached females more often (4.3±0.6 times) than females approached males (0.8±0.2). The time between pairing and mating of newly emerged females (41±5 min) was longer than for 1-day old females (19±4 min). The overall mating success in *D. rapae* was about 70%, and successful mating was largely dependent on female choice. Unlike females, males remained sexually receptive after mating and 56% males displayed courtship to already mated females. Results of this study suggest chemically-mediated mating in *D. rapae*.

Keywords: Mating behaviour, sexual receptivity, multiple mating, *Diaeretiella rapae,* parasitoid, Hymenoptera

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The results of the previous chapter revealed that males emerge before females, and mating occurs only during light period. This chapter investigates sexual receptivity and different behavioural display by male and female *D. rapae* during mating.

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Kant, R., Minor, M., Trewick, S. Sexual receptivity and mating behaviour of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae).

Introduction

In most insects, mating is an integral part of reproduction that directly affects their fitness and population dynamics. Mating behaviour is mediated by signals between the male and the female, and such signals vary considerably among insects (Emlen & Oring 1977; Choe & Crespi 1997). Diversity in insect mating systems suggests that behaviours linked to reproduction, like courtship and mating, evolve rapidly in insects (Gavrilets 2000; Ritchie 2007). Courtship and other behavioural displays by males and females during mating are essential for understanding their mating success (Reitz & Adler 1991; Hardy et al. 2005b). In haplodiploid parasitoids females may choose between mating or oviposition; asexual reproduction results in male offspring whereas females can be produced only by sexual reproduction (Godfray 1994; Kant et al. 2012a). Thus, mating decisions directly affect the maintenance of generation and population sex ratios in these parasitoids (Hardy et al. 2005b).

After emergence, male and female insects search for mates. While searching for mates, adults often display pre-copulatory behaviours (courtship) to attract mates (McNeil & Brodeur 1995; De Freitas et al. 2004; McClure et al. 2007). In some species females release volatile chemicals, including pheromones, which attract males for mating (Decker et al. 1993; McNeil & Brodeur 1995; Abeeluck & Walter 1997; Steiner et al. 2006; McClure et al. 2007). Male courtship behaviours include antennation, wing vibration (fluttering) and waving of forelegs (Reitz & Adler 1991; Abeeluck & Walter 1997; De Freitas et al. 2004). Wing vibration is generally associated with acoustic signalling that induces mating in some hymenopterans (De Freitas et al. 2004; Hardy et al. 2005b). A recent study has shown that male antennae of paper wasps contain secretory cells which release volatiles that help in mating (Romani et al. 2005).

Although mating brings fitness gains to both males and females, their divergent interests in mating often result in sexual conflicts (Chapman et al. 2003). Mating can occur once or many times in parasitoids and can vary between sexes within species (Godfray 1994). Multiple matings allows females to replenish their sperm stock, and some species store sperm from several males and can select which they use for fertilising their eggs. Females that mate only once can store sperm in their spermatheca of only one male to fertilise their eggs (Gordh & Debach 1976; Ridley 1993; Quicke 1997), can become sperm depleted and produce limited number of female offspring. Males can maximise their fitness by mating multiple times (Godfray 1994).

The study of mating behaviour is essential for understanding parasitoid reproductive success, and could be useful during monitoring and manipulation of host-parasitoid dynamics in biological control programmes (Luck 1990; Suckling et al. 2002). Since females are directly responsible for attacking the pest population, female-biased sex ratios are desirable in biological control (Godfray 1994; Ode & Hardy 2008). Overproduction of males during mass production is a major concern in bio-control (Waage et al. 1985; Hardy et al. 2005a), and understanding mating systems can help to develop strategies for increasing female production in insectaries.

We examined courtship and mating behaviour of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae), a parasitoid that attacks more than 60 aphid species (Pike et al. 1999) and has been used as a biological control agent in several countries (Carver & Starý 1974; Vaughn et al. 1996). Unmated females can reproduce but only sons (Vaughn et al. 1996; Kant et al. 2012a), so the proportion of mated females can influence the population sex ratio which, in turn, would influence the success of *D. rapae* as a biological control agent (Ode & Hardy 2008; Kant et al. 2012b). The majority of adult *D. rapae* emerge during early- to mid-photophase where males eclose before females and mate throughout the photophase but not during dark (Kant & Sandanayaka 2009). This study investigated aspects reproductive behaviour of *D. rapae* that have not been previously examined: 1) courtship and mating behaviour; 2) sexual receptivity in males and females; 3) multiple mating.

Materials and methods

Insect colonies

Colonies of *D. rapae* and host cabbage aphids were established from a commercial cauliflower field near Palmerston North, New Zealand. Insects were maintained in the laboratory at 20 ± 2°C, 60-70% RH and 16h photoperiod on cabbage seedlings ('Autumn Pride') in Plexiglas cages (30×30×30 cm). Adults emerged from 6-7 day old parasitised cabbage aphid nymphs were used in this study. After emergence, each adult was transferred to an individual glass vial (7.5 cm, 2.5 cm dia.) and offered 10% honey solution before being used for mating experiments. All mating experiments were conducted during photophase.

Courtship and mating behaviour

Behavioural observations of males before and after pair formation were recorded for 60 min in 1-day old, honey-fed *D. rapae*. "Pair formation" refers to the experimental establishment of a male and female in a test arena. To study the behaviour of males before pair formation, virgin male *D. rapae* (n = 26) were placed in glass vials (as above), and activities including walking, standing, grooming and antennation were observed and recorded. To study the behaviour of males after pair formation, virgin 1-day old honey-fed males and females were placed together in a glass vial. The courtship and mating behaviours of each mating pair (n = 37) were recorded to develop mating ethogram of *D. rapae*. The activities of mating adults were categorised following King et al. (2005):

1.1. Walking: displacement of male per unit time after pair formation
1.2. Standing: when displacement per unit time is zero
1.3. Grooming: rubbing of legs or antennae while standing
1.4. Approaching: when male follows female and vice-versa
1.5. Encounter: male and female come close and touch each other
1.6. Courtship: male briefly moves his wings up and down, usually rapidly, and is often running toward the female at the same time
1.7. Mounting: more than half of male's body is on top of female's body
1.8. Copulation: male inserts its aedeagus in female genitalia
1.9. Copulation time: period between start and termination of copulation
1.10. Antennation (antenna tapping): vertical movement of male antenna during copulation
1.11. Pre-mating period: period between male-female pair formation and copulation

Those pairs that mated within the 60 min period were considered successful. Post mating behaviours for males and females (courtship, mounting and remating) were also observed.

Multiple mating in male and female Diaeretiella rapae

A random selection of 18 males and 18 females that successfully mated in the above experiment was used to examine multiple mating in male and female *D. rapae*. The males and females were first provided with 10% honey solution and rested for

60 min, and then paired for 60 min with virgin females and males, respectively. If the males and females mated during the second mating, they were again offered a virgin mate until completion of the 60 min period. The number of matings by males and females during this time was recorded.

Sexual receptivity in male and female parasitoids

The time required for males and females to become receptive for mating after emergence was examined. Male and female *D. rapae* of four different ages (1, 2, 6 and 24 h) were individually paired with a 24h old adult of the opposite sex in a glass vial, and the behaviours of individuals recorded until mating. Between 30 and 37 individuals were tested in each age group, and none were used in more than one experiment. *Diaeretiella rapae* that did not mate within 60 min after pair formation were considered unreceptive.

Statistical analyses

A goodness-of-fit test was used to test the distribution of the data, and non-normal data sets were transformed prior to analysis. Differences in behavioural activities were tested using ANOVA Generalised Linear Models (PROC GLM, SAS 9.2). Pair-wise comparisons were made using Tukey’s studentised range (HSD) test. T-tests were used to compare the activity of males before and after introduction of a female to the mating arena, and to compare the number of contacts initiated by the male or the female during mating. To analyse the relationship between the pre-mating period and copulation time, data from all mating experiments of this study were pooled and analysed using Ordinary Least Squares (OLS) regression. Mating success in the sexual receptivity experiment was analysed using logistic regression (PROC LOGISTIC, SAS 9.2). The relationship between male courtship display and the time between pair formation and mating (pre-mating time) was analysed using the S-function of PASW (SPSS) Statistics 18. Values in the results are untransformed means ± SE. All analyses used $\alpha = 0.05$ level of significance.

Results

Courtship and mating behaviour of Diaeretiella rapae

Male *D. rapae* were less active before they were paired with females. The activity of males, including wing vibration and upward and downward movement of the antennae, increased significantly after they detected the presence of a female. About 55% of males spread their wings, and 75% showed antennal movement within 1 min of a female being introduced. The percentage of time males spent walking and grooming (47 ± 9%) increased significantly (85 ± 6%) following the introduction of a female ($F_{1, 46} = 76.83$; $P < 0.001$).

When placed in a test arena, both male and female *D. rapae* walked around the arena and waved their antennae. The first encounter between the male and the female occurred on average 4.8 ± 0.5 min after they were paired. A large proportion of males (91%) displayed courtship behaviour upon encountering the female or just before (logistic regression: $\chi^2 = 5.72$; $P = 0.016$). Males followed females and attempted to mount them for copulation. However, females appeared to initially avoid mating by walking away from the encounter site. The detailed behavioural sequences and transition between the behaviours of *D. rapae* during courtship are illustrated in Figure 1.

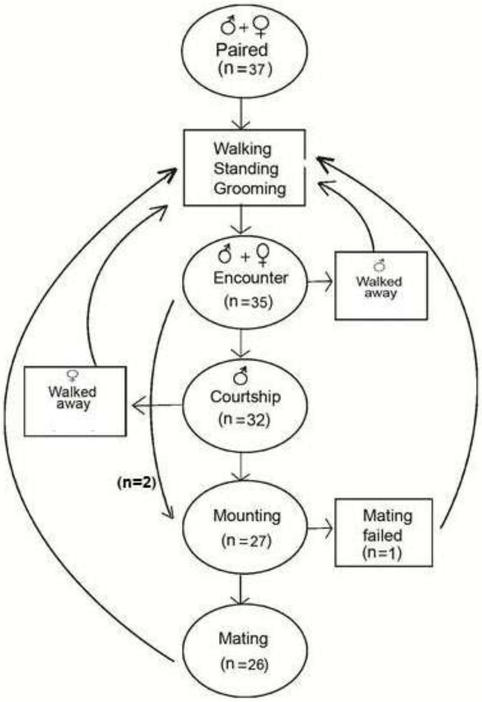

Figure 1: The ethogram of behavioural transitions displayed by *Diaeretiella rapae* during mating after pair formation (n=37). The number of males which displayed particular behaviour or events is shown in parentheses.

The number of times males approached females for mating (4.3 ± 0.6) was significantly greater than the number of times females approached males (0.8 ± 0.2) ($F_{1, 47} = 20.61$, $P < 0.001$). In 73% of cases male courtship behaviour on encountering a female resulted in male mounting on female which led to successful mating except one pair separated without mating. The mean pre-mating time (time period between pair formation and mating) for successful mating was 17 ± 3 min (from 1 to 56 min). The number of courting displays increased during the initial period after pair formation and reached a plateau (Fig. 2a, $R^2 = 0.51$, $P < 0.001$).

The intensity of male courtship decreased when the females rejected male mating attempts. However, males continued to display courtship behaviour until they mated successfully. The number of courtship displays per minute decayed with time before reaching a plateau (Fig. 2b, $R^2 = 0.46$, $P < 0.001$).

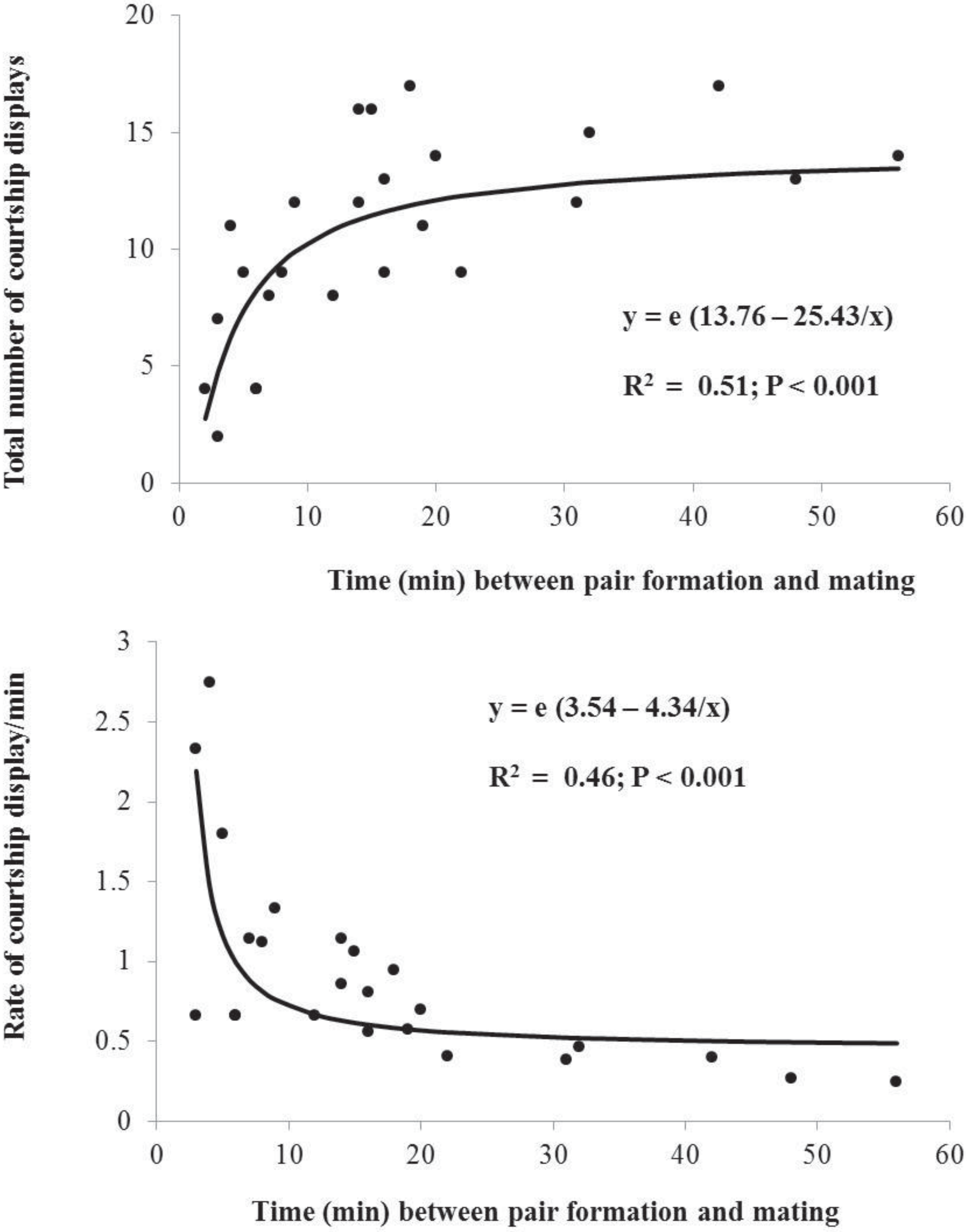

Figure 2: (a) Relationship between the total number of courtship displays by male *Diaeretiella rapae* and time between pair formation and mating (pre-mating time), n=25; (b) relationship between rate of courtship displays (per unit time) by male *Diaeretiella rapae* and the pre-mating time, n=25. Regression analysis was done using S-function of PASW Statistics.

The number of *D. rapae* pairs that successfully mated within the 60 min period (70%) was greater than the number of unsuccessful pairs (logistic regression: $\chi^2 = 5.72$; P = 0.016). Only two males mounted and mated with the female without showing courtship behaviour. To mate with females, males generally followed the female and attempted to mount it from behind or from the side. About two thirds of mounting occurred from the side. Females rejected initial mating attempts by males but 13% of females mated with the same males after initial rejections. Male *D. rapae* attempted mating on average 4.5 ± 0.6 times before being successful, while the unsuccessful males made more attempts 10.3 ± 0.9 times during the 60 min observation period ($F_{1, 32} = 31.07$, $P < 0.001$) (Fig. 3).

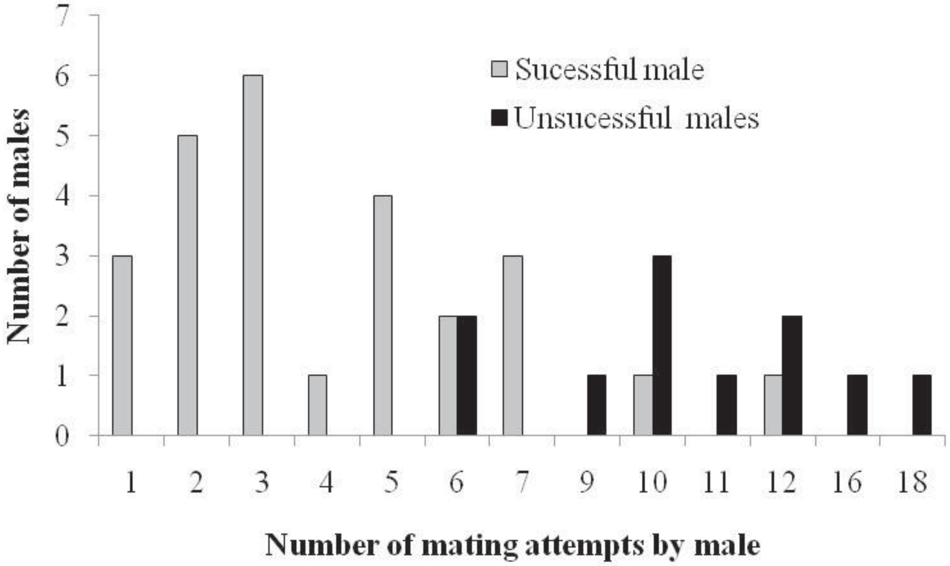

Figure 3: The number of mating attempts by successful and unsuccessful male *Diaeretiella rapae*. Males were regarded as successful if they mated within 60 min of pair formation. Mating attempts males were recorded until their mated successfully or until the end of 60 min period.

After mounting the female, males grasped them with their legs. Males then aligned their body with the dorsum of the female and slowly slid behind the female until the tip of the male's abdomen reached the female's ovipositor. The males then extended their genitalia to copulate with the female. During copulation, females raised their antennae backward and were stationary while the mating males used their antennae to females throughout the copulation. Copulation in *D. rapae* lasted on average 58 s and copulation time was not affected by the length of the pre-mating period (Fig. 4, $R^2 = 0.0047$, $P < 0.57$). Once copulation finished, the pair separated and the males started walking, while the females were observed standing and grooming.

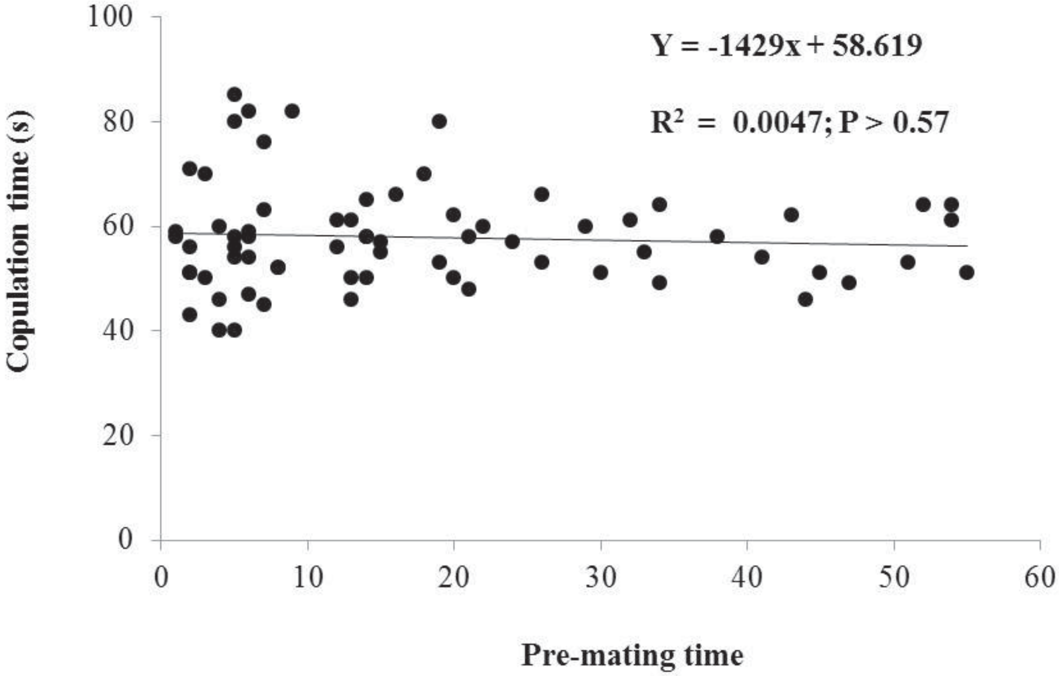

Figure 4: Relationship between pre-mating time (time between paring and copulation) and copulation time in *Diaeretiella rapae* was analysed using Ordinary Least Squares regression.

Multiple mating in male and female Diaeretiella rapae

Females were no longer receptive to mating after copulation. In multiple mating experiments, about 56% males displayed courtship to females that had already mated, and 20% of those who displayed courtship to mated females also mounted and attempted to copulate with them. However, multiple mating did not occur in females with either previously mated males or with virgin males. Unlike females, males remained sexually receptive after their first mating and mated on average with 1.2 ± 0.2 females during the 60 min period.

Sexual receptivity in males and females

Both male and female *D. rapae* were able to mate within 24h of emergence. Younger (1h old) males were more successful in mating than the younger (1h old) females (logistic regression: $\chi^2 = 4.03$; $P = 0.041$). Males attempted to copulate earlier after emergence than females. Younger male *D. rapae* took significantly less time to mate (19 ± 4 min) pairing with 24 h old females, than younger females took (41 ± 5 min) to mate with 24 h old males ($F_{1,\ 23}$ = 12. 3; $P < 0.002$). Variation in mating success in different aged males was not significant ($P = 0.56$), but mating success was significantly lower in younger (1h and 3h old) compared to older (6h and 24h old) females ($P < 0.001$) (Fig. 5).

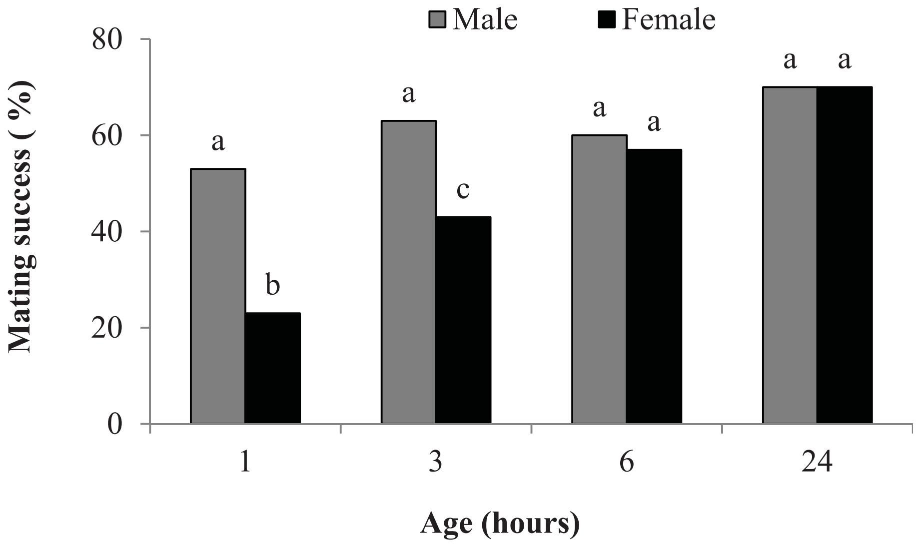

Figure 5: Mating success (percentage) male and female *Diaeretiella rapae* at different ages when paired with a 24 h old mate. Males that failed to mate within 60 min after pair formation were regarded unsuccessful. Bars with the same letters in each age group are not significantly different (Tukey's HSD at α=0.05).

Discussion

Male *D. rapae* were more active than females during mating. Both males and females approached for mating, but males approached females more often. Mating success was largely dependent upon the female's decision to mate. Increased courtship activities (antennal movement and spreading of wings) of males after introduction of a female to the mating arena indicates the presence of a female is first detected by olfaction. After detecting the presence of females, males oriented themselves towards females and made mating attempts. This suggests that both olfaction and visual cues are employed by males during mate finding and mating. Evidence of pheromone-mediated mating in *D. rapae* has been suggested in previous studies (Askari & Alishah 1979; Vaughn et al. 1996), as have long and short distance pheromones in other aphid parasitoids (McNeil & Brodeur 1995; Ruther et al. 2000; Steiner et al. 2006). Some males that encountered empty aphid mummies presented in the glass vial (from which the female parasitoid emerged), attempted to copulate with the mummies (R. Kant, pers. obs.), which further supports the role of chemicals in mate location by *D. rapae* males. Furthermore, antennation by male *D. rapae* on the female body during mating is consistent chemically-mediated mating system (Romani et al. 2005). Therefore, male *D. rapae* probably do not need to visually recognise the females in order to begin courtship towards them (Van den Assem 1996).

Males appeared to respond to female chemical signalling by displaying courtship behaviour such as wing fanning, antennation, and approached female for mating. Wing vibration sometimes also produces acoustic signals (Michelsen 1983), that may help induce sexual receptivity in *Nasonia vitripennis* females (Miller & Tsao 1974). Wing vibration by males upon detection of females was observed in *Aphidius ervi* (McClure et al. 2007). In contrast, presence of a male never results in overt female receptivity in parasitoids (Hardy et al. 2005b). The males need to display courtship to entice female(s) to mate, which is common in sexually reproducing organisms and is an important investment of time and energy by the male (Hardy et al. 2005b). A Female, in turn, responds to courtship behaviour by becoming still and allowing the male to mate, or by walking away and rejecting the male (Abeeluck & Walter 1997). Male *D. rapae* followed females for mating even after being rejected, and our results suggest that initial refusal to mating by female *D. rapae* might not be permanent. The reason for initial denial by females could be due to females not being prepared for mating at the time of the encounter, or needing time to assess males before mating (Eberhard 1996).

Male *D. rapae* showed persistent mating attempts despite initial rejection by females (Fig 3). Male aggression during mating has also been observed in other parasitoid species (Dewsbury 2005; Leonard & Boake 2006), and could be due to male-biased operational sex ratio or the haplodiploid nature of reproduction. A female-biased population sex ratio (2:1) in *D. rapae* has been observed in previous studies (Vaughn et al. 1996; Kant et al. 2012b), but ratio of males and females ready to mate at a given time (operational sex ratio) (Emlen & Oring 1977) is affected by the mating system of *D. rapae*. Multiple mating in males and single mating in females result in a male-biased operational sex ratio in *D. rapae* populations. Additional matings increase male's reproductive fitness (Godfray 1994) and also increases its mating success by limiting access of other males to the females. Therefore, it may be advantageous for a male to be aggressive when virgin and sexually receptive females are available (Emlen & Oring 1977; Choe & Crespi 1997).

Most matings in *D. rapae* occurred immediately after pair formation, and if the mating did not occur quickly the chances of mating success of the pairs decreased. The monandrous and "coy" behaviour of females suggests that they assess the suitability of males before mating, playing a critical role in deciding whether mating will occur or not. The fitness of a female parasitoid is not generally decided by the number of matings she performs, but by the number of offspring she produces in her life (King 2008). Since female *D. rapae* mate only, they are expected to be more selective than males during mate choice.

For successful mating, both the male and the female have to be sexually developed and receptive. Male *D. rapae* were receptive immediately upon emergence, but females took slightly longer to become sexually receptive. In some species (e.g., *Bracon hebetor*), neither males nor females are receptive to mating immediately after emergence (Ode et al. 1996). The early receptivity of male *D. rapae* might contribute to an increase chance of their mating. Female *D. rapae* mated only once and probably turned off their sexual receptivity after the first mating. Multiple mating could be costly to females in a number of ways including time and energy consumption, risk of injury during mating and increased risk of predation (Thornhill & Alcock 1983; Stockley & Seal 2001; Maklakov et al. 2005). Males mated multiple times and did show some courtship to mated females, which may indicate that females take some time after mating to stop releasing olfactory signals. Studies on *Cotesia fleviceps* found that monandrous females continued to release pheromones after mating, and males could not distinguish mated and unmated female during mate searching (De Freitas et al. 2004).

The results show that male *D. rapae* are aggressive during mating, which is their only way to maximise their mating frequency and fitness. Results of this study could also be used to increase the efficiency of *D. rapae* in biological control programmes of aphids. Releasing *D. rapae* adults in the field or in glasshouse immediately after their emergence should be avoided because females are not receptive to males immediately after emergence. Unsuccessful mating may lead to overproduction of males due to female *D. rapae* reproducing before mating (only male offspring) (Kant et al. 2012a). To ensure production of more females during mass production for field release, it is important to maximise mating success. This could be achieved by keeping males and females together for 24h after emergence, before offering them aphids for parasitism.

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Chapter Four

Effects of mate choice and mate density on mating success in *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae)

Abstract

Intrasexual competition and intersexual selection are important drivers in the evolution of mating strategies in sexually reproducing organisms, and in parasitoids where mating is essential for producing female offspring but not males. Effects of mate choice was investigated in males and females of the parasitic wasp *Diaeretiella rapae*, and considered how mate density, sex ratio and operational sex ratio (OSR) affect mating success in this species. Results suggest strong intrasexual competition and intersexual selection in *D. rapae*. Age, body size and mating status affected mate selection in both male and female *D. rapae*. Females were found to be more selective in choosing their mates, whereas males were more active during mating and selected their mates quickly. Virgin female *D. rapae* preferred to mate with virgin males, while males were rejected by mated females. In terms of mate body size, large males did not discriminate females on their body size when offered a large and a small female, and more than 40% of them mated with both. In mate-age choice, younger males mated with younger females. However, older males and females did not show age preference in choosing mates. Asymmetric effect of male and female densities was found on mating success. Increased female density enhanced multiple mating probabilities of males, while increased male density resulted in mating interference. Male *D. rapae* showed courtship to other males in the absence of females. This study revealed that intrasexual competition among males, where males eliminate other males from competition by mounting them, reduces the other male's courting and future mating probabilities.

Keywords: *Diaeretiella rape*, mate selection, operational sex ratio, body size, age.

Mating behaviour and mating success was studied in mate choice tests in *Diaeretiella rapae*. Females were found choosier than males and they discriminated their mates on age, body size and mating status.

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Introduction

Intersexual competition and intersexual selection are important drivers in the evolutionary process (Moore 1990). In systems where mate choice exists, one sex is competitive with same-sex members and the other sex is choosy (selective when it comes to picking individuals to mate with). Mate choice, or intersexual selection, generally depends on attractiveness of trait(s). Darwin (1871) was the first to introduce the ideas on sexual selection but great progress has been seen in this field with the advances in genetic and molecular techniques. In most insects, generally the female expresses mate choice by intersexual selection, while intrasexual competition is common in males (Bonduriansky 2001). However, there are insect species where males, or both males and females, are choosy (Martel et al. 2008). Several studies have documented that males or females choose their partner to gain reproductive fitness (Godfray 1994; Bonduriansky 2001; King et al. 2005; Bailey & Zuk 2009).

The body size is an important factor that affects fitness of the parasitoid species, where larger males and females have greater reproductive potential in terms of sperm and egg count, respectively (Alcock 1979; Cloutier et al. 2000). Larger adults also live longer and perform better (Sagarra et al. 2001). Age is another factor that affects the reproductive fitness of insects, where reproductive potential of males and females decreases with their age (Brooks & Kemp 2001; Kaltenpoth & Strohm 2006). Mating status of the mating partner that varies in natural populations is another factor that decides mating success, and can affect parasitoid fitness (Thornhill & Alcock 1983; King 2000; Chevrier & Bressac 2002). In monogamous females, where a female mates once in its life, selection of the male is critical. If a female mates with multiple-mated male, which would be sperm-depleted, then the female would experience sperm shortage quickly, and would lose fitness by producing more male offspring than required for inseminating all the female offspring (Smith 1984; Marcotte et al. 2007). However, a polyandrous female in a similar situation can replenish its spermatheca by mating multiple times to regain its sperm stock (Boivin et al. 2005). Furthermore, polyandrous females can also choose a good quality sperm from the sperm of different males stored in the spermatheca from multiple matings (Smith 1984).

Density and sex ratio of adults vary with place and time in a population (Comins & Wellings 1985; Lawrence 1986; Krupa & Sih 1993) and can affect their mating success (Comins & Wellings 1985; Godfray 1994). In response to this variability, parasitoids adjust their mating strategies to optimise their reproductive output. High adult density might increase intersexual selection and intrasexual competition and mating interferences. The ratio of males to females in parasitoids is generally skewed, or divergent from Fisher's (1930) sex ratio (Godfray 1994). the operational sex ratio (OSR) which is defined as the ratio of females and males that are ready to mate in a population at a given time (Emlen & Oring 1977), is generally different from the actual ratio of females to males physically present in the population. The OSR depends on the mating system in the species, for example OSR of a species with monandrous females and polygamous males will be highly male-biased despite a greater number of females than males present in the population (King 1996; Shuker et al. 2005).

This research investigated mate choice and mating success in the parasitoid *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae) which parasitises more than 60 different aphid species that attack cruciferous and non-cruciferous plants (Pike et al. 1999). *D. rapae* is the only parasitoid of the cabbage aphid *Brevicoryne brassicae* (L) (Homoptera: Aphididae) (George 1957), which is one of the major pests of cruciferous plants including cabbage, cauliflower, lettuce, brussel sprout, canola, mustard and swede (Ayal 1987; Costello & Altieri 1995). The cosmopolitan *D. rapae* has been introduced for biological control of different aphid species in several countries including Australia and USA (Carver & Stary 1974; Vaughn et al. 1996). *D. rapae* was inadvertently introduced into New Zealand probably in the early 20th century (Carver & Stary 1974), and is now established throughout New Zealand. Use of the parasitoid for biological control has not been explored in New Zealand, but it can be a potential candidate for biological control of some aphid species including cabbage aphid, potato-peach aphid and lettuce aphid, which cause damage to New Zealand agriculture (Cottier 1953; Gerard et al. 2010). Hardly any information is available on mating or mate selection in *D. rapae,* despite the fact that information on mating is crucial for increasing efficiency of a parasitoid in biological control programmes (Godfray 1994; Hardy et al. 2005).

Adult *D. rapae* emerge after the onset of a light period (photophase), the males eclose before females (protandry), and mating occurs throughout the photophase but not during darkness (Kant & Sandanayaka 2009). Male *D. rapae* are sexually receptive after emergence and mate multiple times (polygamous), on average three times a day, while females become sexually mature after 12 h (Kant et al. 2012c). A study carried out in an unmanipulated environment found that about 40% of mating males of *D. rapae* were mounted on another male even in the female-biased population (Kant et al. 2012b). Female *D. rapae* can oviposit immediately after emergence but prefer to mate first before oviposition if males are readily available in the population (Kant et al. submitted). Female *D. rapae* also prefer to lay fertilised eggs in larger nymphs (Kant et al. 2008a), and the size of adults emerging from parsitised nymphs is proportional to the size of nymphs at the time of parasitism (Kant et al. 2012a).

This study investigated mating efficiency in male and female *D. rapae* in mate-choice tests. Choice of age (young vs. old), body size (small vs. large) and mating status (virgin vs. mated) was offered to male and female *D. rapae*. The study also tested the effects of density, sex ratio and OSR on mating success in *D. rapae*.

Material and methods

Insect colonies

Colonies of *D. rapae* and host cabbage aphid *B. brassicae* were established from insects originally collected from a commercial cauliflower field near Palmerston North, New Zealand. Insects were maintained in the laboratory for several generations at 20±2°C, 60-70% RH and 16 h photoperiod on cabbage seedlings (Summer Globe Hybrid) in Plexiglas cages (30×30×30 cm). All mating experiments were carried out in the same conditions. Adults were first transferred to a glass vial (7.5 cm, 2.5 cm dia.) containing 10% honey solution before being used in the experiments.

Impact of mating status on mating success in male and female Diaeretiella rapae

Choice between virgin and mated males was given to male and female *D. rapae*. In the first part of the experiment, a single virgin male (n=25) was offered a choice of a virgin and a mated female. Similarly, a single virgin female (n=25) was given a choice of a virgin and a mated male. To avoid the effects of age and size on mate choice, all adults used in the mating status experiments were 1-day old and emerged from 5-6 day old cabbage aphid nymphs. To get mated males, newly emerged males were placed with five 1-day old, virgin females for 30 h. All mating trails were run for 30 min, and observations on courtship, mounting and mating were recorded. Same size glass vials were used as the ones used for keeping the adults after emergence.

In order to visually distinguish between virgin and mated adults in choice trials, individuals were sprinkled with radiant green and orange colour (Magruder Color Co. Inc., Holmdel, NJ) using a fine paint brush. Colours for each male and each female were selected randomly. No effect of colour on mate selection in *D. rapae* was found (P=0.79).

Impact of body size on mate selection in male and female Diaeretiella rapae

Effects of mate body size on mating success were tested in two body sizes of male and female *D. rapae*. Adults emerged from 1-2 day old parasitised cabbage aphid nymphs were considered “small”, while the adults emerged from 5-6 day old parasitised nymphs were considered “large”. All adults used in body size choice tests were virgin, naive and 1-day old. A choice of a large and a small female was given to small and large males in separate experiments. Similarly, small and large females were given a mate choice of a small and a large male. All experiments run for 30 min and observations on courtship, mounting and mating were recorded. In each category 35 individuals were tested.

Impact of age on mate selection in male and female Diaeretiella rapae

Effects of mate age on mating success were examined in two age groups of male and female *D. rapae*. One day old adults were considered “young”, while 5-day old adults were considered “old”. All adults used in mate age choice tests emerged from 5-6 day old parasitised nymphs, and were naive and virgin. Choice of a young and an old mate was offered to males and females in separate experiments. All experiments run for 30 min and observations on courtship, mounting and mating were recorded; 35 individuals were recorded in each category. In order to distinguish young and old individuals, the adults were sprinkled with radiant green and orange colour as described for the mating status choice tests.

Effects of adult density and sex ratio on mating success

To examine the effects of density and sex ratio on mating success, male and female *D. rapae* emerged from 5-6 d old parasitised cabbage aphid nymphs were used. All adults used in this experiment were unmated, naive, honey-fed and 1-2 day old. Three densities of 1, 2 and 5 male and female *D. rapae* were examined. Mating success in all nine possible combinations of male and female densities was tested, with 30 replications. All experiments run for 60 min and observations on courtship, mounting and mating were recorded. A separate higher density (5 males and 25 females together) experiment (n=30) was conducted to compare the effects of density on numerical and operational sex ratio. OSR of *D. rapae* was calculated based on the mean number of mating occurred when 1 male and 10 female were kept together for 60 min. Mating success was considered 100% when the highest possible number of matings occurred in each replicate.

In another experiment, behaviour of males was observed in the absence of female(s). Ten males of mixed sizes (adults emerged from 1-day to 7-day old parasitised aphids) were kept together in the same size glass vial as used earlier. The experiment was replicated 10 times. Male-male mounting pairs were observed, and 3-4 mounting pairs were collected from each trail. Size of mating males was measured using hind tibia length (HTL) and correlated with the occurrence of each male on the top or bottom of the mating pair.

Statistical Analysis

All data were subjected to normality tests and the data found not normal were arcsine transformed. The data found not normal after transformation were subjected to nonparametric tests. Logistic regression (PROC LOGISTIC, SAS 9.2) was used to calculate mating success in different mate choice experiments. Mann-Whitney U test (MWT) was used to analyse the effects of adult density and sex ratio on the mating success. Analysis of variance (PROC GLM, SAS 9.2) was used to compare the means (copulation length, number of courtships displayed by males, length of pre-mating period), and means were further compared by Tukey's studentised range (HSD) test. Effect of male and female densities on mating success were analysed by Kruskal-Wallis test (KWT).

Results

Impact of mating status on mating success in male and female Diaeretiella rapae

When virgin males were given a choice of a virgin and a mated female, the virgin males mated with virgin females only. However, 28% of virgin males mounted on an already-mated female, but none of the mounting resulted in a successful copulation. Mated females rejected already mounted males to copulate by continuously moving their abdomens. When virgin females were given choice of a virgin and a mated male, only 55% of females mated successfully. Presence of two males in the arena caused mating interference in *D. rapae*. A significantly larger number of successful matings occurred between a virgin female and a virgin male (Logistic regression: $\chi^2 = 5.3$; df = 1; $P < 0.021$) (Fig.1).

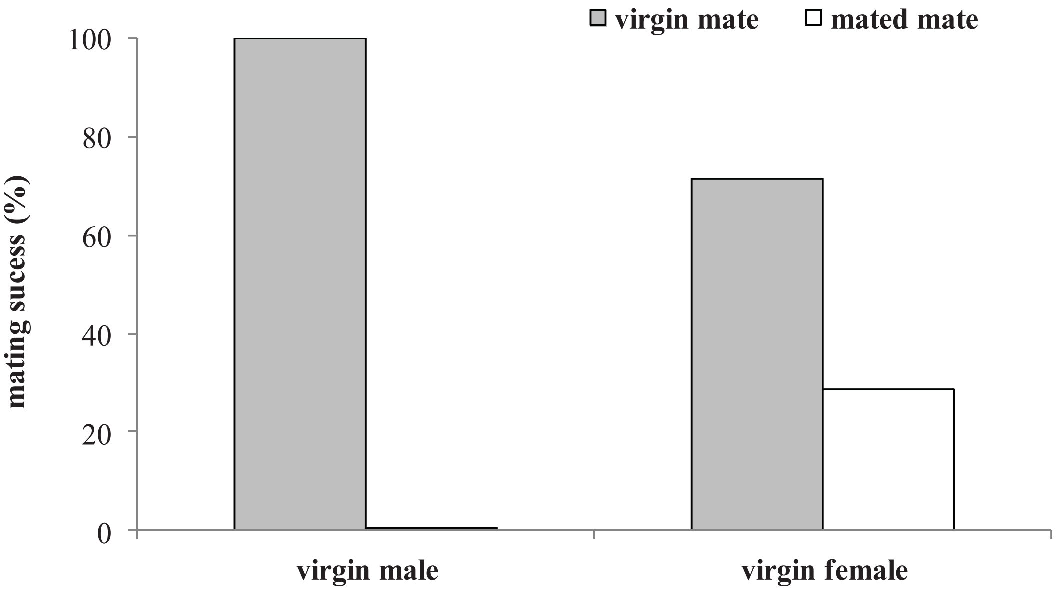

Figure 1: Mating success in virgin male and virgin female *Diaeretiella rapae* when given a mate choice of virgin and mated females and males, respectively.

Impact of body size on mate selection in male and female Diaeretiella rapae

When large males were offered a small and a large female, males did not show any size preference in choosing mates (Logistic regression: $\chi^2 = 1.05$; $P < 0.30$) (Table 1). However, when a small male was offered a small and a large female, the number of matings between the small male and the small female was significantly greater than between the small male and the large female (Logistic regression: χ^2 = 8.23; P < 0.01) (Table 1).

Table 1: Number of successful matings when large and small males (selectors) were separately given the choice of a small and a large female (selectees) in mate choice tests. Males emerged from 1-2 day old parasitised aphid nymphs were "small" and those emerged from 5-6 day old nymphs were "large". All adults were 1-day old, honey-fed, naive and unmated, and each mating experiment was run for 30 min (n=35).

	Selectors			
	Large male		Small male	
Selectees	First mating	Second mating	First mating	Second mating
Small female	13	11	24	9
Large female	20	16	7	4

More than 62% of males mated with both females. The mean (±SE) time between the pairing and the first mating (5.4±0.7 min) was shorter than the mean time between the first and the second mating (13.6±1.2 min) (P<0.001).

When large females were offered the choice of a small and a large male for mating, 80% of the females successfully mated with larger males (P < 0.01). Although mating of larger females with small males was less successful, small males mated with the females quickly (4.7 ± 0.3 min) after pairing, while larger males took longer (9.4±1.1 min) (P<0.001). When small females were offered a small and a large male for mating, the probability of the female to mate with small vs. large male was not significantly different (P>0.09). Intrasexual competition between males played an important role in mating success. Large males often chased the small males after a female was introduced in the arena. However, smaller males were more active in courting and chasing females than large males. The small males were able to mount on the females, but after the mounting larger males also mounted on top of the mating pair, and disrupted the mating between the small male and the female by trying to insert their aedeagus into the abdomen of the small male. Females also escaped mating during male-male contests (Fig 2). The small males mounted by large males were found to be less active during further mate searching.

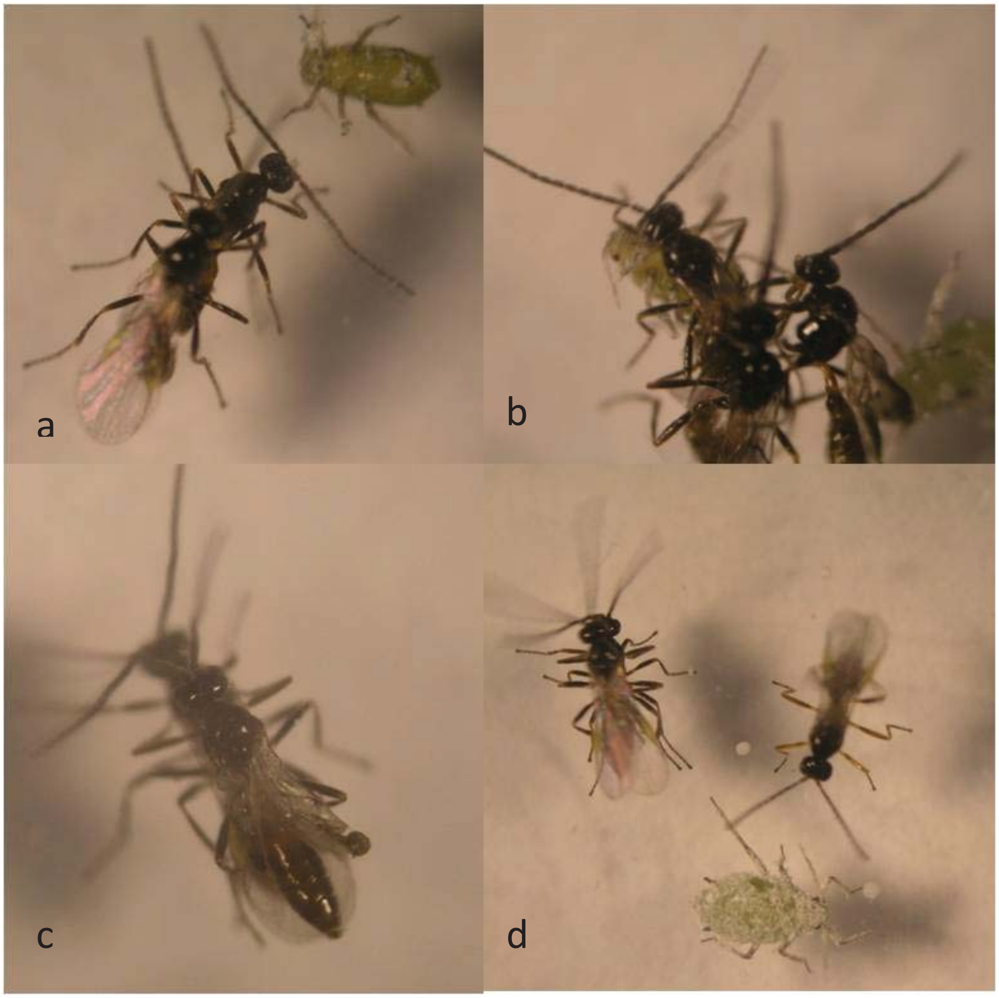

Figure 2: Mating sequence in *Diaeretiella rapae* when more than one male was present during mating: (a) a mating pair, (b) another male approaches and disrupts the mating, (c) second male mounted on top of the mating pair and (d) the two males mounted and struggling with each other, while the female has escaped during the contest.

Mate guarding after mating was not observed either in large or small *D. rapae* males. The males who could not mate with females first, showed courtship, chased, mounted on the mated females and tried to copulate, but none of the mated females accepted a second mating.

Impact of age on mate selection in male and female Diaeretiella rapae

When young (1-day old) males were offered the choice of a young (1-day old) and an old (5-day old) female, a significantly large number of young males mated with young females first (Logistic regression: $\chi^2 = 4.28$; $P = 0.03$) (Table 2). However, when old males were offered a young and an old female, males did not discriminate between the females, and mating was equally successful with young and old females (Logistic regression: $\chi^2 = 0.31$; $P = 0.58$) (Table 2). Mating success in younger and older males was the same ($P = 0.34$), however, younger males were more successful in mating with the second female than older males (Logistic regression: $\chi^2 = 7.52$; $P < 0.01$).

Table 2: Number of successful matings when young and old males (selectors) were given the choice of a young and an old female (selectees) in mate choice tests. Adults of age 1-day old were considered "young", while 5-day old were considered "old". All adults used in this experiment emerged from 5-6 day old parasitised cabbage aphid nymphs, were honey-fed, naive and unmated, and each mating experiment was run for 30 min (n=35).

	Selectors			
	Young male		Old male	
Selectees	First mating	Second mating	First Mating	Second mating
Young female	22	8	16	5
Old female	10	7	13	4

The copulation period was longer (72.1 ± 1.8 s) in older than younger (58.8 ± 1.9 s) males ($F_{1, 40}=24.89$; $P<0.001$). Unlike male *D. rapae*, females did not show any aggression towards the other female while competing for mates.

When young females were given the choice of a young and an old male, 71% of the matings occurred with young males (Logistic regression: $\chi^2 = 3.97$; $P < 0.04$). When older females were offered the choice of a young and an old male, females indiscriminately mated with younger and older males (Logistic regression: $\chi^2 = 0.067$; $P < 0.79$). Strong intrasexual competition existed between the males. Younger males were more active than older males, with higher frequency of courting and chasing the females in younger (7.8 ± 0.4 times) than in older males (5.1 ± 0.4 times) ($F_{1, 68} = 23.3$; $P < 0.001$).

Effects of adult density and sex ratio on mating success

Adult (male and female combined) density affected mating success in *D. rapae* (MWT: $U_o = 56.27$; $P < 0.01$). The mating success was greatest at the lowest adult density, i.e. when one male and one female were present (Fig. 3). Presence of more than one adult reduced the mating success, where the presence of more than one male caused significantly more mating inferences compared to the presence of more females ($P<0.01$).

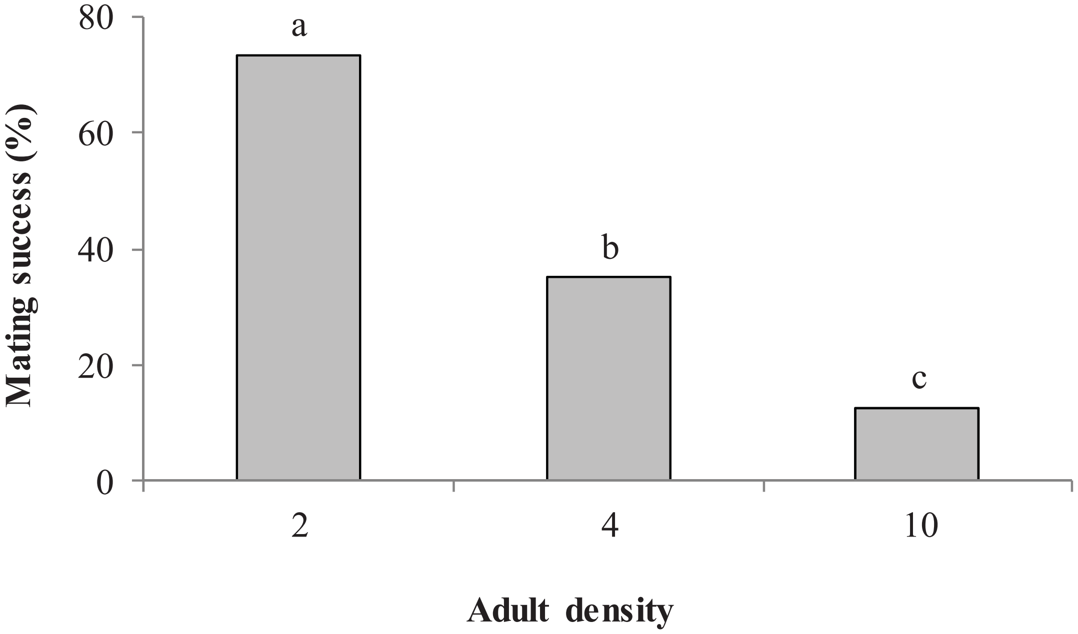

Figure 3: Mating success (%) in *Diaeretiella rapae* at different adult (male and female combined) densities when the same proportion of males and females (1:1) was present during mating. Bars with the same letters are not significantly different, α=0.05.

Increase in female density did not affect the mating probability of male *D. rapae* (P= 0. 09). However, increase in female density increased the multiple mating in males (KWT: $\chi^2 = 30.01$; $P < 0.001$) (Fig. 4). Time between the pairing and the first mating decreased from 11.2±1.4 min to 4.3±0.6 min when the density of females increased from 1 to 5 ($P<0.001$).

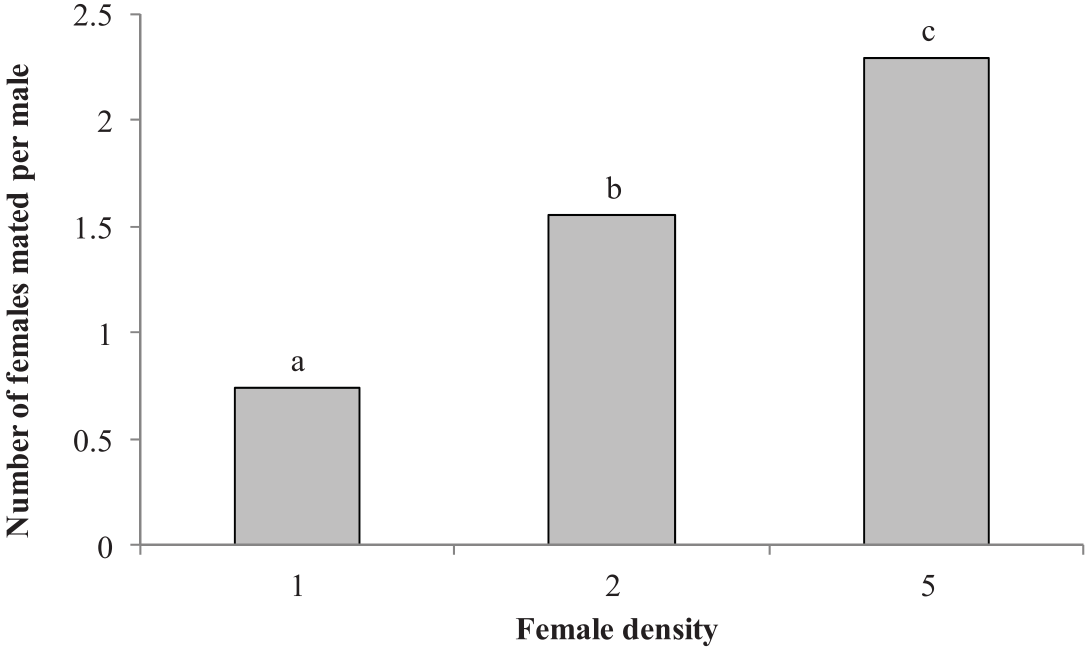

Figure 4: Mean number of females mated per male at different female densities. In each mating experiment only one male was present. Since male *D. rapae* mate multiple times, in several cases a male mated more than once during the 60 min period. Means with the same letters are not significantly different, $\alpha=0.05$.

When more than one male were present in the arena, mating success decreased significantly (KWT: $\chi^2 = 38.13$; $P < 0.001$) (Fig. 5). The low mating success was due to mating interference created by males. When a male mounted a female, another male would mount on top of the mating pair and try to insert its aedeagus into the first male. During such male-male interactions females escaped mating.

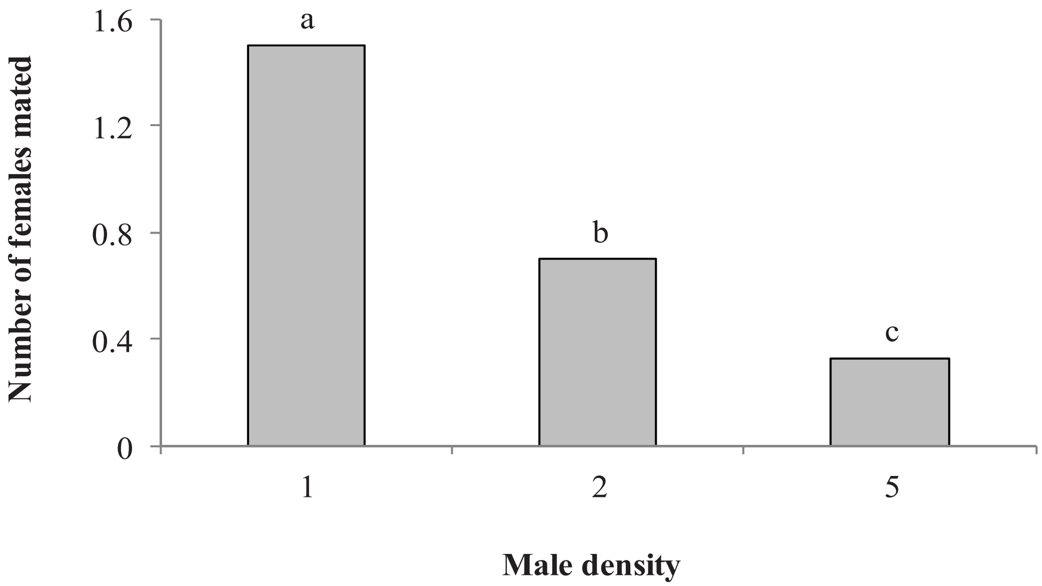

Figure 5: Mean number of *Diaeretiella rapae* females mated at different male densities. The number of females at each density was fixed (two individuals). Means with the same letters are not significantly different, α=0.05.

When a male *D. rapae* was offered several females at a time, the male mated multiple times during the given time. The maximum number of females a male mated with was 5, but on average (±SE) a male mated with 2.2 ± 0.1 females. The operational sex ratio (female: male) of each mating pair was calculated based on the assumption that a male can mate 2.2 times in 60 min period (Table 3). The probability of mating in *D. rapae* decreased with decrease in OSR of the adults present together during mating (MWT: U_o = 348. 1; P<0.001)

Table 3: Mating success at different sex ratios and operational sex ratios (ORS) in *Diaeretiella rapae*. Operational sex ratio was based on the experimentally-derived assumption that a male mates on average with 2.2 females in 60 min. Expected number of matings was calculated based on the assumption that a male mates 2.2 times. Actual number of matings is the number of matings observed during the experiment. Same letters within a column indicate no significant difference (Mann-Whitney test, α = 0.05) (n=30).

Number of individuals		Sex ratio		Number of matings		
Male	Female	Actual	Operational	Expected	Actual	Success (%)
1	5	5	2.3	66	59	89.38 a
2	5	2.5	1.1	132	33	25.00 b
1	2	2	0.9	60	46	76.67 a
5	25	5	2.3	165	22	13.33 b

When virgin males were kept together, they started showing courtship by vibrating their wings. Males showed strong intrasexual interaction even without the presence of any female. One male would chase another male, mount on him, and try (unsuccessfully) to insert their aedeagus into the abdomen of another male. The larger males mounted on smaller males more often (78%) than smaller males mounted on larger males.

Discussion

Mate age, body size, mating status and density affected mate selection and mating success in both male and female *Diaeretiella rapae*. Females were found more selective than males in choosing their mates, whereas males were more active than females during mating and quickly selected their mates. Density of males and females affected mating success in *D. rapae* differently. Increase in female density enhanced multiple mating probabilities of males, while increase in male density created mating interference in *D. rapae*.

Female *D. rapae* mate once (monandrous), so they might be more selective for their mates. Choosing an inappropriate mate could adversely affect their reproductive fitness (Godfray 1994; Bissoondath & Wiklund 1996). Female *D. rapae* preferred previously unmated males. The preference for unmated (virgin) males over mated males decreases their probability of being sperm depleted. The mated males, especially those who have already mated multiple times, generally have low sperm stock (King 2000; Chevrier & Bressac 2002), and they might transfer insufficient amount of sperm during mating, less than required by the female to fertilise all her eggs (Chevrier & Bressac 2002). A female mated with a sperm-deplete male might not produce enough female offspring, resulting in an imbalance in the sex ratio of the population (Chevrier & Bressac 2002). Female *D. rapae* were unreceptive after mating which has been reported in some hymenopterans (Godfray 1994; Hardy et al. 2005). Female *D. rapae* produce highly female-biased offspring from a single mating (Kant et al. 2012a), so probably they do not need to mate with multiple males to replenish sperm. Male *D. rapae* showed courtship to mated females, but never succeeded in copulation with the mated females. By not allowing the males to mate, a female can use the time for host searching and oviposition (Heimpel et al. 1998).

Effects of body size on mate selection were found in both male and female *D. rapae,* but the mate preferences varied in males and females. When offered females of different size, large males showed initial preference for large females, but after the first mating males mated with females irrespective of their sizes. When females were given a choice of large and small males, more matings were observed between large females and large males despite the high courtship display by small males. Preferences for larger mates probably increase fitness of both male and female *D. rapae* because large amount of sperm transferred by larger male could produce more females in the population. Preference for large body size has been demonstrated in other parasitoids (McNeil & Brodeur 1995; Sagarra et al. 2001; McClure et al. 2007). However, smaller female *D. rapae* did not show any mate preference. Small males were rejected by large females so mating between small males and small females was high (Ramadan et al. 1991). When more than one male was present in a mating arena, an intrasexual conflict occurred between the males which caused delay in mating.

Mate age also affected the mate selection in *D. rapae*, where larger numbers of matings occurred between younger males and younger females. The younger females attracted males more often than the older females. Earlier studies on *D. rapae* have indicated pheromone-mediated mating (Askari & Alishah 1979; Vaughn et al. 1996). Age of females affects pheromone release in the aphid parasitoid

Aphidius ervi Haliday (McClure et al. 2007), so it is possible that older female *D. rapae* were less attracted to males than younger females. Male *D. rapae* were less selective and mated with both old and young females. Younger males were preferred over older ones probably because younger males transferred more sperm than older ones. In this study, mate preference was not clearly demonstrated by older *D. rapae* adults.

The density of male and female *D. rapae* affected their mating decisions. Multiple mating in males and single mating in females shifts the female-biased numerical sex ratio to male-biased operational sex ratio (ORS). Increased density and OSR of males caused unsuccessful mating in *D. rapae,* whereas when OSR is female-biased, male–male competition and mating interference rates are expected to decrease, and females are less likely to reject male mating attempts (Hartley & Matthews 2003; Smith & Sargent 2006). Thus, a female bias in the population increases the likelihood of multiple mating or mating success in *D. rapae* which might cause females to be more selective (Emlen & Oring 1977).

With more than one male present in a mating arena, intrasexual conflict occurred between the males causing a delay in mating. Male-male contests in *D. rapae* have previously been demonstrated in an open system where thousands of males and females were present (Kant et al. 2012c). About 40% of the mating males of *D. rapae* were mounted on another male even in the female-biased population (Kant et al. 2012c). Males showing courtship to other males and mounting them suggests that male-male contest is an adaptive strategy. Larger males were observed to mount on top of smaller ones which is consistent with the situation in other insects (Hartley & Matthews 2003; Polak 1993). Smaller males were found to be less active after being mounted by larger males in our experiments. Therefore, male-male mounting behaviour in *D. rapae* and some other parasitoids could be a strategy to reduce mate competition (Hartley & Matthews 2003; Smith 2007).

The results of this study supports the hypothesis that the presence of more females than males in the field or during their mass production would be important for successful biological control by *D. rapae* and other parasitoids showing similar behaviour. Density and sex ratio of *D. rapae* varies in the field and in glasshouses over time; early season (spring) populations generally have a low proportion of females (Kant et al. 2012c). Therefore, releasing more female parasitoids during the early season could bridge the gap between the natural and the desirable sex ratios of the parasitoid, in order to use them effectively in suppressing the pest population.

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Chapter Five

Effects of multiple matings on reproductive fitness of male and female *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

Abstract

Mating frequency and the amount of sperm transferred during mating have important consequences on progeny sex ratio and fitness of haplodiploid insects. Production of female offspring may be limited by the availability of sperm for fertilising eggs. This study examined multiple mating and its effect on fitness of the cabbage aphid parasitoid *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae). Female *D. rapae* mated once, whereas males mated with on average more than three females in a single day. The minimum time lag between two consecutive matings by a male was 3 min, and the maximum number of matings a male achieved in a day was eight. Sperm depletion occurred as a consequence of multiple mating in *D. rapae*. The number of daughters produced by females that mated with multiple-mated males was negatively correlated with the number of matings achieved by these males. Similarly, the proportion of female progeny decreased in females that mated with males that had already mated three times. Although the proportion of female progeny resulting from multiple mating decreased, the decrease was quicker when the mating occurred on the same day than when the matings occurred once per day over several days. Mating success of males initially increased after the first mating, but then males became ‘exhausted’ in later matings; their mating success decreased with the number of prior matings. The fertility of females was affected by mating with multiple mated males. The study suggests that male mating history affects the fitness of male and female *D. rapae*.

Keywords: multiple mating, sperm depletion, sex ratio, mating history

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This chapter discusses the multiplicity of mating and its effects on sperm depletion and fitness in *Diaeretiella rapae*.

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Introduction

Mating is an integral part of reproduction in most insects, and it is essential for the production of viable offspring in sexually reproducing species. However, in parasitic wasps in which males are haploid and females are diploid, mating is needed only for producing female offspring (Godfray, 1994). Therefore, females of haplodiploid organism have a choice between ovipositing and mating, and the decision affects their fitness (Kant et al., 2012a; Steiner & Ruther, 2009). After mating, females store sperm in a specialised organ, the spermatheca, and use the sperm throughout their lives facultatively to fertilise their eggs for producing females (Thornhill & Alcock, 1983). Therefore, the amount of sperm transferred during mating and stored in spermatheca is an important determinant of the population sex ratio and the population dynamics of the species (Chevrier & Bressac, 2002; King, 2000).

The reproductive fitness of a polyandrous female can depend on the number of mating and oviposition activities she undertakes during her life (Bissoondath & Wiklund, 1996; Godfray, 1994). For monandrous females, which mate only once in their lifetime, fitness depends on the number of ovipositions and on the mating history of the male she mates with (how much sperm transferred during copulation) (Marcotte et al., 2007). After mating with a sperm-depleted male, a polyandrous female might be able to replenish sperm stored in her spermatheca by remating, whereas a monandrous female will remain in shortage of sperm throughout her life. Thus, reproductive success in monandrous females is influenced both by the acquisition of mating and by the quality of the male (Smith, 1984). The number of female offspring produced by the mother provides an indirect estimation of the amount of sperm transferred during mating (Henter, 2004).

Reproductive fitness of males depends on the number of times they mate and/or the amount of sperm transferred during each mating (Bissoondath & Wiklund, 1996; King & Fischer, 2010). A male is capable of inseminating more than one female, because in most cases sperm stock of a high-quality virgin male is significantly greater than the number off eggs a single female fertilizes (Bressac et al., 2008). In some species, males emerge with already developed or fixed quantity of sperm (prospermatogeny), whereas in other species males can replenish their sperm once it has been exhausted after mating (synspermatogeny) (Boivin et al., 2005). The synspermatogenic males should be able to increase their fitness by transferring a large amount of sperm, so that the mated female does not experience sperm shortage and does not need to re-mate. The prospermatogenic males have a limited sperm stock and may enhance their fitness by transferring their sperm partially during each mating. By limiting the amount of sperm transferred during each mating, a male can avoid rapid depletion of sperm and eventually inseminate more females. However, this is only successful if females are monandrous; otherwise, a female can re-mate with a different male once she faces sperm shortage to replenish her spermatheca. The amount of sperm transferred to the female during each mating and the number of female offspring produced are used as measures of male fitness (Bressac & Chevrier, 1998; Foster & Ayers, 1996; Henter, 2004).

Diaeretiella rapae McIntosh (Hymenoptera: Aphidiidae) is an important paraitoid of the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae), and several other aphid species across the world (Pike et al., 1999). The parasitoid has been used in various biological control programmes globally, including in Australia for cabbage aphid (Carver & Stary, 1974) and in the USA for controlling Russian wheat aphid (Bernal & Gonzalez, 1993). Previous studies on this parasitoid suggested that mating occurs throughout the photophase but not during the scotophase (Kant et al., 2012b; Kant & Sandanayaka, 2009). Although the sex ratio of *D. rapae* is female-biased (Kant et al., 2012b), multiple mating of males, females, or both, could alter the operational sex ratio of the population. Higher operational male/female sex ratio – specifically, more males being present in the population than the number required to inseminate all the females – can cause mating interference (Kant et al., 2012b) and leave some females unmated. As *D. rapae* is haplodiploid, oviposition by unmated females further contributes to overproduction of males in the population. Therefore, the study of mating in *D. rapae* is important for understanding reproductive strategies of the parasitoid, which could help improve their manipulation for biological control (Godfray, 1994). The current study investigated (1) the capacity for multiple mating in *D. rapae* males and females, and behavioural display during multiple mating, and (2) the effects of multiple mating on reproductive fitness of *D. rapae*.

Materials and methods

Insect colonies

Colonies of the cabbage aphid *B. brassicae* and its parasitoid *D. rapae* were established from insects originally collected from a cauliflower field near Palmerston North, Manawatu, New Zealand. Insects were reared in the laboratory on cabbage seedlings (Summer Globe Hybrid) in Plexiglas cages (30 × 30 × 30 cm). The colonies were maintained at 22 ± 2 °C, 60-70% r.h., and L16:D8 photoperiod. Honey-fed adult *D. rapae* that emerged from 5- to 6-day-old cabbage aphid nymphs were used in the experiments. All mating experiments were done during the light period, because mating in *D. rapae* does not occur in the dark (Kant & Sandanayaka, 2009).

Multiple mating in female and male Diaeretiella rapae

Multiple mating in females was tested by pairing 1-day-old virgin females with 1-day-old virgin males in separate glass vials (7.5 × 2.5 cm) with a ventilated cap. The behaviours of each pair were observed and recorded up to 60 min or until copulation occurred if sooner. The male and female from each pairing were separated; after 1 h, the mated female was again paired with a 1-day-old virgin male and observed for a maximum of 60 min period or until copulation occurred. The mated females were then each offered 50 aphids per day. The mated and oviposited female *D. rapae* were paired again after 1 or 2 days with 1-day-old virgin males. Twenty five females were examined for multiple mating.

Two experiments were conducted to examine multiple mating in males. First, multiple mating was examined every hour (hourly mating) where 1-day-old virgin males were paired with 1-day-old females for 60 min or until copulation occurred if sooner. Successful males were offered another virgin female after a gap of 1 h, and the process continued for the entire photoperiod. Behaviours of the males and the females, such as walking, standing still, grooming, and mating attempts, were observed in each mating pair until copulation occurred. Mating attempt is defined as when a male started vibrating its wings in the presence of a female and chased the female for mating. The unsuccessful males were discarded. A total of 37 males were examined for hourly-multiple mating success. In the second experiment, multiple mating was examined once every day (daily mating). Males and females were paired for 18-24 h in daily mating. On the following photophase males were taken out of the mating arena and paired with another virgin female, and the process continued for the entire life of the males. Mating success in daily-mating males was analysed. Fifteen males were examined in daily multiple mating.

Progeny production and sex ratio from multiple-mated males

The effects of hourly- and daily-multiple mating of males on progeny production and progeny sex ratio was examined using the insects from multiple mating experiments described above. The mated females from hourly-mating experiments were each offered 15 fresh aphids for 2 h in a Petri dish per day for three consecutive days. The parasitised aphids were transferred to a cabbage seedling to feed and develop. Once the aphids were mummified, they were transferred to individual 2-ml microfuge tubes. The mummies were observed daily for adult emergence. The total number of adults and the proportion of female resulting from each mating were examined.

Similarly, progeny production and sex ratio of the progeny from daily mating was tested. Mated females were each offered 30 aphids on cabbage seedlings in a 2-l transparent and ventilated plastic container for 24 h. Fresh 30 unparasitised aphids were offered to the mated females for three consecutive days. The parasitised aphids were allowed to feed and develop on cabbage seedlings and the process was followed as above. The total number and sex ratio of adults emerged from hourly and daily mating experiments were compared. Ten males that mated at least 5× were used in each hourly and daily mating experiment.

Statistical analysis

Binomial logistic regression was used to analyse mating success during daily and hourly multiple mating. When modelling the proportion of male wasps that were successful during mating, we use the model:

$$y_{ij} = m_i + f_j + (mf)_{ij} + \grave{o}_{ij},$$

where y_{ij} is proportion of mating success, m_i is the mating number, f_j is the indicator for the hourly/daily mating frequency, $(mf)_{ij}$ is the interaction of these effects, and $\grave{o}_{ij}$ is the random error term for each observation. A general linear model (ANOVA) was used to compare the number of mating attempts made by successful and unsuccessful males during first and fifth matings. The sex ratio, among the *D. rapae* offspring (proportion of female offspring) resulting from different matings was analysed by logistic regression (Wilson & Hardy, 2002). The log linear model was used to understand the relationship between the mating history of males and the number of female offspring produced (Crawley, 1993). The model was fitted as a generalised linear mixed model using penalised quasilikelihood (glmmPQL) (Venables & Ripley, 2002):

$$y_{ijk} = m_i + f_j + (mf)_{ij} + w_k + \grave{o}_{ijk},$$

where m_i is the mating number, f_j is the indicator for the daily/weekly mating frequency, $(mf)_{ij}$ is the interaction of these effects, w_k is the random effect for each male wasp, and $\grave{o}_{ijk}$ is the random error term for each observation. The response y_{ijk} and the error $\grave{o}_{ijk}$ change for the last three models. When modeling the proportion of females as the response, a logistic regression was used. For modeling the counts of females and then the total offspring, the response and the error term were assumed to be Poisson distributed. All analyses were done at $\alpha = 0.05$ level of significance by SAS (v9.2) and R (v2.11.1) statistical software.

Results

Multiple mating in female and male Diaeretiella rapae

Females mated only once during their lifetime. Females did not accept a male for re-mating on the same day or after 1-2 days after first mating. Furthermore, female *D. rapae* did not remate even after oviposition. In contrast, male *D. rapae* remained sexually receptive after the first mating and showed courtship to previously mated as well as virgin females.

Mating success of males depended on their mating history [sequential analysis of deviance: G = 7.42, d.f. = 15, P = 0.006] (Figure 1). The proportion of mating success also depended on mating approaches, whether mating was hourly or daily [G = 9.54, d.f. = 14, P = 0.002] (Figure 1). As the number of male mating sessions increased, the mating success decreased. This was true for both hourly- and daily-mating sessions; however, the rate of decline was greater in hourly mating [G = 5.01, d.f. = 13, P = 0.025] (Figure 1). All males died by the 9th day in the daily multiple mating. The mean (± SE) number of times males mated in the single day was 3.4 ± 0.5. The minimum time gap between two consecutive matings was 3 min, and the maximum number of successful matings a male achieved in a day was eight.

When activities of males (walking and mating attempts) during mating were compared in early (first) and late (fifth) matings, males were less active in the late mating bouts. Before copulation in the fifth mating, males spent 35% of their time walking around the arena and the rest of the time standing still or grooming, whereas in the first mating they spent 85% of the time walking. The mean number of mating attempts by males that were unsuccessful during their first mating was significantly higher than the number of mating attempts by males that were unsuccessful during their fifth mating (10.9 ± 0.9 vs. 4.6 ± 1.3) ($F_{1,15} = 13.65$, $P < 0.002$). The mean number of mating attempts by successful males during the first mating (4.0 ± 0.6) and fifth mating (4.8 ± 0.8) were similar ($F_{1,23} = 0.60$, $P = 0.45$).

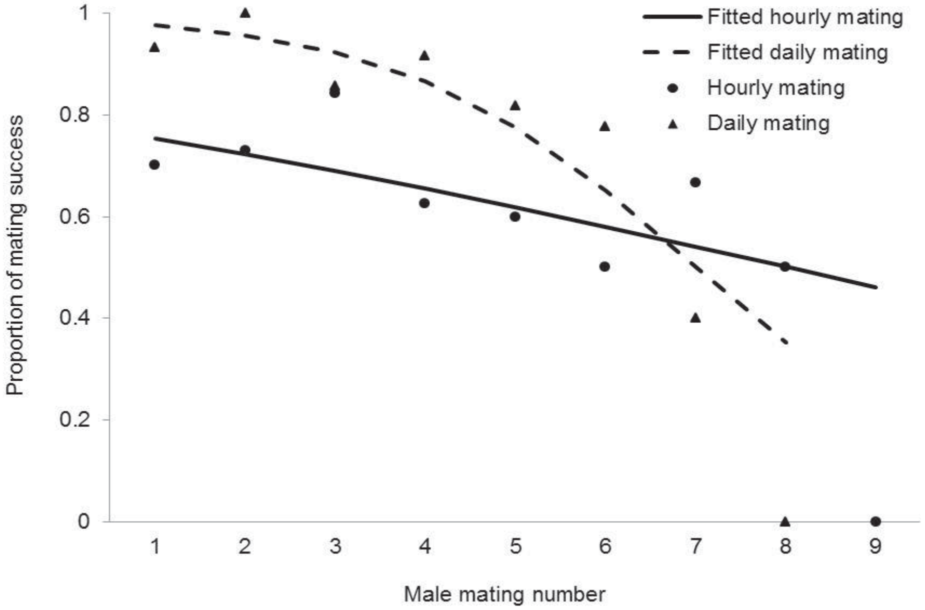

Figure 1 The effects of hourly and daily multiple matings on mating success of *Diaeretiella rapae*.

Progeny sex ratio from multiple-mated males

The proportion of female offspring from multiple-mated males decreased irrespective of whether the multiple mating occurred hourly in a single day (glmmPQL: t = 2.66, d.f. = 85, P = 0.009) or daily over a period of several days (t = 2.51, d.f. = 85, P = 0.014) (Figure 2). However, the decrease in the proportion of female offspring was significantly quicker in hourly mated compared to daily mated males (t = 2.35, d.f. = 18, P = 0.03) (Figure 2).

Effect of male-mating history on progeny production of mated females

The fertility of females was affected by the mating history of their mates (glmmPQL: t = 2.02, d.f. = 18, P = 0.057) (Figure 3). Females that were mated with multiple-mated males produced fewer progeny. The reduction in the total number of offspring produced by females also depended on whether the females were mated in hourly or daily mating sessions (t = 2.49, d.f. = 86, P = 0.014) (Figure 3). The total offspring resulted from hourly mating was much less than daily mating.

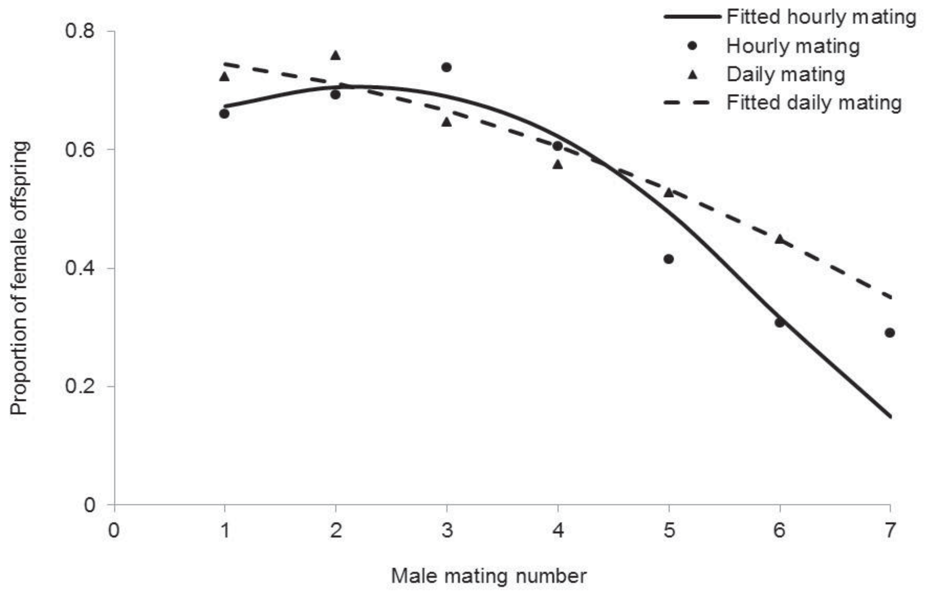

Figure 2 Sex ratio (proportion of female offspring) of the offspring produced by female *Diaeretiella rapae* that mated with multiple-mated males. Logistic regression model was used to analysis the decrease in the proportion of female offspring.

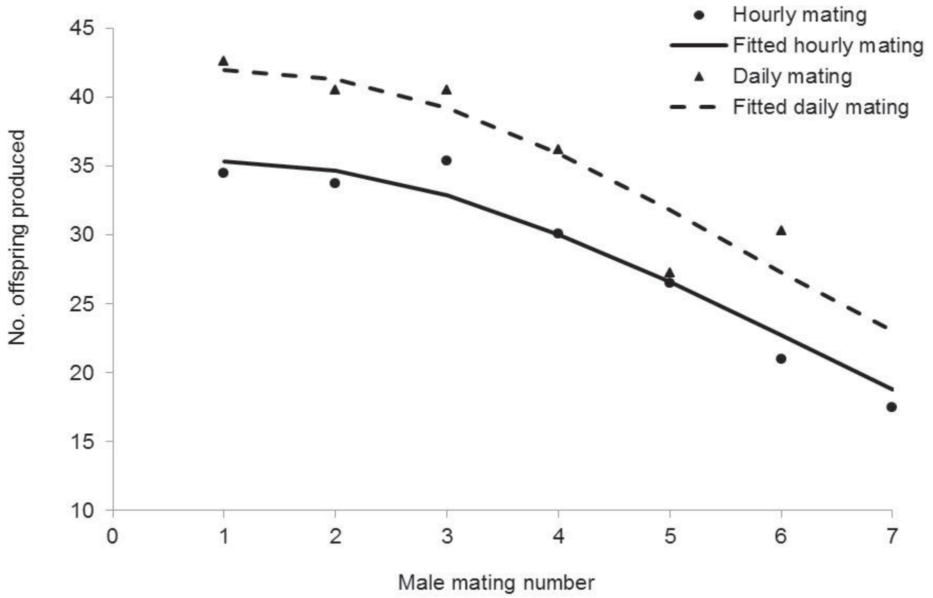

Figure 3 Total number of offspring produced by *Diaeretiella rapae* females that mated with multiple-mated males. The log linear model was used to understand the relationship between the mating history of males and the number of offspring produced.

Females also produced significantly fewer female offspring when they mated with multiple-mated males (glmmPQL: t = 3.11, d.f. = 18, P = 0.006) (Figure 4). The number of female offspring produced also depended on whether the females were mated in hourly or daily mating sessions (t = 2.53, d.f. = 85, P = 0.013) (Figure 4).

The number of female offspring produced by sperm-constrained females (mated with males in their fifth and sixth mating) was greater in their first oviposition bouts than in their second and third oviposition bouts ($F_{2,84} = 21.59$, $P < 0.001$) (Figure 5). A similar number of female offspring were produced by females mated with sperm-depleted males during their second and third oviposition bout (t = 1.28, d.f. = 67, P = 0.41) (Figure 5).

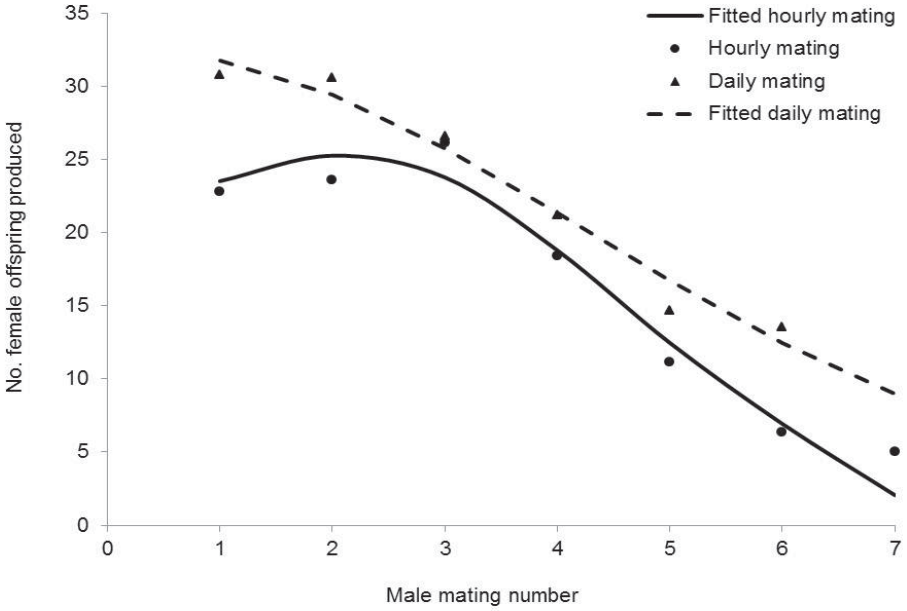

Figure 4 Mean number of female offspring produced by female *Diaeretiella rapae* that mated with multiple-mated males. The log linear model was used to understand the relationship between the mating history of males and the number of female offspring produced. The model was fitted as a generalised linear mixed model using penalised quasilikelihood (glmmPQL).

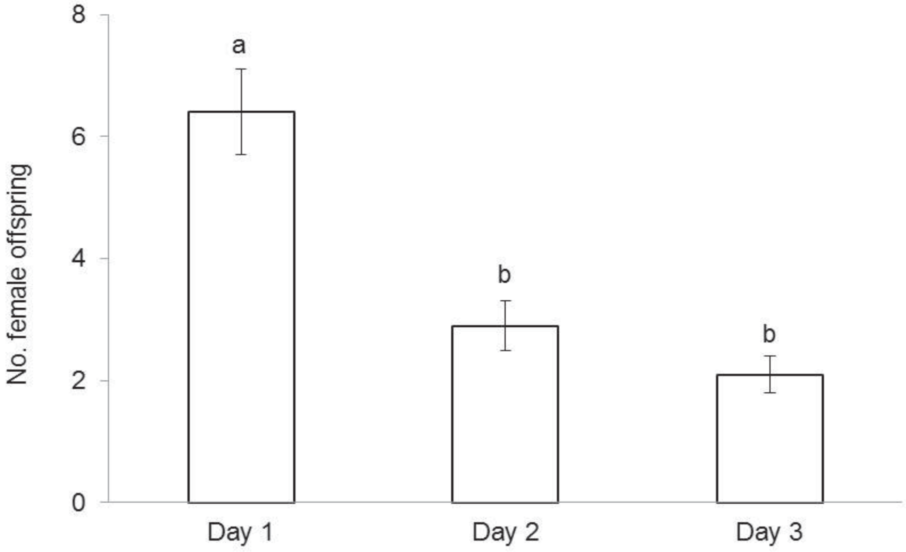

Figure 5 Mean (± SE) number of female offspring produced from three consecutive ovipositions (Day 1-3) by female *Diaeretiella rapae* that mated with 4 or 5 times already-mated males. Means with same letters are not significantly different (Tukey's HSD: P > 0.05).

Discussion

Multiple mating occurred in male but not in female *D. rapae*. Multiple mating in males affected the reproductive performance of females. Sperm depletion as a consequence of multiple mating by male *D. rapae* probably caused the low proportion of female offspring. Multiple mating was more exhaustive in a single-day (hourly) mating than daily mating. Males that mate frequently or in quick succession deplete their sperm stock more rapidly than males mating after longer intervals (Hardy et al., 2005a). The number of female progeny resulting from matings involving a virgin male was higher than the number of females produced from matings with multiple-mated males. This suggests that multiple-mated males become sperm depleted and transfer a reduced amount of sperm during copulation. Reduction in sperm transfer with successive mating has been documented in other species of Hymenoptera (Bressac et al., 2008; King, 2000; Nadel & Luck, 1985).

From the female's perspective, mating with a sperm-depleted male might result in sperm shortage in her spermatheca, which could reduce the number of fertilised eggs and hence the number of female offspring. Because female *D. rapae* mate once and do not replenish their sperm supply by remating, they may ultimately produce more sons than are required for inseminating all the females of the subsequent generation, and enhance imbalance in the sex ratio in the parasitoid population (Damiens & Boivin, 2005). Thus, monandrous females are expected to evolve mechanisms acting against the male multiple-mating strategy (Chapman et al., 2003). If a female can recognise male-mating history, it would allow her to avoid mating with a sperm-depleted males (Harris & Moore, 2005). In our experiments, less than 20% of males were able to reach the fifth mating and the remaining males were rejected by females. This suggests that female *D. rapae* might be able to recognise this aspect of male quality. The strategy of the female to initially avoid mating might explain the large number of unsuccessful matings and longer pre-mating period for the pairs who mated successfully. It should be noted that in our experiments females had no option to choose between a virgin and a multiple-mated male but could only accept or reject the male provided. Furthermore, in this study, opportunity and time for females to make mating decisions was limited.

Although the sex ratio of New Zealand populations of *D. rapae* has been found to be female-biased in laboratory as well as in open systems (Kant et al., 2012b; Kant et al., 2012c), the monandrous and polygynous nature of *D. rapae* (R Kant, unpubl.) biases the operational sex ratio in favour of males. Thus, in order to inseminate all the females in a population, a male would on average mate 3-4× in its life. Large numbers of males could also lead to an increase in male-male interactions, reducing mating success in females, and further decreasing the efficiency of *D. rapae* to suppress aphid populations (Kant et al., 2012b). In this study, we found that sperm depletion in male *D. rapae* (as reflected by production of male offspring) did not occur until the third mating in hourly matings. However, sperm depletion in daily multiple matings is obvious in third matings which is probably because of the age factor. Age of male *D. rapae* negatively affects the sperm production ability (R Kant, unpubl.). Furthermore, under natural conditions, where females are abundant in the population, it is less likely that a male would mate with only one female per day. Therefore, under natural/field conditions the chances of females encountering sperm-depleted males should be low (Godfray, 1994; Hardy et al., 2005b).

We found that female *D. rapae* mate only once in their life. Mating with a sperm-depleted male did not increase the tendency of females to mate again. This could be due to some physiological constraint on re-mating in female *D. rapae*. During copulation, males might transfer certain chemicals with the spermatozoa that might prohibit females from mating again (Avila et al., 2011; Davey, 1985; Neubaum & Wolfner, 1999). By doing so, sperm-depleted males would increase their reproductive success by inseminating more females and preventing the females from mating with other males (Damiens & Boivin, 2006; Gardner & West, 2004). Absence of re-mating in females experiencing sperm shortage has also been reported in other species (Damiens & Boivin, 2006; Gardner & West, 2004).

In some parasitoid species, the longevity of adult females is relatively longer than the offspring developmental time which provides the opportunity for females mate with their own sons and produce female offspring (Greeff, 1996; King & Copland, 1969). However, in *D. rapae*, development takes longer than the lifespan of adult females (Kant et al., 2012c), thus, there is no opportunity for inbreeding (females to mate with their own sons) in this species. Therefore, the strategy for females that live for a shorter period of time is probably to mate once and spend more time on oviposition. In contrast, females of longer lived species have more options; they can mate several times if they become sperm deprived in later life and/or choose best sperm stored in their spermatheca (Jacob & Boivin, 2005). As *D. rapae* is a monandrous species, there is no opportunity for sperm competition.

The results of this study suggest that male *D. rapae* continue to mate even after sperm-depletion. They probably increase their reproductive fitness by optimising the amount of sperm transferred per female to maximize the number of matings. Ageing negatively affected the insemination capacity of males. Female *D. rapae* rejected multiple-mated males, and the females mated with multiple-mated males received less sperm and produced fewer offspring.

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Chapter Six

Impact of adult nutrition on longevity and reproductive potential of parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

Abstract

Parasitoids feed on their hosts as larvae to acquire nutrients, and gain additional resources by feeding on either host or non-host resources during their adult stage. This study investigated the importance of adult feeding patterns on longevity and reproductive potential of *Diaeretiella rapae*, a parasitoid of cabbage aphid *Brevicoryne brassicae*. Three feeding treatments, (1) honey, (2) water, and (3) no food or water were assessed in this study. Female D. rapae emerged with developed eggs and did not require additional food for egg maturation or for successful oviposition. The females were able to produce viable offspring immediately after emergence, and the number of eggs left in the ovaries of females after oviposition decreased with each oviposition bout, suggesting that ovigeny of D. rapae is inclined towards the pro-ovigenic condition. The longevity of *D. rapae* adults increased significantly when offered honey or water compared to when they were starved. When unmated males and females were paired in the presence of honey, females were attracted to honey, whereas males began courtship for mating and did not feed until after mating. The positive effects of honey-feeding on longevity were greater in females than in males. The honey-fed females that were deprived of hosts lived longer than those offered hosts regularly. Fecundity and fertility of female *D. rapae* increased significantly when they fed on honey compared to when they were starved. Honey-fed males gained fitness by increasing their mating frequency.

Keywords: longevity, fecundity, feeding, parasitoid, *Diaeretiella rapae*

This chapter discusses the effects of food provisioning on the fitness of *Diaeretiella rapae*. Food supply significantly affected longevity and reproductive potential of *D. rapae*.

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Kant R, Minor MA, Trewick SA and Sandanayaka WRM. Impact of adult nutrition on longevity and reproductive potential of parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae).

Introduction

Nutrient acquisition and allocation are important components of parasitoid life. Parasitoid larvae acquire nutrients from their hosts, whereas adults can gain additional nutrients by feeding on host or non-host resources (Heimpel & Collier 1996). Moreover, the nutrients acquired in the two life stages differ: larvae generally acquire lipids and proteins, while adults generally obtain carbohydrates and sometimes proteins (Strand & Casas 2008). These nutrients probably have different functions in the growth and development, reproduction, and somatic maintenance of parasitoids. The resources acquired by parasitoid larvae by feeding on hosts are employed directly for their growth and development while the nutrients acquired by adult females can be used for physical maintenance (including longevity) or reproduction, or both, depending upon the egg status of emerged females (Jervis et al. 2001). Egg load indicates the reproductive capacity of a female parasitoid. Females of some parasitoid species emerge with developed eggs (pro-ovigenic) and can parasitise their host immediately after emergence, whereas other females emerge with immature eggs and require some time and often additional resources (Jervis et al. 2008) to mature their eggs before oviposition (synovigenic) (Flanders 1950).

Longevity and fecundity of parasitoids are directly linked to their efficiency in pest suppression (Godfray 1994). Studies have suggested an interaction between cost of reproduction and longevity in parasitoids (Sevenster et al. 1998; Ellers et al. 2000; Francisco 2001). The basis for this trade-off is encompassed by the nutrient limitation theory (Francisco 2001). The physiological and physical characteristics of a parasitoid could be affected by resource acquisition during the two-phase feeding. Thus, patterns of nutrient allocation, utilization, and acquisition are important in understanding reproductive strategies of parasitoids (Bernstein & Jervis 2008).

Diaeretiella rapae McIntosh (Hymenoptera: Aphidiidae) is the only reported parasitoid of cabbage aphid *Brevicoryne brassicae* L. (Homoptera: Aphididae). However, it attacks several other aphid species worldwide, including green peach aphid *Myzus persicae* (Sulzer), mustard aphid *Lipaphis erysimi* (Kaltenbach) and Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Pike et al. 1999). Previous studies have shown that longevity of *D. rapae* varies with their body size, where larger adults live longer than smaller (Kant et al. 2012a). Female *D. rapae* also live longer than males (Kant et al. 2012a). The sex ratio of *D. rapae* populations was observed to be female-biased in a laboratory and in a wild population (Kant et al.

2012a; Kant et al. 2012b). However, multiple mating in males (polygamy) and single mating in females (monandry) change the operational sex ratio of *D. rapae* towards male-biased (Kant & Sandanayaka 2009). In this situation, male longevity could directly affect reproductive fitness within and between sexes in *D. rapae*. The longer living male *D. rapae* can maximise their mating frequency, while longer living females can increase their oviposition. The present study examined how food availability to adults affected: (1) life expectancy of male and female *D. rapae* and (2) egg load and reproductive potential of females.

Materials and methods

A laboratory culture of D. rapae was established from individuals that emerged from mummified cabbage aphids B. brassicae (L) collected from a cauliflower field in Palmerston North, New Zealand. The culture was maintained at 20 ± 2^{o}C, 65 ± 5% RH, 16 h light. Parasitoids emerged from the mummies were reared on cabbage aphids feeding on cabbage seedlings (cv. Summer Globe Hybrid). Adult D. rapae emerged from 5-6 day old parasitised aphid nymphs were used in this study.

Adult feeding and adult longevity of Diaeretiella rapae

Longevity of male and female *D. rapae* was tested in three separate feeding trials: (1) honey (honey-fed); (2) water; (3) no food or water (starved). Honey refers to 10% honey-water solution throughout the article. Each feeding trial was replicated 16 times for both males and females. Each male and female was placed in an individual glass vial (2 x 5 cm) with vented lid, and honey or water was provided where appropriate. Adults were checked every day, and honey and water were supplemented if required. Feeding behaviour and longevity were recorded. In a separate trial, male and female *D. rapae* pairs (n=12) were offered honey together in a similar size vial for 10 min, and their feeding behaviour and feeding time was recorded.

Adult feeding and egg load in Diaeretiella rapae

The egg load of *D. rapae* females was examined in three feeding treatments as described above. Females of five age groups, 2-4 h, 1-2 day, 3-4 day, 7-8 day and 10-11 day were dissected without exposing them to the aphids. Egg loads of 2-4 h old females were assessed without offering them food or water. Starved females did not live longer than 4 days, so only females that were offered honey or water were recorded in the last two age groups. At least ten females in each feeding/age combination were dissected under a stereomicroscope (Olympus SZX12). Ovaries were excised from the abdomen of each female on a glass slide in insect saline and a cover slip placed gently on the top to burst the ovaries so that the eggs could be counted.

Effect of feeding on oviposition in Diaeretiella rapae

The number of aphid nymphs parasitised by female *D. rapae* in the three feeding treatments was examined. Newly emerged unmated females were first exposed to one of the feeding treatments for 24 h, before being offered 15 aphid nymphs (5-6 day old) for 2 h each day for three consecutive days. Eight females were tested in each feeding treatment. Parasitised aphids were allowed to develop until mummies were formed. Each mummified aphid was then transferred to a 2 ml microfuge tube until adult emergence, and the numbers of adults emerged from each treatment were recorded and treatments compared.

Effect of oviposition bouts on egg load of honey-fed Diaeretiella rapae

The number of eggs remaining in the ovaries of honey-fed females after oviposition was examined. Honey-fed females were offered 15 aphids for 2 hours each day. After a known number of oviposition bouts, the females were frozen and the eggs remaining in their ovaries were counted. The number of eggs remaining in the ovaries of females that oviposited for 1, 2, 3, 4 and 5 days consecutively was compared. Eight females were dissected in each oviposition group.

Statistical analysis

Longevity of males and females, and the egg-load of females in the three feeding treatments were compared using a General linear model (PROC GLM). Means were compared using Tukey’s studentised range (HSD) tests. Feeding duration in males and females was compared by T-tests. Relationship between egg-load and number of oviposition bout was analysed by ordinary linear regression (PROC REG). All the analyses were done using SAS (version 9.2, SAS Institute) at $\alpha = 0.05$ level of significance.

Results

Adult feeding and adult longevity of Diaeretiella rapae

When newly-emerged females and males were offered honey, 90% of females started feeding on honey immediately (within 3 min) after they were placed in the glass vials, while only 25% of males fed on honey by that time ($P<0.001$). The average honey feeding duration was significantly longer in females (2.7±0.3 min) than in males (1.2±0.2min) ($P<0.001$). When newly emerged, unmated and unfed males and females were paired in a glass vial and offered honey, 90% of the females fed on honey before mating, compared to only 18% of males feeding on honey before mating. Males often tried to mate with females while females were feeding on honey; the females generally walked away from the feeding site to escape the initial mating attack.

Food provisioning prolonged the life expectancy of both male and female *D. rapae*. Honey-fed males and females lived significantly longer compared to the other groups ($P<0.001$), and honey-fed females lived longer than honey-fed males ($P<0.001$) (Fig. 1). Starved females died sooner than starved males ($P<0.001$). A slight increase in the longevity of water-fed female *D. rapae* was observed compared to starved females. However, longevity of water-fed and starved males did not differ significantly ($P=0.47$) (Fig. 1).

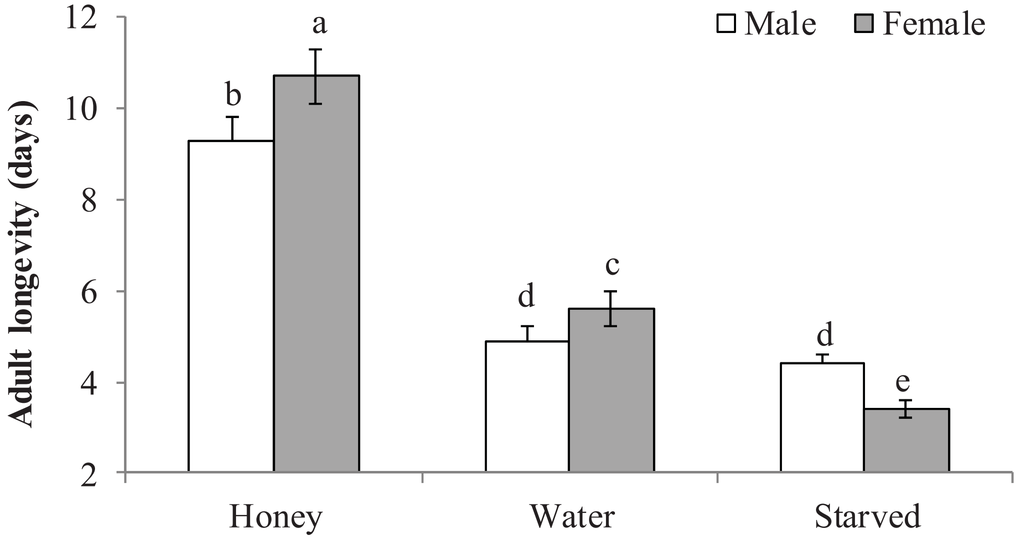

Figure 1: Mean (±SE) longevity of male and female *Diaeretiella rapae* fed with 10% honey, water, or neither. Means with the same letters are not significantly different at α = 0.05 (Tukey's HSD).

Adult feeding and egg load in Diaeretiella rapae

All female *D. rapae* dissected within 4 h of emergence had developed eggs in their ovaries. After emergence, the egg load of females initially increased with age, irrespective of feeding (Fig. 2). Feeding had no significant effect on the egg load of females up to the age of 1-2 days old (P=0.118). Females fed honey or water achieved maximum egg load when they reached age 3-4 day; in contrast, the egg load of starved females was decreasing by that age. The egg load of females in each feeding treatment eventually decreased even without any oviposition (P<0.001), however, the decrease in egg load was slower in females fed honey than (P<0.001).

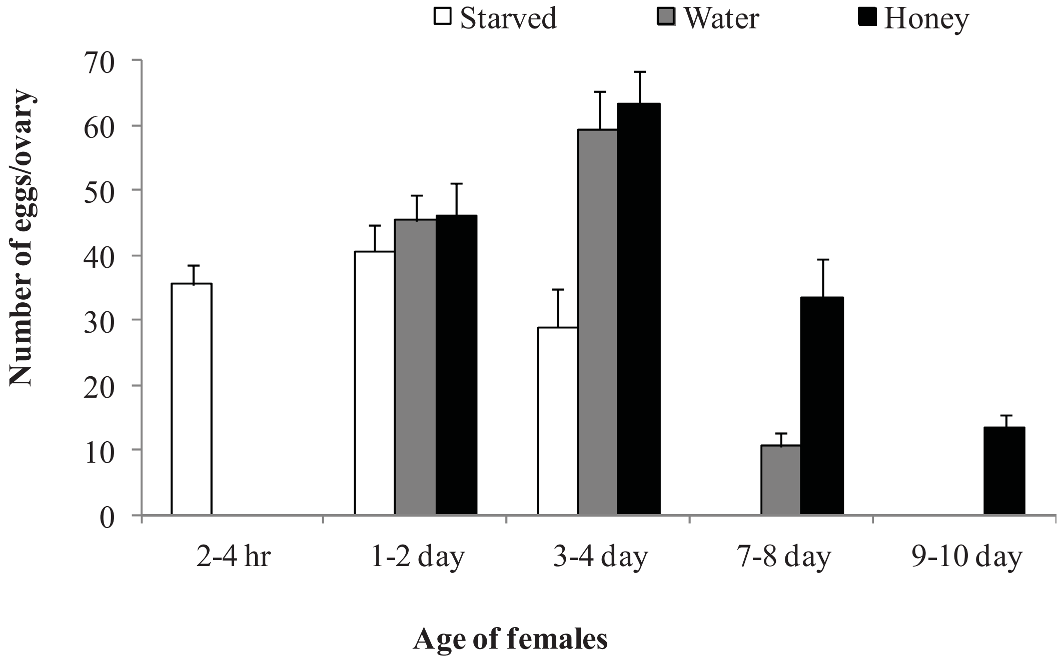

Figure 2: Effects of age on mean (±SE) egg load of female *Diaeretiella rapae* fed with honey, water, or starved.

Adult feeding and oviposition of Diaeretiella rapae

Parasitism in *D. rapae* was affected by food provisioning to the females. Honey-fed females parasitised significantly more aphids (28.1±0.9) than starved females (24.7±0.9) (P=0.03). The number of aphids parasitised by honey-fed and water-fed females did not differ significantly (P=0.9).

The pattern of daily oviposition was also affected by feeding. Honey-fed females produced a similar number of offspring during the three oviposition bouts (P=0.23), but the number of offspring produced by starved and water-fed females decreased after day 1 (P<0.001) (Table 1). The total number of aphids parasitised during three oviposition bouts by honey-fed *D. rapae* females was greater than by females in the other two treatments (P<0.001) (Table 1).

Table 1: Mean (±SE) number of offspring produced during three oviposition bouts by *Diaeretiella rapae* offered different feeding treatments – honey and water (“Honey”), water only (“Water”), or neither. Columns with the same letters are not significantly different (α=0.05).

Feeding treatment	Oviposition bout		
	Day 1	Day 2	Day 3
Honey	10.3±0.7 a	9.3±0.7 a	8.9±0.5 a
Water	11.2±0.5 a	7.6±0.6 b	8.8±0.8 a
No food or water	7.4±0.3 b	10.5±0.9 b	6.9±0.4 b

Number of oviposition bouts on egg load of honey-fed Diaeretiella rapae

The number of eggs remaining in the ovaries of honey-fed females decreased with the number of oviposition bouts (P<0.001). However, there was no significant difference in the mean number of eggs left in the ovaries of females that had oviposited 2 and 3 times, and 3 and 4 times (P=0.38)

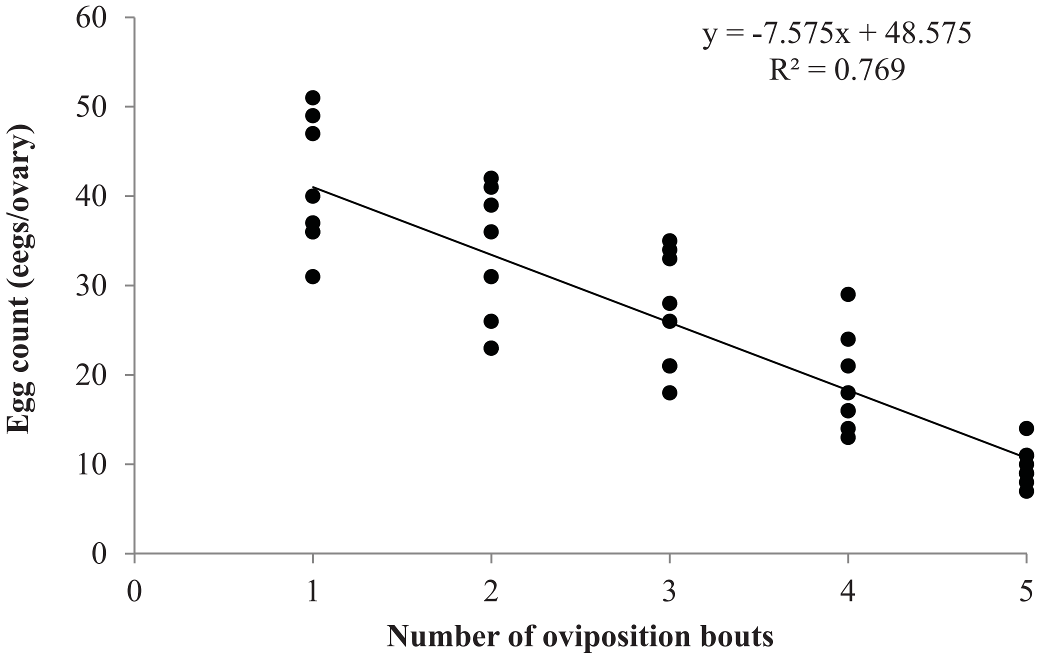

Figure 3: Number of eggs left in the ovaries of honey-fed *Diaeretiella rapae* after a series of ovipositions.

Discussion

Food provisioning has an asymmetric effect on longevity and reproductive potential of male and female *D. rapae*. Urgency to feed after emergence was observed more in females than in males. Female *D. rapae* attained the highest egg load 3-4 days after emergence without any host contact. This indicates that the ovigeny index (Jervis et al. 2001) of *D. rapae* is inclined towards pro-ovigenic status (Bernal & González 1997), otherwise defined as weakly synovigenic (Jervis et al. 2001). This also suggests that, unlike some other parasitoids (Papaj 2000; Wu & Heimpel 2007), *D. rapae* does not require hosts for egg maturation. Pro-ovigenic species feed on external resources (honey solution in this case) during adult life and allocate these resources for somatic maintenance and to promote longevity (Jervis et al. 1993). However, the egg load of honey-fed *D. rapae* decreased after a series of ovipositions, suggesting that even with nutrient supplementation, females were not able to replenish their egg load like synovigenic parasitoids. *Diaeretiella rapae* females were not observed feeding on their hosts during oviposition.

The presence of developed eggs in the ovaries and the production of viable offspring by *D. rapae* immediately after emergence indicate that the resources acquired during larval stage are sufficient not only for growth and development of the immature stages, but also for the initial reproduction in adult *D. rapae*.

When newly emerged males and females were paired in the presence of honey, males showed courtship behaviour immediately, whereas females avoided mating before feeding. Females also fed on honey longer than males. This result indicates that female *D. rapae* need more resources than males after emergence (Godfray 1994). The energy adult females acquire by feeding is used for somatic maintenance, locomotion and future egg production (Rivero & Casas 1999; Visser & Ellers 2008).

Male *D. rapae* lived longer than females in the absence of food. When males and females were offered honey, longevity of both males and females increased significantly, but the effect of honey on longevity of females was more substantial. Increase in longevity was observed in both male and female *D. rapae* after feeding on water compared to those offered nothing. Provisioning of water protected the insects from dehydration. These results indicate that nutrients acquired during adult life (income resources) help in body maintenance and enhance the reproductive fitness of male and especially female *D. rapae*. Insect parasitoids acquire resources during the larval stage, which is supplemented during adult feeding from host or non-host sources (Rivero & Casas 1999; Jervis et al. 2008).

The egg load of 1-2 day old females was higher than the egg load of 2-3 h old females irrespective of feeding treatment. The egg load of 4-5 day old females did not differ significantly whether fed on honey or water. However, egg load was much higher in 7-8 day old honey-fed females than in the water-fed females of similar age. We can speculate that either honey-fed females were able to develop more eggs than water-fed individuals, or water-fed females were nutritionally stressed and resorbed their eggs (Jervis & Kidd 1986; Guo et al. 2011). The egg load in both groups of females was similar on days 4-5. Since there was no significant increase in the egg count between 4-5 day old and 7-8 day old honey-fed females, it is likely that water-fed females had resorbed their eggs.

Nutrients acquired during larval stages, mostly proteins and lipids, are largely used for the production of eggs or sperm (Olson et al. 2000; Rivero & West 2002). Female parasitoids that emerge with all (pro-ovigenic) or most (weakly synovigenic) eggs developed, generally produce relatively yolk-deficient (hydropic) eggs in relatively high numbers (Mayhew & Blackburn 1999). Adults of this type generally lack lipogenesis, and the absence of lipids in the body indicates that ovigenesis cannot occur (Olson et al. 2000; Visser & Ellers 2008). However, if additional carbohydrates can be gained through supplementary feeding, these nutrients can contribute to longevity and physical activity (Strand & Casas 2008), preserving the larval resources for egg production. In contrast, the parasitoids which feed upon their host (generally idiobionts) acquire lipids and are, therefore, able to produce more eggs during the adult stage.

Results show that adult *D. rapae* emerge with mature spermatozoids and developed eggs, and can produce viable offspring immediately after emergence, but the extra nutrients gained during the adult stage are essential, and are used for body maintenance. Honey clearly provided some additional nutrition that helped increased longevity, and provided resources for somatic maintenance in *D. rapae*. Newly emerged *D. rapae* females have enough resources in their body for initial reproduction and body maintenance. However, they require food later in life, and if they are nutrient limited, they may resorb egg resources (Heimpel et al. 1997). Water on its own increased the longevity of females, which indicates that water helps in metabolism of reserved (capital) resources in *D. rapae*. Therefore, it is important that females and males have access to food during adult stages for increasing their efficiency in biological control programmes. Supplemental feeding of wasps in glass houses, or food provisioning in the field by growing nectar-producing plants (Landis et al. 2000) could increase the longevity, egg production and biocontrol efficiency of this parasitoid.

Acknowledgements

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Chapter Seven

Mating or ovipositing? A crucial decision in the life history of cabbage aphid parasitoid *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae)

Abstract

Reproductive fitness of a parasitoid depends on its mating and oviposition success. Virgin haplodiploid females can reproduce but produce only males, and females might lose fitness by producing more male offspring than required. Therefore, females must make a decision whether to mate or oviposit first. This study was conducted to assess mating versus oviposition decision, and its impact on reproductive fitness of *Diaeretiella rapae,* an endoparasitoid of the cabbage aphid, *Brevicoryne brassicae*. When the newly emerged females were given a choice of mating or oviposition, about 62% of *D. rapae* females preferred to mate before oviposition. Those females who oviposited before mating, parasitised only 10% of the available aphids. After mating, females superparasitised their hosts with fertilised eggs, which resulted in a highly female-biased sex ratio of the offspring. Mating success rate was very high (91%) in the presence of the host (cabbage aphid nymphs) compared to when the aphids were absent. However, mating success was not influenced by the quality (size) of the hosts present in the mating arena, despite parasitoid preference for larger hosts during oviposition. The time between pairing and mating was also shorter in the presence of host aphids. The mean number of aphids parasitised, and parasitism rate were significantly greater after mating.

Keyword: reproductive fitness, mating, oviposition, sex ratio, superparasitism

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This chapter examined the preference for mating or oviposition and its consequences on the progeny production and sex ratio in *Diaeretiella rapae*. Female *D. rapae* preferred to mate before oviposition which allowed female to produce female-biased offspring.

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Introduction

In sexual reproduction, females must mate to produce viable offspring. However, in haplodiploid Hymenoptera, where males are haploid and females are diploid, virgin females can produce viable male offspring, but need to mate and fertilize their eggs to produce female offspring (Godfray, 1994). After mating, sperm transferred by the male is stored in the spermatheca; subsequently, throughout their life females fertilise their eggs facultatively with the stored sperm, adjusting the sex ratio of their offspring in response to the prevailing conditions (Charnov, 1982; Fauvergue et al., 2008). Therefore, the sex ratio and ultimately the reproductive success of parasitic wasps are directly linked to the mating status of ovipositing females.

When a female parasitoid emerges from its host, it has a choice of searching for a host and immediately producing sons, or searching for a mate and producing both male and female offspring later. Mate searching may differ in gregarious and solitary parasitoids. In gregarious parasitoids where a number of males and females emerge from one host, they may immediately mate with one another (Greeff, 1996; King & Copland, 1969). However, solitary parasitoids may actively search for mates (Hardy et al., 2005). For example, virgin females of aphid parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) attract males for mating while foraging, with males being attracted to host volatiles (Fauvergue et al., 2008).

The decision by a virgin female to mate or oviposit first can affect her fitness. If the female lays eggs before mating, she could end up producing more males than are required for inseminating all available females (Ode et al., 1998). Females that emerge with full or partial complement of developed eggs (pro-ovigenic or weakly synovigenic, respectively) are generally egg-limited and might therefore be cautious in choosing to oviposit before mating, as they run the risk of reducing overall fitness by depleting their egg supply producing too many male offspring (Jervis & Ferns, 2004). However, females that can produce eggs throughout their life span (synovigenic parasitoids) are not constrained in this way. If they oviposit before mating, they might be able to compensate for pre-mating oviposition (production of only males) by increased oviposition after mating (production of female and males). Thus, they are capable of manipulating the overall sex ratio of their offspring by producing more fertilised eggs after mating later in their life.

Diaeretiella rapae (M'Intosh) (Hymenoptera: Aphidiidae) is the only parasitoid of the cabbage aphid (*Brevicoryne brassicae*) although it also parasitises several other aphids of cruciferous and non-cruciferous plants (Pike et al., 1999). It is a solitary endoparasitoid where a single adult emerges per host, irrespectively of number of eggs laid in a host (Bernal & Gonzalez, 1997; Kant et al., 2011). Previous studies of *D. rapae* have shown that males of this species mate several times while females mate only once (R Kant, submitted). In species where this is the case, the production of additional females is considered as a fitness increment (Godfray, 1994). Multiple mating in males and single mating in females alters the operational sex ratio of a population (the proportion of males and females ready to mate at a given time - (Emlen & Oring, 1977) to male-biased, despite the numerically female-biased population sex ratio. Females are weakly synovigenic (Bernal & Gonzalez, 1997), and can be considered egg-limited (R. Kant, submitted).

Male *D. rapae* emerge before the females, and mate anytime during daylight but not in the dark (Kant & Sandanayaka, 2009). Our research on mating and oviposition of *D. rapae* in an open environment found a high proportion of male-male mating attempts in a female-biased population (Kant et al., 2012b), suggesting that despite the high proportion of females in a population, the number of mating males at any time might exceed the number of females available for mating. When males compete for mating with fewer females, it interferes with the male-female mating success (Kant et al., 2012b). Lack of, or even a delay in mating could force virgin females to oviposit unfertilised eggs, exacerbating overproduction of males, and further affect the gender imbalance in the subsequent generation.

The present study investigated the mating and oviposition preferences of *D. rapae* under laboratory conditions. After emergence, the females have a choice of mating or ovipositing, and have to respond to the environmental conditions and related trade-offs. We hypothesise that the weakly-synovigenic state of *D. rapae* females (Bernal & Gonzalez, 1997) would result in a preference for mating before oviposition. The specific questions of this research were: (1) Do females show preference for mating or for oviposition in the presence of males and of aphid hosts? (2) How does the mating/oviposition sequence affect fitness gain in *D. rapae*? (3) Does the presence or absence of hosts affect the mating success rate in *D. rapae* females?

Material and methods

Insect culture and preparation

Cabbage aphid and its parasitoid *D. rapae* were collected from cabbage plants in Palmerston North, New Zealand. Aphids were reared on live cabbage plants (cv. Summer Globe Hybrid) grown in pots in cages and the parasitoids used in this research were reared on 5-7 day old cabbage aphid nymphs in a controlled laboratory environment at 20 ± 2 °C, 60-70 % RH and 16 h photoperiod. Parasitised aphid mummies were removed from the plants and kept individually in 2 ml microfuge tubes until adult emergence. The emerged *D. rapae* adults were sexed based upon the presence or absence of ovipositor under a stereomicroscope (Olympus SZX12) and offered 10 % honey solution for feeding. All bioassays were conducted under the similar environmental conditions during the daylight hours.

Experimental set-up

Two experiments were conducted to investigate the effects of host quality on mating vs. oviposition choice. In the first experiment, individual females (n=15) were offered larger (6-7 day old) aphid nymphs, while in the second experiment the females (n=14) were offered smaller (1-2 day old) aphid nymphs. In both experiments, a 1-day old, honey-fed virgin female *D. rapae* was introduced into a closed Petri dish containing ten cabbage aphid nymphs and a 1-day old honey-fed virgin male *D. rapae*. The number of aphid nymphs parasitised by the females was recorded and it was noted whether female parasitoids were standing or grooming (rubbing their legs) during their foraging. The pairs that copulated within 30 min after pairing were considered successful; the unsuccessful pairs were discarded from the experiment. Earlier results showed that females most females make mating decision within 30 min of pairing. The time between pairing and mating (pre-mating time) was recorded for all successful matings. The females were further observed, and the time between mating and first probing (pre-oviposition time) was recorded. The females were allowed to continue oviposition until 60 min after the start of the experiment (i.e. since beginning of male-female pairing), irrespective of how quickly they mated.

The number of aphid nymphs parasitised between the first host-encounter after parasitoid pairing and the start of mating was recorded as pre-mating oviposition. Similarly, the number of aphids parasitised after mating (post-mating oviposition) was recorded between the first host-encounter after mating and the end of the experiment (60 min since pairing). After the experiments, aphids were transferred to a cabbage seedling leaf for feeding and development. Subsequently, all mummified aphids were transferred to individual 2 ml microfuge tubes until the emergence of parasitoids. The number of emerged adults was recorded, and their sex determined under the stereomicroscope.

In order to compare the effects of presence and absence of hosts on mating success, a separate experiment was conducted where a male and a female (n=37) were paired in the absence of host aphid nymphs for 30 min, but in all other respects the configuration of the experiment was as above. The pairs that copulated within 30 min were considered successful. Pre-mating time was recorded for each successful pair.

Statistical analyses

Logistic regression was used to analyse the mating success in *D. rapae* adults in the presence and absence of aphid host. We compared pre-mating time in absence and presence of hosts and parasitism in large and small hosts using analysis of variance. Paired t-tests were used to compare the parasitism rate before and after mating. To understand the relationship between the length of the oviposition period and the number of aphids parasitised during pre-mating and post-mating oviposition, we used regression analyses. The sex ratio of the number of offspring resulting from each experimental exposure to parasitoids was compared using Chi-square test. All statistical analyses were performed using the software SAS v9.2.

Results

Mating in Diaeretiella rapae in the presence and absence of host aphids

More *D. rapae* mating pairs were successful when paired in the presence of host cabbage aphids than in the absence of hosts ($P < 0.001$) (Fig. 1). There was no significant difference in mating success of *D. rapae* adults paired in the presence of small or large hosts ($P = 0.58$). Therefore, data on mating success in the presence of large and small hosts were pooled for comparing the mating success in the presence and absence of aphid hosts (Fig. 1).

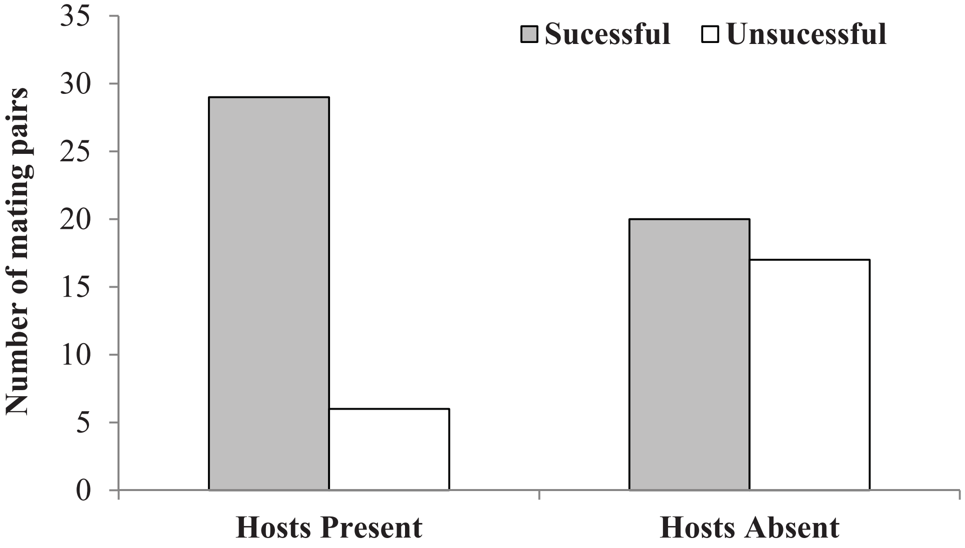

Figure 1: Number of male and female *Diaeretiella rapae* pairs which successfully copulated within 30 min of pairing in presence and absence of the host cabbage aphid nymphs (*Brevicoryne brassicae*).

Mating in *D. rapae* occurred throughout the allocated 30 min period. The pre-mating time was significantly less (6.9 ± 1.2 min) for the pairs which mated in the presence of host aphids, compared to those that mated in the absence of aphids (11.9 ± 1.5 min) (P=0.01) (Fig. 2). The minimum time between pairing and mating was observed to be 1 min in a pair that mated in the presence of hosts. How quickly the pairs mated was independent of the size of the aphid host (P=0.78) (Fig. 2).

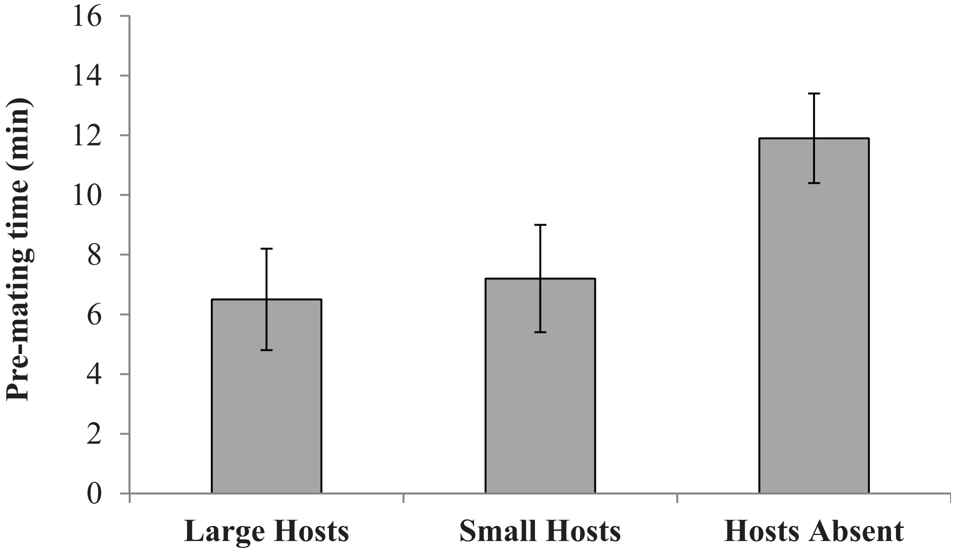

Figure 2: Mean (± SE) pre-mating time (duration between pairing and copulation) of mated pairs of *Diaeretiella rapae* in the presence of large, small or no cabbage aphid nymphs (*Brevicoryne brassicae*).

Mating choice and oviposition before and after mating

When female *D. rapae* were given a choice of mating or ovipositing, 62% of them chose to mate first, hence most parasitism occurred after mating. The mean number of hosts parasitised before mating was significantly lower than the number of hosts parasitised after mating (P<0.0001) (Fig. 3). The female *D. rapae* that oviposited before mating, parasitised only 10% of the aphids present in the arena during pre-mating oviposition. The number of aphids parasitised before mating was not affected by the size of the aphids offered (P=0.73) (Fig. 3).

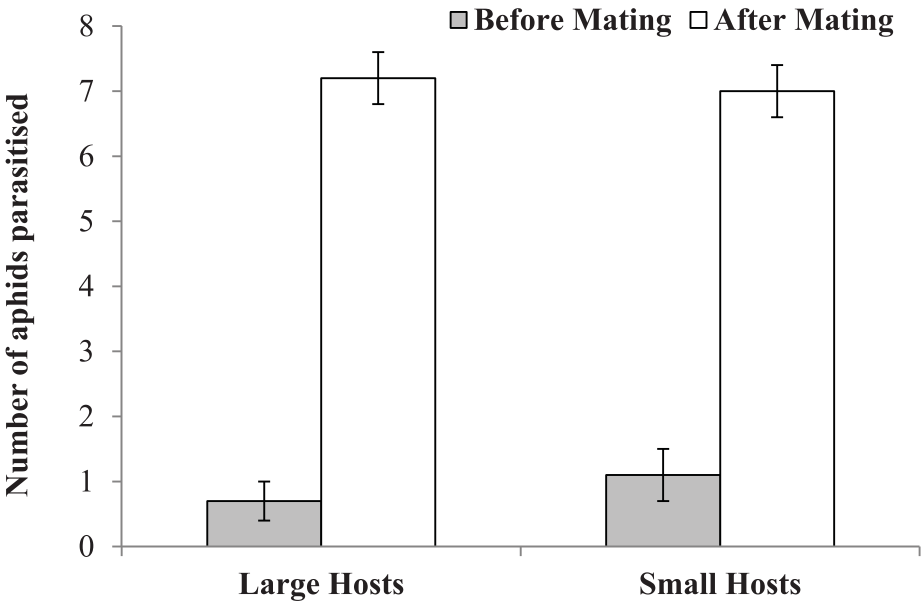

Figure 3: Mean (± SE) number of cabbage aphid nymphs (*Brevicoryne brassicae*) parasitised per *Diaeretiella rapae* female before and after mating when provided with small and large host individuals.

Time between mating and oviposition

The female *D. rapae* did not parasitise aphids immediately after mating. Instead, after mating, the females spent time grooming before starting to oviposit. The mean (± SE) time between mating and oviposition (pre-oviposition time) was significantly longer (24.4 ± 1.8 min) than the mean time between pairing and mating (6.9 ± 1.2 min) (P<0.001). However, there was no correlation between the pre-mating time and the pre-oviposition time (Pearson correlation= -0.12, P=0.50).

Parasitism rate

The mean number of aphids parasitised per unit time before mating (0.12 ± 0.04 hosts/min) was significantly less than the number of aphids parasitised per unit time after mating (0.33 ± 0.03 hosts/min) ($P<0.001$). The relationship between oviposition period and the number of aphids parasitised was positive and linear during pre-mating oviposition (number of aphids = 0.1219x - 0.0436, where x=length of pre-mating oviposition period), while positive and curvilinear during post-mating oviposition (number of aphids = $-0.0043x^2 + 0.3493x + 1.1877$, where x=length of post-mating oviposition period) (Fig. 4). The virgin females were found standing and grooming for longer period of time in their pre-mating oviposition, than did mated females during post-mating oviposition.

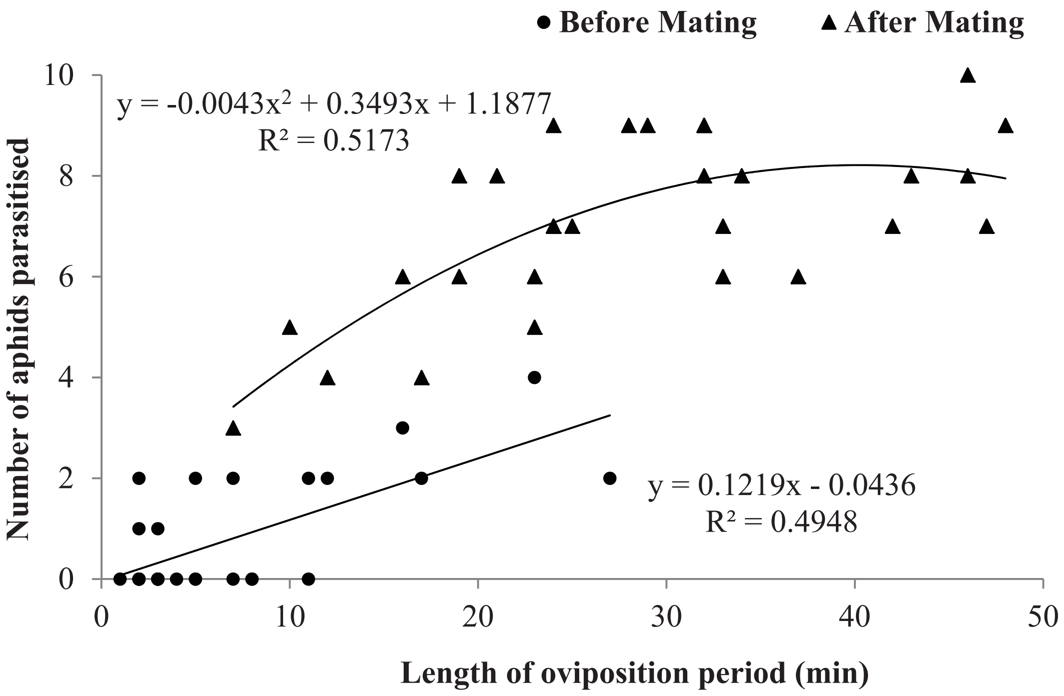

Figure 4: The relationship between oviposition duration and the number of cabbage aphid nymphs parasitised by *Diaeretiella rapae* during pre-mating and post-mating oviposition. Length of oviposition period for "pre-mating" is the time between the first host encounter after pairing and the mating; for "post-mating" it is the time between the first host encounter after mating and the end of the experiment (60 min since pairing).

Adult emergence and sex ratio

The number of aphids from which adult *D. rapae* emerged was greater when oviposition occurred both before and after mating (9.0 ± 0.3), compared to when oviposition occurred only after mating (7.3 ± 0.5) ($P<0.01$).

More than half of the females that oviposited before mating subsequently superparasitised the host with fertilised eggs after mating, causing a highly female-biased sex ratio among emerged offspring. The mean number of females emerged from parasitism of 10 cabbage aphids was significantly greater than the number of males ($P<0.001$) (Fig. 5). There was no significant difference in emergence of adult parasitoids from small and large host aphids ($P>0.45$), but there was a significant interaction between host size and the sex of emerging offspring, such that a greater proportion of females emerged from large aphids ($P<0.004$).

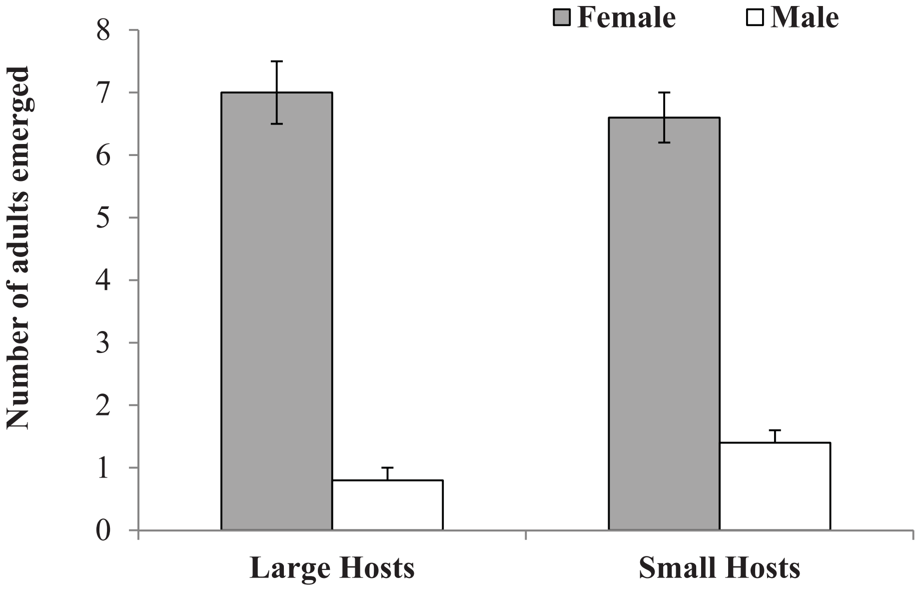

Figure 5: Mean (± SE) number of male and female *Diaeretiella rapae* offspring that emerged from large and small size cabbage aphid nymphs (*Brevicoryne brassicae*).

Discussion

Mating and oviposition are the two components of reproduction in insects. Female *D. rapae* emerge after males (Kant & Sandanayaka 2009) so they generally have a choice between mating and oviposition. When a choice between aphids for oviposition and a male for mating was available to a virgin *D. rapae* female, mating was preferred before oviposition. Those females that did not mate, probed (ovipositor insertion into host) few aphids before mating. However, it is known that *D. rapae* and some other parasitoids sometime probe their hosts not only for oviposition but also to assess its quality as well as temporarily paralyse the host appendages to reduce the host defence (Phillips 2002; Kant et al. 2008; Kant et al. 2011).

In the presented experiment, females were 10 times more likely to encounter an aphid than they were to encounter the male parasitoid, because one and 10 aphids were present in the arena; this is assuming that the male was not actively searching for the female. Despite the high probability of encountering an aphid, females probed very few hosts. Given a choice of different size hosts, mated *D. rapae* females preferred to attack and allocate fertilised eggs to larger hosts (Kant et al. 2008; Kant et al. 2012a). By mating first, females gain the option of choosing the sex of their offspring, and of allocating fertilised eggs to higher quality hosts for producing female offspring (Godfray 1994a; Bernal & González 1997).

The mating success of *D. rapae* was significantly greater in the presence of aphids than in their absence. This suggests that mating and oviposition are interdependent in *D. rapae*. The presence and ready access to ovipositional resources, such as hosts, is known to induce sexual receptivity and mating in some insects (Ringo 1996; Carsten & Papaj 2005; Harano et al. 2006). The longer pre-mating time in the absence of aphid hosts suggests that female *D. rapae* choose when they are receptive to males**.** Females rejected males more often when mating occurred in the absence of aphid hosts. Sexual receptivity in female wasps is usually induced only a limited number of times, and the opportunity for further mating tends to be low (Hardy et al. 2005). Presence of a host also increases the male efficiency in locating virgin females (Metzger et al. 2010). Our results suggest that in *D. rapae*, a female's decision to mate first when she has access to both a host and a mate might help in optimising her reproductive fitness. However, it is possible that females do not choose between mating and ovipositing, but rather between ovipositing and not ovipositing depending on whether or not they have mated.

The sequence of mating and oviposition could be further influenced by the egg-load of females, which could also affect the sex ratio of the parasitoid population. Species in which females emerge with developed eggs, including *D. rapae,* are generally egg-limited (Bernal & González 1997); therefore, it is advantageous for females to mate before ovipositing. When given the choice to mate or to oviposit, about a third of females in this study chose to oviposit first. However, these females attacked aphids at a lower rate during pre-mating oviposition than after mating, and parasitised only 10% of hosts offered to them before mating. More males in a population increases competition among female's sons to find mates (Ode et al. 1998), which caused mating interference in *D. rapae* (Kant et al. 2012b). The increase in parasitism up to 90% after mating suggests the adaptive nature of oviposition in *D. rapae*, and that mating preferentially precedes oviposition in *D. rapae*.

Female *D. rapae* did not parasitise aphids immediately after mating but spent time grooming before starting oviposition. The time lag between mating and oviposition decreased the oviposition time available to the females in our experiment. The time gap might be due to physiological process necessary for females to store the sperm in their spermatheca and to fertilise eggs. The length of this period between mating and oviposition has been shown to vary substantially between insect species (Hardy et al. 2005). Our previous studies suggested that females were not able to fertilise their eggs immediately after mating (R. Kant, unpublished data), therefore, by delaying oviposition after mating, females might be able to produce more female offspring.

The number of *D. rapae* adults that emerged was slightly greater when females oviposited both before and after mating. The sex ratio of the *D. rapae* adults emerged from parasitised hosts was highly female-biased, irrespectively of whether oviposition occurred only after mating or both before and after mating. The proportion of females that emerged from larger and smaller hosts was similar, which is contrary to our previous findings where females preferred to allocate fertilised eggs to larger hosts in a host-size choice test (Kant et al. 2011a). This also suggests that female parasitoids adjust their oviposition and vary their sex allocation strategies in host choice and no-choice conditions (Godfray 1994a; Murray et al. 2010).

Another reason for the finding of uniform sex allocation in small and large hosts in this study could be the low density of aphids promoting parasitoids to deposit more fertilised eggs in hosts regardless of their quality.

Parasitoids regulate clutch size and progeny sex ratio to maximize overall fitness for a given host density (Godfray 1994a; Yu et al. 2003). In our experiments, the females that oviposited their hosts before mating, later superparasitised some of their hosts with fertilised eggs to produce female offspring. Superparasitism in *D. rapae* could be considered an adaptive strategy of females to maximise their fitness (and balance the sex-ratio) in low density host population (such as in our experiment), where *D. rapae* females have the opportunity to attack the same host again (Kant et al. 2011).

This research suggests that mating and oviposition choices are not mutually exclusive, and can complement each other for maximising reproductive fitness. A trade-off exists between mating and oviposition, and the choice made by females probably depends on the immediate availability and density of hosts for oviposition, or males for mating. In addition, the sequence of the two activities plays an important role in fitness gain, and eventually in population dynamics. Mating first enables the females to utilise the host resources more efficiently to produce female-biased sex ratio in their offspring. Efficiency of parasitoids can be increased by releasing mated females during augmentative field/glasshouse release. As the increase in proportion of female is a primary requirement for *D. rapae* to suppress the host population (Kant et al. 2011b), releasing mated females would probably enhance the proportion of female in the next generation, and offer better suppression of aphids in biological control programme.

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Chapter Eight

Effect of mating and oviposition delay on parasitism and sex allocation behaviour of *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

Abstract

Mating and oviposition complement each other in parasitoid reproduction; delay in either of these activities has disparate consequences for the fitness of the parasitoid. Effect of mating and oviposition delay on parasitism rate and progeny sex ratio was examined in a parasitic wasp *Diaeretiella rapae*. Egg dynamics in the ovaries of females of different ages, and females which completed different number of oviposition bouts were also examined. Delay in mating and oviposition adversely affected reproductive performance of both male and female *D. rapae*. Delayed oviposition resulted in reduced parasitism by both mated and unmated *D. rapae* females. The progeny sex ratio was female-biased, but the proportion of female offspring decreased towards the end of *D. rapae* life. Although females emerged with developed eggs, the egg load in the ovaries of unmated and naïve females initially increased then continuously decreased with age, suggesting reabsorption of eggs towards the end of a female life. Oviposition rate of unmated females was low before mating. The length of mating delay experienced by females affected their offspring sex ratio, causing overproduction of males. Delayed mating did not affect the courting capacity of males, but reduced the number of their offspring and proportion of female offspring. The result of this study suggests the use of 1-2 days old *D. rapae* for best results in biological control programmes.

Keywords: oviposition delay, mating delay, egg count, sex ratio

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This chapter investigates the effects of mating and oviposition delay on progeny production by *Diaeretiella rapae*.

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Introduction

Mating and oviposition are two essential activities in sexually-reproducing insects. In parasitoids, reproductive efficiency is directly linked to their ability to suppress the pest population. For example, in solitary parasitoids where females oviposit one egg per host, fecundity is generally equivalent to the expected number of hosts killed by the parasitoid unless females superparasitise, laying more than one egg per host. Although the two activities complement each other in reproduction of parasitoids, their timing and sequence can affect reproductive fitness (Damiens et al., 2003; Fauvergue et al., 2008; Kant et al., 2012c). Oviposition or mating could be delayed when hosts or mates, respectively, are limited.

When female parasitoids emerge, they have a choice between oviposition and mating (Godfray, 1994; Kant et al., 2012c). Females can oviposit before or without mating, but pre-mating oviposition in arrhenotokous parasitoids will result in haploid male progeny and could imbalance the sex ratio of the population in the next generation (King, 1962). Mating is required for producing fertilised eggs which result in female offspring (Godfray, 1994). Mating is, therefore, an essential component of parasitoid fitness in terms of the ability of parasitoids to carry on their generation, and for maintaining the sex ratio of the population. High sex ratio (female/male) is always desirable for effective biological control of insect pests, because females are directly responsible for reducing the pest population.

The number of eggs a female parasitoid carries is an indicator of its reproductive output (Godfray, 1994). In pro-ovigenic parasitoid species, females emerge with all their potential eggs ready to be oviposited, while in synovigenic parasitoids females emerge with immature eggs and can produce new eggs throughout their adult life (Flanders, 1950). Females of these two types of parasitoids may face disparate consequences of oviposition delay (Jervis et al., 2001) – synovigenic females can regulate their egg load by reabsorbing and replenishing eggs depending upon the availability of hosts, but if a pro-ovigenic female reabsorbs her eggs, the eggs may not be replenished. Parasitoids are time-limited and also egg-limited if they are pro-ovigenic or weakly synovigenic. When females emerge with mature eggs, even a short delay in oviposition could reduce their reproductive potential. Females also reabsorb their eggs in the absence of hosts (Guo et al., 2011; Richard and Casas, 2009), and this reabsorption could adversely affect their reproductive potential.

The current study examined the effects of mating and oviposition delay on reproductive fitness of *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Aphidiidae). *Diaeretiella rapae* is a solitary endoparasitoid of more than 60 aphid species (Homoptera: Aphididae) infesting cruciferous and non-cruciferous plants (Pike et al., 1999) and it is the only recorded parasitoid of the cabbage aphid *Brevicoryne brassicae* (George, 1957). Cabbage aphid is an important agricultural pest, causing severe damage to crops in New Zealand and other countries (Hughes, 1963; Lammerin and Morice, 1970).

Female *D. rapae* live longer than males, and their longevity depends on their body size (Kant et al., 2012a) and food. The status of ovigeny index of *D. rapae* is not clear; Bernal et al. (1997) suggested *D. rapae* as a pro-ovigenic species as female emerge with abundant developed eggs and they reproduce during first half of their life. However, another study suggests *D. rapae* a synovigenic species (Tylansik et al 2004). Male *D. rapae* emerge earlier than females (Kant and Sandanayaka, 2009), which increases the probability of females being mated quickly upon emergence. Female *D. rapae* prefer to oviposit after mating (Kant et al., 2012c). However, even if the females are mated upon emergence, they may not find suitable hosts for parasitism. Therefore, understanding the impact of mating and/or oviposition delay under deprived conditions on the efficiency of *D. rapae* can be useful during mass production and release of parasitoids in biological control programmes. This study sought to (1) assess the effects of oviposition delay on parasitism in mated and unmated female *D. rapae*; (2) examine the effect of age on egg-count in *D. rapae*, and (3) investigate the effect of mating delay in male and female *D. rapae* on the progeny sex ratio.

Materials and methods

Insect cultures

Colonies of *D. rapae* and cabbage aphid were established in the laboratory from insects originally collected in the field in Palmerston North, Manawatu, New Zealand. Colonies were maintained in a controlled environment at 20±2°C, 65±10% RH and 16 h photoperiod. The same environmental conditions were used for mating and oviposition experiments. The cabbage aphid nymphs of preferred age and size (5-6 day old) were used in the experiments. The parasitoids used in the experiments were fed with 10% honey solution after emergence.

Oviposition delay in unmated females

Newly emerged, honey-fed, unmated female *D. rapae* were exposed to 60 cabbage aphid nymphs per day on a cabbage seedling for three consecutive days. The parasitised aphids were allowed to feed and develop on the seedling. Once the aphids were mummified, they were transferred to individual 2 ml microfuge tubes. The mummies were observed daily for adult emergence. To understand the effect of oviposition delay on parasitism, similar oviposition experiments were performed with three age groups of unmated females (1, 3 and 6 days old) not exposed to aphids since their emergence. The number of offspring (all of which were males) emerged from each group of females was compared with the number of offspring produced by newly emerged females. Each female group was replicated 10 times.

In a separate test, the egg-load of newly emerged (2-4 h old) *D. rapae* females and females of age 1-2, 3-4, 7-8 and 9-10 days, not exposed to hosts, was examined under a stereomicroscope (Olympus SZX12). Ovaries were excised from the abdomen of each female on a glass slide in saline solution, and a cover slip was placed gently on the ovaries. Pressure on the cover slip made the ovaries burst so that the eggs could be counted. In each group 10-15 females were dissected.

Oviposition delay in mated females

Newly emerged female *D. rapae* were each paired with a 1-d old virgin male and their mating was observed. Each mated female (n=10) was offered 60 cabbage aphid nymphs per day on a cabbage seedling for 3 consecutive days. The same experimental protocol (from parasitism until adult emergence) was followed as in the unmated females experiment. The parasitism and progeny sex ratio of three female age groups (1, 3 and 6 days old) not exposed to aphids were compared with those of newly emerged females. Each female group was replicated 10 times.

Mating delay in male D. rapae

To check whether newly emerged male *D. rapae* are able to mate and transfer sperm or not during mating, males less than 1 h old were individually paired with 1-day old, honey-fed, virgin females. Females that did not mate within 30 min were discarded. The mated females were provided with honey for 6 h before being offered 15 aphids per female for parasitism. The number and the sex of the offspring emerged from parasitised aphids were recorded. Mating delay was tested in 10 different males.

Similarly, the parasitism and sex ratio of offspring produced by the females mated with the three age group males (1-2 days, 4-5 days and 7-8 days old), not exposed to females since their emergence were investigated. Individual virgin males of each group were offered two honey-fed, 1-day old, virgin females for 24 h. Each of the mated female was then offered 15 unparasitised aphids per day for three consecutive days. The number of female offspring produced by the females mated with males of each age group was compared. The number of female offspring produced was used as an indirect estimate of sperm transfer (Godfray 1994a; Henter 2004a). More than ten males were tested in each male-age group treatment.

Mating delay in female D. rapae

Effects of different periods of mating delay– 1-2 days, 4-5 days and 7-8 days were tested on parasitism and sex ratio of the female’s offspring. All females were honey-fed and offered 15 aphids for parasitism from the first day of emergence. When the females reached the desired mating age for the experiment, they were each paired with 1-day old, honey-fed, virgin male for 24 hours. After mating, each female was again offered 15 aphids per day for three consecutive days, irrespective of the mating delay period. This means that in 1-2 day delay treatment, the females mated prior to oviposition, while the group of females which were not exposed to males for the first four days of their emergence already oviposited for 3 days prior to mating; similarly, the group of females which were not exposed to males for the first

7-8 days of their emergence, oviposited for 6-7 days prior to mating The total number of offspring and the proportion of female produced by each group of females were compared, replicated 9-10 times.

Statistical analysis

A goodness-of-fit test was used to assess the distribution of the data. The number of offspring produced by mated and unmated females in oviposition and mating delay experiments, and the number of eggs found in the ovaries of females of different age were analysed using analysis of variance (ANOVA). The means of different treatments were compared using a Tukey's studentised range (HSD) test. Logistic regression with binary response variable was used to analyse the offspring sex ratio in different treatments. The data were analysed using SAS Enterprise Guide 4.2 at $\alpha=0.05$ level of significance

Results

Oviposition delay in unmated females

The number of offspring produced by female *D. rapae* was negatively affected by the duration of oviposition delay (number of offspring produced = 41.03 - 4.49x, where x=delay in number of days, $R^2 = 0.48$, $P< 0.001$). Unmated females that were not allowed to oviposit for 6 days, produced less than a third of the total number of offspring they would have produced if their oviposition started soon after emergence ($F_{3,\ 36} = 11.31$, $P<0.001$) (Fig. 1). There was no significant reduction in the number of offspring produced by females whose oviposition was delayed for a single day.

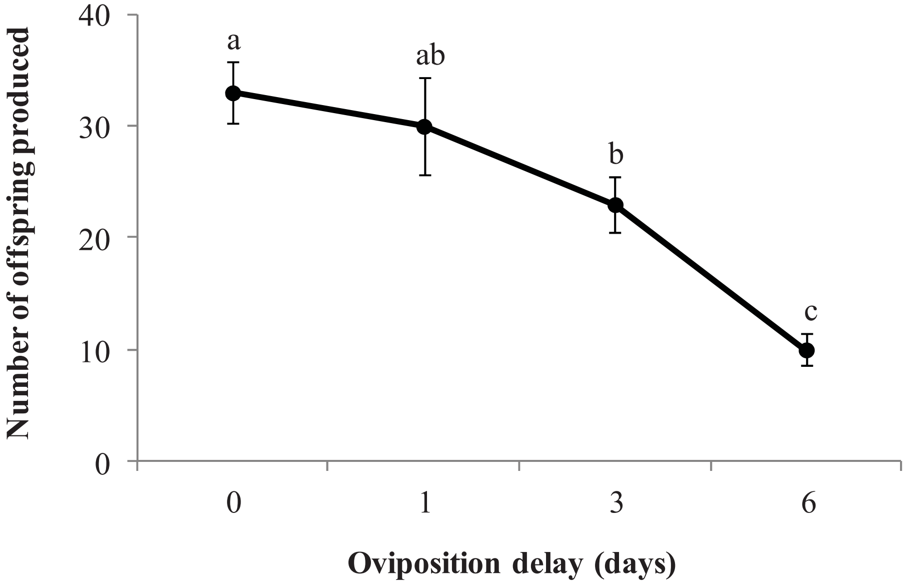

Figure 1: Total number of offspring (mean±SE) produced by unmated *Diaeretiella rapae* females which were not allowed to oviposit for different periods since their emergence. Means with the same letter are not significantly different at α=0.05 (Tukey's HSD).

The egg count in the ovaries of unmated and naïve female *D. rapae* changed with age (Fig. 2). Although females emerged with developed eggs, their egg count initially increased with age, reaching a maximum 3-4 days after emergence, before decreasing ($F_{4, 63} = 62.23$, $P<0.001$) (Fig. 2).

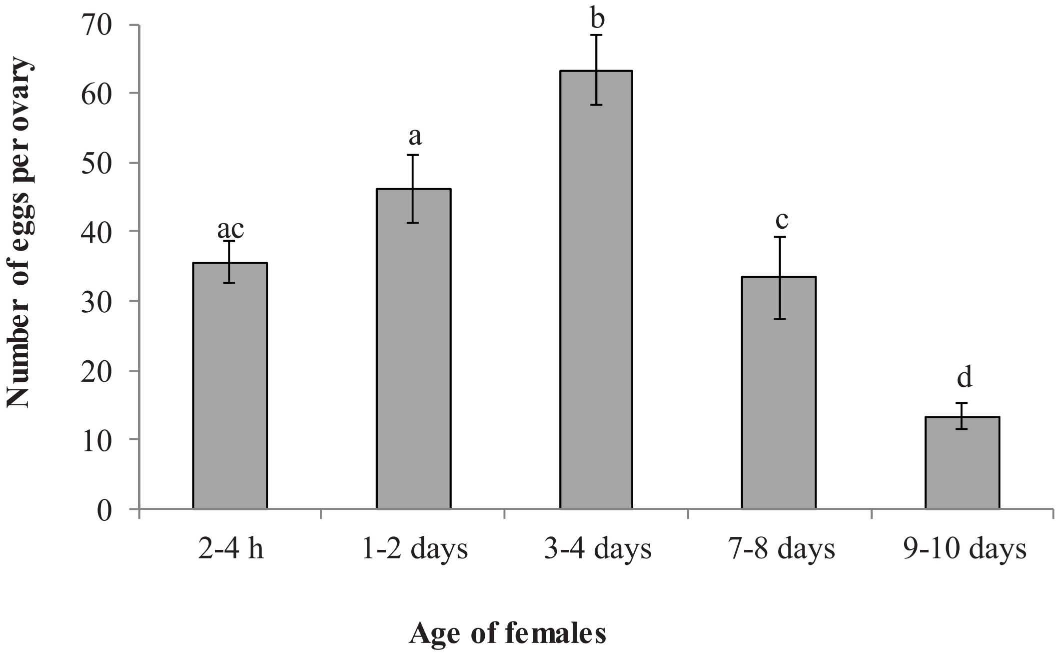

Figure 2: Mean (±SE) number of mature eggs found in the ovaries of unmated naïve female *Diaeretiella rapae* of different age groups. Means with the same letter are not significantly different at α=0.05 (Tukey's HSD).

Oviposition delay in mated females

Mated female *D. rapae* that were refrained from oviposition for the longest period (6 days) produced the least number of offspring ($F_{3,\ 36} = 30.99$, $P<0.001$) (Table 1). Mated females whose oviposition was delayed for 6 days also produced the least number of female offspring (7.0 ± 0.63) compared to other mated females ($F_{3,\ 72} = 20.83$, $P<0.001$) (Table 1). The progeny sex ratio (female-to-male) declined significantly when *D. rapae* oviposition was delayed for 6 days, but not until 3 days delay (Logistic regression: $\chi^2 = 4.71$, $P<0.05$) (Table 1).

Table 1: Mean (±SE) and sex ratio of offspring and their sex ratios produced by mated female *Diaeretiella rapae* which were not allowed to oviposit (oviposition delay) for three different periods since their emergence. Means with the same letters within a column are not significantly different at α=0.05 (Tukey's HSD).

Oviposition delay (days)	Mean number of offspring	Progeny sex ratio (female: male)
nil	41.1±3.17 a	2.6 ab
1	49.2±3.20 b	3.9 b
3	35.2±2.59 a	2.3 a
6	14.4±1.31 c	1.3 c

Mating delay in males and females

All females that were mated with newly emerged males produced female offspring. This confirms that male *D. rapae* are able to successfully mate and transfer viable sperm immediately upon emergence. Delay in mating did not affect the courtship capacity of males. When 7-8 day old males were paired with females, they displayed courtship immediately after pairing in a way similar to the newly emerged males, and 80% of them mated with both females. However, the duration of copulation was longer in males whose mating was delayed for 7-8 days (72.1 ± 1.8 s) compared to newly emerged males (58.8 ± 1.9 s) ($F_{1,40} = 24.89$; $P<0.001$).

Mating delay adversely affected the reproductive potential of male *D. rapae*. Females mated with older males (7-8 days old) produced significantly fewer offspring than females mated with younger males ($F_{2,31} = 4.31$; P=0.022) (Fig. 3). The mean number of female offspring produced by females was also affected by the age of the males they mated ($F_{2,31} =$ 7.99; P=0.002) (Fig 3). However, the number of male offspring produced by females was not significantly affected by the age of their mating partner ($F_{2,31} =$ 1.14; P=0.33) (Fig 3).

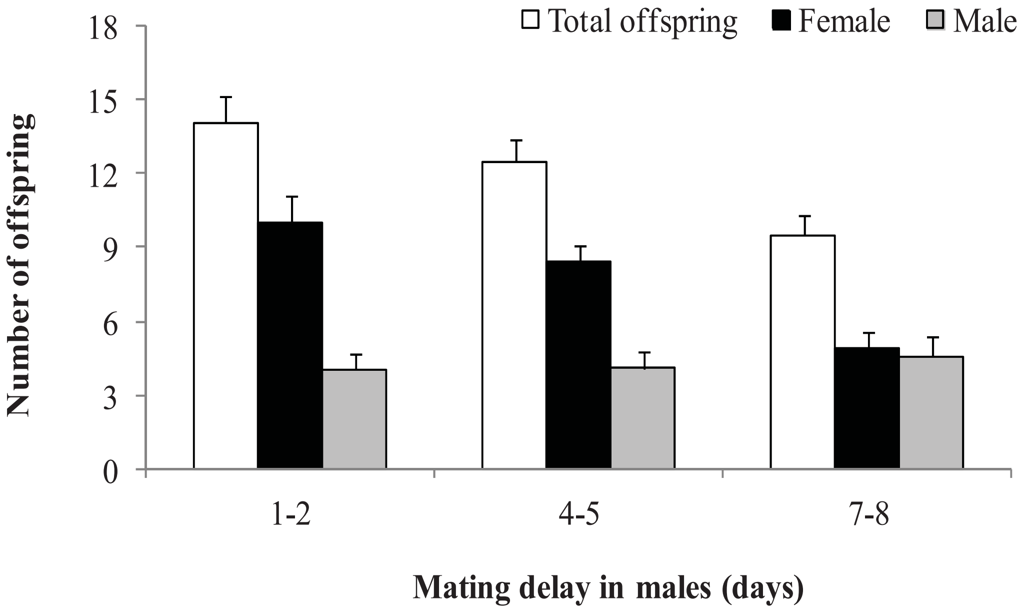

Figure 3: Mean (±SE) number of male and female offspring produced by female *Diaeretiella rapae* mated with males whose mating was delayed for different period since their emergence. Means with the same letter are not significantly different at α=0.05 (Tukey's HSD). Capital letters compare the means of total offspring produced, while small letters compare the means of male and female offspring.

The reproductive capacity of female *D. rapae* was negatively affected when their mating was delayed. The sex ratio (female-to-male) of their offspring decreased significantly when their mating was delayed ($F_{2, 27} = 24.89$; $P<0.001$) (Fig. 4).

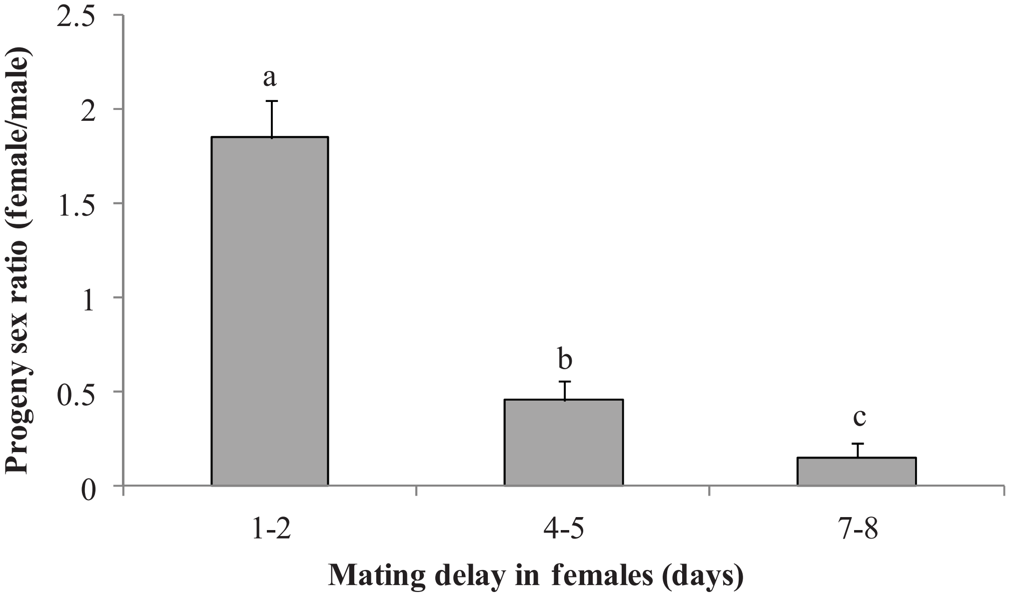

Figure 4: Progeny sex ratio of female *Diaeretiella rapae* subjected to different periods of mating delay since their emergence. Means with the same letter are not significantly different at α=0.05 (Tukey's HSD).

Discussion

Delay in mating and oviposition affected the reproductive fitness of *Diaeretiella rapae*. The number of offspring produced by female *D. rapae* decreased when their mating was delayed. Older male *D. rapae* transferred less or lower quality sperm, which was reflected by the decrease in the resulting female offspring. Female *D. rapae* produced viable male offspring through ovipositing unfertilised eggs immediately after emergence. However, the number of offspring produced by female *D. rapae* prior to mating was less than the number of offspring produced after mating. Pre-mating oviposition can cause overproduction of males in the following generation, so the reduced oviposition rate by unmated female *D. rapae* could be a strategy to minimise the overproduction of males. Results also revealed that the number of subsequent ovipositions was also reduced when the females were deprived of hosts for a period of time, and the number of ovipositions decreased with increasing length of delay, irrespectively of the mating status of the female.

Although females emerged with developed eggs, their egg load increased during their early life, suggesting that *D. rapae* is a weakly-synovigenic species (Jervis et al., 2001). The reduced oviposition and decreasing egg count in females whose oviposition was delayed suggests either deterioration or resorption of some eggs by the females towards the end of their life. Resorption of eggs occurs in various parasitoid species, and females usually have full control over the resorption process (Guo et al., 2011; Richard and Casas, 2009). The number of eggs in the ovaries of females whose oviposition was delayed for 1 or 2 days remained same, suggesting that the egg resorption occurs at a late stage of female life that could be due to nutritive stress at old age (Bell and Bohm, 1975; Rosenheim et al., 2000). Resorption of eggs reduces fecundity and is a last resort that could help the female to live longer (Jervis and Kidd, 1986). The increase in egg count early in the life of *D. rapae* females followed by late decrease, suggests that females can mature eggs but cannot produce new eggs.

Sex ratios of the progeny of mated *D. rapae* females were female-biased irrespectively of the length of oviposition delay. However, the number of females and the progeny sex ratio decreased with increasing oviposition delay. Since female *D. rapae* mate once in their life time while males mate multiple times (Kant et al. unpublished), the operational sex ratio of *D. rapae* becomes male-biased even when numerically more females than males are present in the population (Kant et al. 2012b). In ideal conditions (without any delay) a female produces more female progeny in order to have only enough males to ensure that all her daughters will be mated (Ode et al. 1998). This reduces competition among her sons in finding mates, and increases the number of mates for each of her sons (Frank 1998; Reece et al. 2004). Therefore, reduction in the number of female offspring as a result of oviposition delay causes decrease in the relative fitness of *D. rapae*. The decrease in the proportion and the number of female offspring in older mothers suggests that with increasing age, female *D. rapae* may lose their capacity to fertilise their eggs, and this might be due to deterioration in quality of sperm stored in the spermatheca of females (Reinhardt & Meister 2000). The decrease in the egg count with age of female *D. rapae* would lead to decrease in total number of fertilised eggs produced by those females compared to young females. A similar result was found in the pupal parasitoid, *Nasonia vitripennis*, which produced more males when oviposition was delayed for a longer period of time (King 1962). The progeny sex ratio of the egg parasitoid *Trichogramma brassicae* did not change when its oviposition was delayed for a small proportion up to 25% of life time (Fleury & Bouletreau 1993). Similarly, the results of this study also indicate that the short delay in oviposition (10-20% of their total life) does not affect the overall reproductive capacity of female *D. rapae*.

All females mated with newly emerged males produced female offspring, indicating that male *D. rapae* can successfully mate and transfer viable sperm immediately after emergence. Males were active during late mating and successfully mated with the females despite a delay. Females mated with males whose mating was delayed, also produced fewer and lower proportions of female offspring, suggesting that reproductive potential of male *D. rapae* deteriorates with age. The capacity of males to transfer sperm during mating decreases with their age (Bissoondath & Wiklund 1996), and the decline in female offspring indicates a decline in sperm transfer in males (Henter 2004). The longer copulation duration reported from male *D. rapae* whose mating was delayed further supports the inference of decreased efficiency of the sperm transfer.

Mating delay in females affected their reproductive capacity. Although the average daily oviposition was low in *D. rapae* before mating, a longer delay in mating could result in oviposition of unfertilised eggs. Thus, longer delay in mating resulted into production of more males in the next generation. Despite the production of a highly female-biased offspring from the post-mating oviposition, this did not compensate for the overproduction of males during pre-mating oviposition, so the overall sex ratio (female/male) remained low.

The results of this research indicate that female *D. rapae* produce fewer offspring and fewer females when oviposition or mating is delayed for a relatively long period. The decrease in number of total offspring suggests reabsorption of eggs in *D. rapae*. The decrease in the proportion of female offspring produced by mothers whose oviposition was delayed suggests sperm stored in the spermatheca might deteriorate by the end of female's life. However, one day delay in oviposition increased the number of offspring and proportion of females in *D. rapae*. Thus, in biocontrol programmes, knowing the age group of the parasitoids being released in glasshouses or in the field is important to increase the aphid suppression by *D. rapae*. This research suggests the use of 1-2 days old adult *D. rapae* for best results in biological control programmes.

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Chapter Nine

Effect of host age on searching and oviposition behaviour of *Diaeretiella rapae* McIntosh (Hymenoptera: Aphidiidae)

Abstract

Diaeretiella rapae is an important biological control agent of cabbage aphid. Host searching, handling and oviposition behaviour were investigated in relation to host age. The parasitoid spent 61% of her foraging time on searching for hosts. Host handling time of the parasitoid decreased with increased number of host encounters. The females were more successful in finding older hosts (7 days old) and spent more time (94.9 ± 20.5 s/encounter) and did more stings (9.9 ± 1.4/encounter) on them than on the younger hosts. They preferred to sting the abdomen over the thorax, head and legs of the host. The average number of eggs laid per host was highest (1.4 ± 0.2 eggs) in 7-day-old hosts. When attacking 7-day-old hosts, they gained 42% success in ovipositing the host compared to 10, 18 and 30% success in 1-, 3- and 5-day-old hosts, respectively. Results suggest that *D. rapae* may have adaptive preference for larger hosts and mass production could be more efficient by using 7-day-old aphids.

Keywords: *Diaeretiella rapae*, *Brevicoryne brassicae*, host age, foraging, oviposition, searching, encounter.

This chapter investigates host-searching and oviposition behaviour of *Diaeretiella rapae*. Female *D. rapae* attacked all body parts of aphids including head, thorax and abdomen but oviposited in the rear part of the aphid's abdomen.

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http://www.nzpps.org/journal/61/nzpp_613550.pdf

Introduction

Cabbage aphid, *Brevicoryne brassicae* (L) (Homoptera: Aphididae), is a cosmopolitan pest of cruciferous crops (Hughes 1963c). *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Aphidiidae) is the most common natural enemy of the cabbage aphid (George 1957a) and can also be used for controlling other aphids like *Myzus persicae* (Sulzer) and *Diurpahis noxia* (Mordvilko). It is solitary species, i.e. lay one or more than one eggs in a host but only one develops into an adult (Godfray 1994a), and females emerge with fully developed eggs (Flanders 1950c). *D. rapae* allows its hosts to feed and develop after becoming parasitised. Foraging behaviour, including searching, handling and oviposition, varies both among and within species (Lewis et al. 1990). Parasitoids may use chemical cues (semiochemicals) and/or physical features like host size to select hosts for oviposition (van Driesche & Bellows 1996).

Once a parasitoid encounters a host, the amount of time it spends on the host depends on host quality (Godfray 1994a). Host age or body size may reflect the quality of the hosts for parasitoid development (Charnov et al. 1981a; He & Wang 2006). Although in some parasitoids, host age or body size has no effect on oviposition decisions (Mackauer 1973; Phillips 2002), many parasitoids prefer a particular host stage for oviposition (Vinson & Iwantsch 1980). Larger hosts contain more resources and may give rise to large offspring (Bennett & Hoffmann 1998) and are often preferred by parasitoids for oviposition (Charnov 1982a; Harvey et al. 2004). Host size can also affect a parasitoid's attack rate (Charnov 1982a; Islam & Copland 1997) and its host handling time (Drost et al. 2000).

Time is considered as a limited resource for parasitoids so host handling time influences the efficiency of parasitoids (Hudak et al. 2003). Understanding the foraging behaviour of parasitoids is important for the success of biological control programmes (Lewis et al. 1990), especially for optimising methods for mass production of biological control agents. Some studies on foraging behaviour of *D. rapae* have been done in relation to host plants (Gentry & Barbosa 2006) and pesticides (Umoru et al. 1996), foraging behaviour of *D. rapae* in relation to host age or size has not been studied in detail. To help develop efficient biological control programmes for cabbage aphid and other hosts, host searching and oviposition behaviour of *D. rapae* was studied in relation to host age.

Materials and methods

Insect colonies

Colonies of *B. brassicae* and its parasitoid *D. rapae* were established from parasitised and unparasitised adults of *B. brassicae* collected from a commercial cauliflower field near Palmerston North. Insects were reared in the laboratory on cabbage seedlings (‘Autumn pride’) in plexiglass cages (30×30×30 cm). Two holes, each (13 cm in diameter) was made on opposite sides of the cage and covered with metal mesh (aperture size of the mesh <0.5 mm) for ventilation. One of the two remaining sides of the cage was fitted with small circular opening (16 cm in diameter) for handling plants and insects in the cage. The colonies were maintained at 22 ± 2°C, 60-70% RH and 16:8 h light:dark and reared for 20 generations before use in the experiments. The sizes of the aphids were measured under a stereomicroscope (Leica MZ12, Germany) using a micrometer with a readability of 0.01 mm. Body length of fifteen aphids of each age class, i.e. 1, 3, 5 and 7 day old, were measured with a mean (± SE) of 0.60 ± 0.01, 1.13 ± 0.02, 1.50 ± 0.02 and 1.92 ± 0.04 mm, respectively (ANOVA: $P<0.001$). The host sizes were positively correlated with their age (analysis of regression: $R^2=0.9$, $F_{1,\,59}=1708.82$, $P<0.0001$).

Searching and oviposition behaviour

The behaviour of *D. rapae* was observed during the light period under a light intensity of 430 Lux supplied by broad-spectrum, high-frequency tube-lights (Osram L36W/72-965). Four aphids, each of a known age (1-, 3-, 5- and 7 days old), were released to a Petri dish (5.5 cm in diameter and 1.3 cm in height). To allow parasitoids to mate, newly emerged male and female parasitoids were put together for 24 h in a glass vial (2.5 cm in diameter and 7.5 cm in height) containing a cotton bud soaked in 10% honey solution. The mated and honey-fed female parasitoid was released in the aphid arena, through a small hole (1 cm in diameter) in the lid of the Petri dish. Before observation, the lid was replaced with an intact lid.

Host searching and oviposition behaviour of female *D. rapae* (n=18) was recorded for 20 min using a video camera (JVC-TK-C1380E, Digital ½ inch CCD) connected to VCR and TV monitor. The behaviour and time budget for host searching and handling were documented manually via TV monitor and stop watch. The time budget of the parasitoid was divided into searching and host handling periods. The documented searching time of the parasitoid included walking, standing, and preening between two host encounters. The host handling time refers to the time period between the first and last stings (inserting ovipositor) on the same aphid. The host handling behaviour of the parasitoid was quantified based on the amount of time the parasitoid spent per host and number of stings done per host. The number of stings on head, thorax, abdomen and legs was also recorded to determine the body part preference by the parasitoids for attack.

After these observations the aphids were transferred to cabbage leaves for feeding and development, and were dissected four days after parasitism to find the number of larvae in the hosts, which was assumed to be equivalent to the number of eggs laid (Bueno et al. 1993). Twenty four parasitoids were tested in this experiment.

Statistical analyses

A goodness-of-fit test was used to test the distribution of the data. The percentage data were arcsin transformed prior to analysis of variance (ANOVA). Analysis of regression (AOR) was used to determine the relationship between the number of hosts a parasitoid encountered in 20 min and time spent per encounter. Data on handling time per encounter, stings per encounter, time taken per sting, body part preference for stings, eggs laid per host and number of stings required per egg laid were not normally distributed and were analysed by non-parametric Kruskal-Wallis test (KWT) followed by multiple comparisons using Bonferroni (Dunn) t test. Searching and oviposition success of parasitoids on hosts of different sizes was estimated by Mann-Whitney U Test (MWT).

Results

Host searching and handling behaviour

The foraging time of *D. rapae* was divided into searching and host handling time. Parasitoids spent 61 ± 3.1% (mean ± SE) of their total foraging time searching for hosts, significantly more than that on handling hosts ($P<0.001$). Host handling times decreased as host encounter rates increased ($P<0.001$) (Fig. 1).

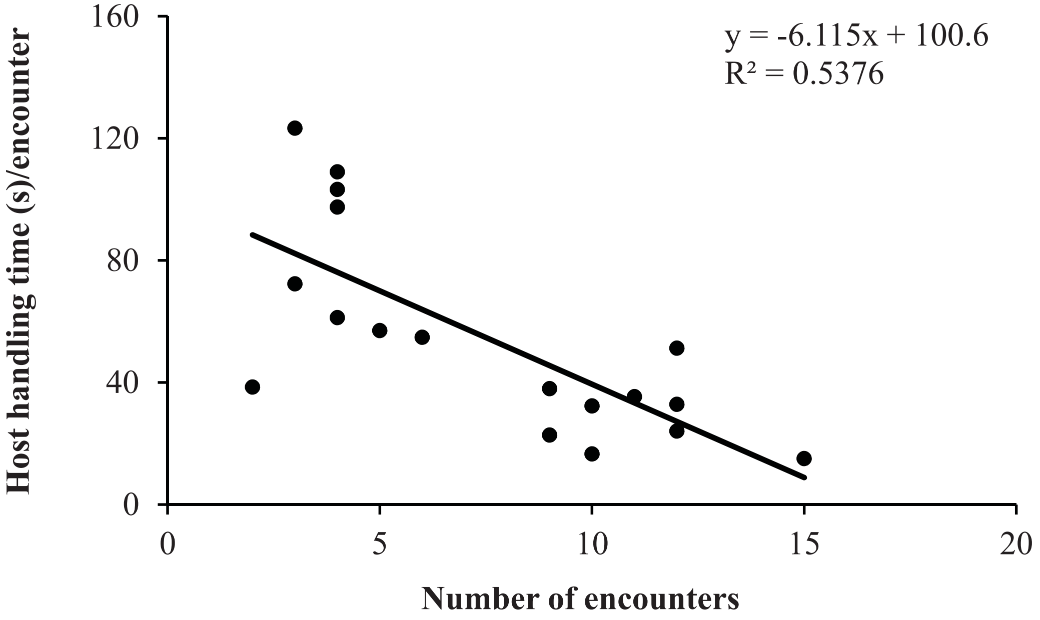

Figure 1: Relationship between the time spent (seconds) on an encounter by *Diaeretiella rapae* and the total number of encounters in 20 min.

During host searching, parasitoids were significantly more likely to encounter older than younger hosts (MWT: U_0=36.76> $\chi^2_{3,0.05}$ =7.82, P<0.0001) (Fig. 2).

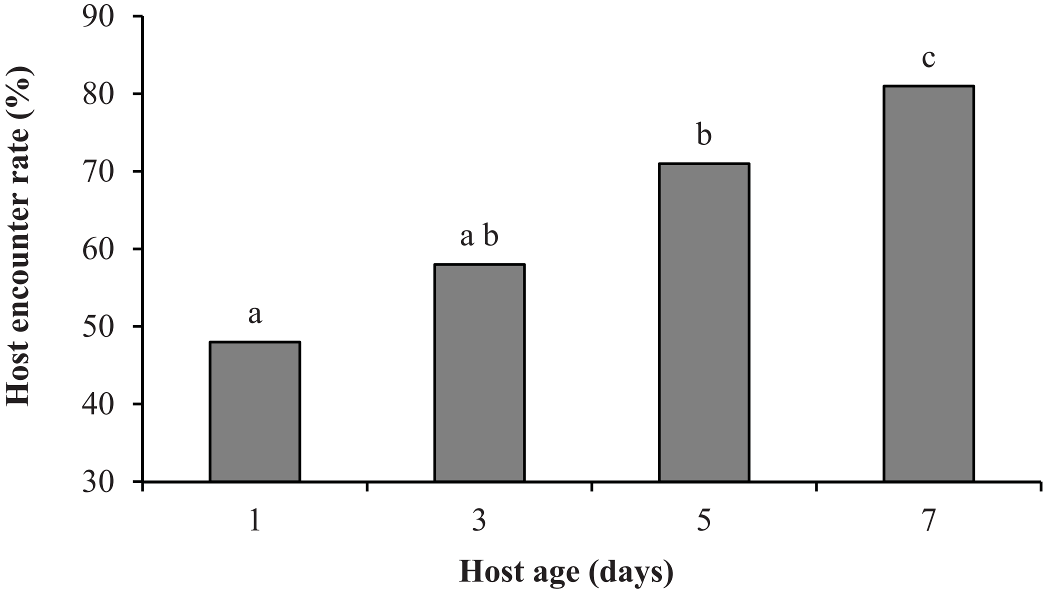

Figure 2: Host encounter rate by *Diaeretiella rapae* in relation to host ages. Columns with the same letters are not significantly different (P>0.05).

Parasitoids spent significantly more time handling older hosts (P<0.0001), and stung them significantly more often than younger ones (P<0.0001) (Table 1). They took significantly longer time to sting 7-day-old hosts than younger ones (P<0.0001) (Table 1). Older hosts appeared to attempt to defend themselves from parasitism by shaking, swinging their antennae and kicking, and these behaviours appeared to disturb parasitoids. With younger hosts, however, these behaviours were not as effective and did not disturb the parasitoid. Parasitoids also appeared to use more power to penetrate older hosts with their ovipositors.

Table 1: Time spent and number of stings by *Diaeretiella rapae* per host and time taken per sting in hosts of different ages.

Host age (days)	Mean time (s) per encounter	Mean number of stings per encounter	Time (s) per sting
1	15.1 ± 3.0 a	2.4 ± 0.3 a	5.5 ± 0.6 a
3	21.4 ± 4.6 ab	4.4 ± 0.9 ab	6.0 ± 0.9 ab
5	42.3 ± 7.2 b	6.5 ± 1.0 bc	8.6 ± 0.8 b
7	94.9 ± 20.6 c	9.9 ± 1.4 c	11.5 ± 2.2 c
H (df = 3)	33.96	26.75	28.43

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

Preference for stinging different body positions in hosts

Parasitoids significantly preferred the abdomen over other body parts of the host for stinging (KWT: H=28.66> $\chi^2_{3,0.05}$, P<0.0001) (Fig. 3). Legs were the body position least preferred by the parasitoids.

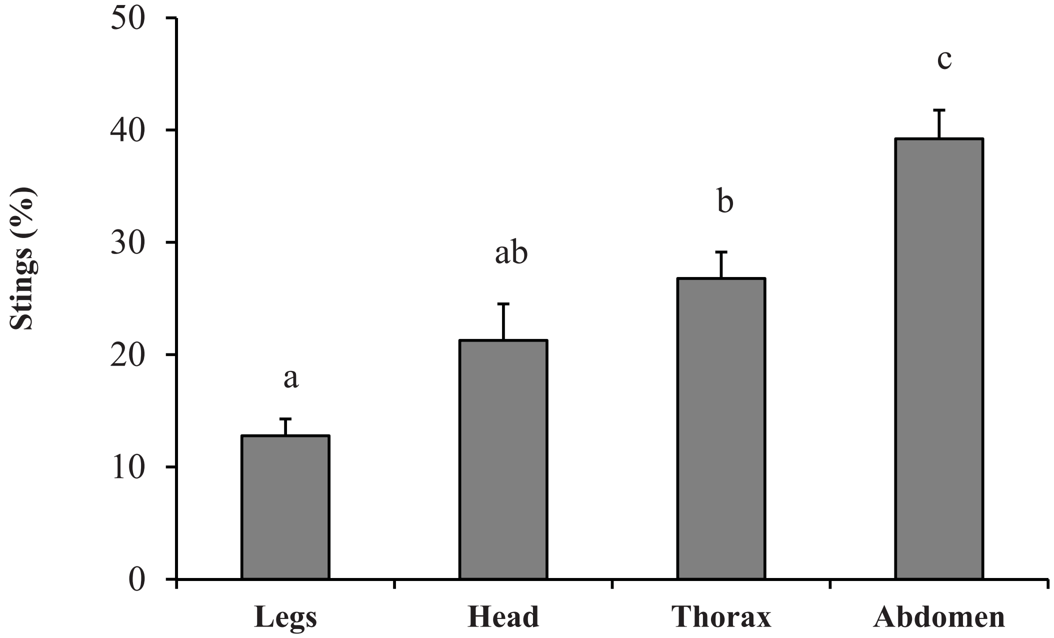

Figure 3: Proportion of stings performed by *Diaeretiella rapae* on different body parts of hosts. Columns with the same letters are not significantly different (P>0.05).

Oviposition success in hosts of different ages

Females laid significantly more eggs in older hosts than in younger ones (P<0.0001). However, the number of stings required by the parasitoid for ovipositing an egg in younger hosts was significantly less when compared to larger ones (P<0.001) (Table 2).

Table 2: Mean number of eggs laid by *Diaeretiella rapae* per encounter and number of stings required to lay an egg in hosts of different ages.

Host age (days)	Parasitoid larvae found/host	Stings required to lay an egg
1	0.3 ± 0.1 a	3.0 ± 0.9 a
3	0.5 ± 0.1 ab	6.5 ± 1.1 ab
5	0.9 ± 0.1 bc	9.8 ± 2.0 b
7	1.4 ± 0.2 c	21.8 ± 5.2 c
H (df=3)	19.57	15.56

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

Females were significantly more likely to lay eggs upon encountering older than younger hosts (MWT: $U_0=10.38 > \chi^2_{3,0.05}=7.82$, $P<0.001$) (Fig. 4).

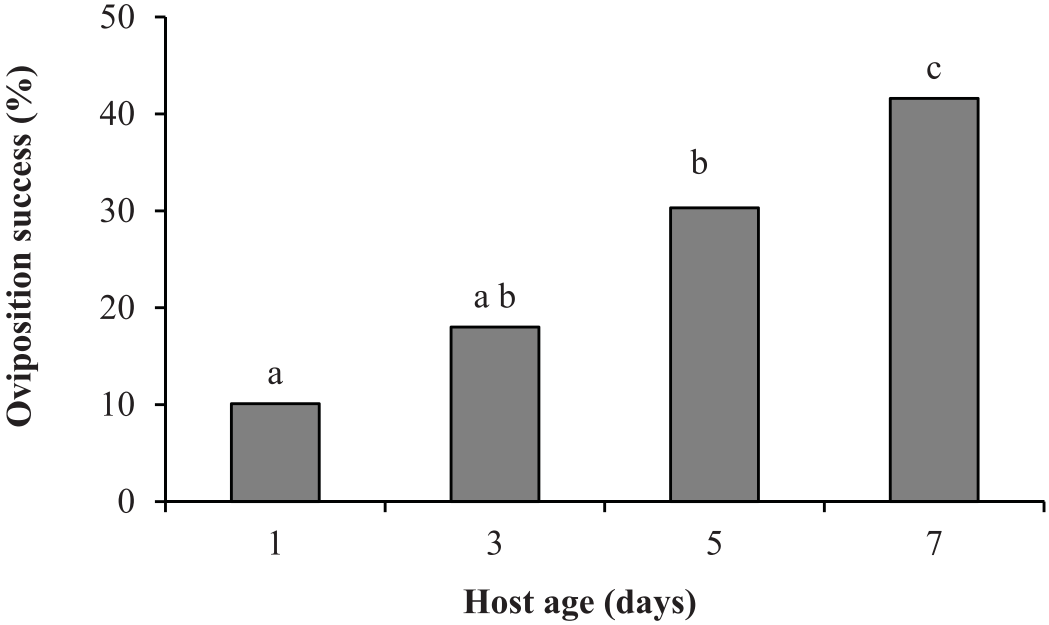

Figure 4: Proportion of *Diaeretiella rapae* laying eggs when encountering hosts of different ages. Columns with the same letters are not significantly different (P>0.05).

Discussion

Most parasitoid species have limited time for foraging (Rosenheim 1999) and they show a great variability in searching and handling times (Vis et al. 2003). Female *D. rapae* spent twice as much time on searching for as on handling hosts. Similar behaviour was observed in *Encarsia Formosa* (Hymenoptera: Aphelinidae) (Sutterlin & van Lenteren 1999) and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) (Hudak et al. 2003). Host handling time of *D. rapae* decreased even more when the female encountered a greater number of hosts. Hudak et al. (2003) have also reported that the host handling time of whitefly parasitoids, *E. mundus* and *Amitus bennetti* (Hymenoptera: Platygasteridae), is affected by the number of hosts encountered.

The chance of finding a host during searching depends upon the size of host (van Roermund et al. 1994). In the present study, *B. brassicae* age was highly correlated with body length, and it was shown that *D. rapae* was more successful in encountering an older host than a younger one. This agrees with the findings of Drost et al. (2000) on parasitoids of *B. argentifolii*. It may be possible that parasitoids can detect larger hosts more easily than smaller ones. Another factor that increases the probability of encountering larger hosts is odours (semiochemicals) emanating from the host body (van Roermund et al. 1994). It is possible that larger hosts release odours in greater quantity.

Parasitoids prefer to oviposit in hosts of specific size to increase the oviposition success (Drost et al. 2000). In the present study, *D. rapae* spent more time and stung more while handling older hosts, which agrees with Godfray's (1994a) suggestion that larger hosts have stronger defence capability. This may be due to frequent kicking and shaking by large hosts. The stronger cuticle of larger hosts probably makes it harder for the parasitoids to penetrate their ovipositors in hosts' bodies, which may eventually result in the longer handling time.

While attacking a host, *D. rapae* preferred to sting the abdomen of the host. A parasitoid of ants, *Elasmosoma* sp. (Hymenoptera: Braconidae), also preferred to sting and oviposit in the abdomen of its host (Poinar 2004). Phillips (2002) also observed the preference of the parasitoid *Microctonus* sp. (Hymenoptera: Braconidae) for the host's abdomen to the head, thorax and legs. Preference for abdomen for sting could be because it is the suitable side for oviposition. Stings on thorax, head and legs could paralyse the hosts, which would reduce their defence capability and facilitate the oviposition process.

Although older hosts show stronger defence, *D. rapae* still prefers them for oviposition. This may be because these hosts are large and thus contain more resources for development (Charnov 1982a) and produce large offspring which increases the fitness of the parasitoids (Bennett & Hoffmann 1998). Female *D. rapae* deposited more than one egg in larger hosts to increase survival and emergence chances of their offspring (Rosenheim & Hongkham 1996). Depositing more than one egg per host by *D. rapae* was previously observed by Lu et al.(Lu et al. 1992). Therefore, when parasitoids encounter hosts of different quality, they adjust their foraging strategy and maximise their reproductive fitness per unit time by attacking larger hosts.

Results of this study indicate that female *D. rapae* has a preference towards older hosts for oviposition with large offspring produced. Older aphids may be provided to *D. rapae* for its mass production in biological control programme of cabbage aphids.

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Chapter Ten

Fitness gain in a koinobiont parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) by parasitising hosts of different ages

Abstract

Host age-dependent fitness of *Diaeretiella rapae* was studied on cabbage aphid *Brevicoryne brassicae*. When given a choice of nymphs of four different ages (1, 3, 5 and 7 days), females showed a strong preference for oviposition in older nymphs, which were also the largest in the body size, and left the majority of younger nymphs unparasitised. More than 70% of offspring emerged from 5-day and 7-day old parasitised nymphs. Parasitoids develop faster in older hosts than younger hosts. Development time of males was shorter than females in older hosts, but the development time of males and females was similar in younger hosts. The body size of adult *D. rapae* was positively correlated with the age of the hosts in which they developed. The sex ratio of offspring was found to be female biased. A higher proportion of females emerged from older than younger hosts. *D. rapae* females emerged from older hosts lived significantly longer. The potential fecundity of the females emerged from older (larger in body size) hosts was significantly greater than those emerged from younger hosts with smaller bodies. Results suggest that *D. rapae* preferred older hosts to maximise its reproductive fitness gain. The oviposition strategy of *D. rapae* is discussed in context of efficiency of this parasitoid in biological control programmes.

Keywords: reproductive fitness, solitary, pro-ovigenic, host age, body size

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This chapter investigated the effect of host age (size) on different fitness parameters like body size, longevity and sex ratio in *Diaeretiella rapae*. Larger hosts brought more fitness gain in *D. rapae*.

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Introduction

Cabbage aphid *Brevicoryne brassicae* (L) (Homoptera: Aphididae) is a worldwide pest of forage, horticultural crops and oil-seed crucifers (Singh & Ellis 1993). It is considered to be the most serious pest of Brassica such as cauliflower, cabbage, broccoli and brussel sprouts, and has a history of destroying entire crops of turnip, rape and swede (*Brassica napus*) in New Zealand (Lammerin & Morice 1970). Direct feeding by the aphid causes yellowing, wilting, distortion and stunting of infested plants. In addition, this aphid transmits more than 20 different plant viruses, including cauliflower mosaic virus (CaMV) and turnip mosaic virus (TuMV) (Broadbent 1957) that result in economically important diseases of crucifers. Cabbage aphid reproduces both sexually and asexually and its population increases very quickly. In New Zealand, the aphid generally reproduces parthenogentically, overwinters in the egg stage and completes more than 15 generations per year.

A number of pesticides are used for control of these aphids. However, these tend to have a short term effect because of the short aphid generation time, and severe pesticide toxicity to their natural enemies (Xu et al. 2009). Moreover, the aphid has developed resistance to several commonly used pesticides (Clark & Yamaguchi 2002). Biological control and Integrated Pest Management (IPM) provide an alternative option for suppressing the aphid populations.

Diaeretiella rapae (M'Intosh) (Hymenoptera: Aphidiidae) is a solitary endoparasitoid of more than 60 different aphid species (Pike et al. 1999). The most common hosts of *D. rapae* in Australia, New Zealand and in other countries are cabbage aphid, green peach aphid *Myzus persicae* (Sulzer) and mustard aphid *Lipaphis erysimi* (Kaltenbach) (Carver & Stary 1974) and it is as a potential candidate for suppressing cabbage aphid populations (George 1957). In the USA, *D. rapae* has been used in classical and augmentative biological control of Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Bernal & Gonzalez 1993). The parasitoid was deliberately introduced in Australia for controlling cabbage aphid (Carver & Stary 1974). *Diaeretiella rapae* is of Western Palaearctic origin and has been accidently introduced, and established in New Zealand (Carver & Stary 1974) but little attention has been given to it as a potential biological control agent of aphid pests.

When parasitoids locate their hosts, they make a series of oviposition decisions in order to maximise their fitness gain (Godfray 1994), which include the number of eggs to lay, ratio of fertilised and unfertilised eggs and whether to superparasitise the host or not. The decisions made by a parasitoid are affected by the quality of the hosts they encounter and host quality generally depends on its age and size. As in many Hymenopteran species, females develop from fertilised eggs and males develop from unfertilised eggs (haplodiploidy) in *D. rapae.* A foraging mother thus controls the sex of her offspring and can produce sons before mating. Because sex ratio directly affects the population dynamics of the species, decisions made on sex allocation are crucial, have strong fitness outcomes and therefore, have evolutionary implications. In the case of pro-ovigenic species (such as *D. rapae*), which have a limited number of eggs, the oviposition decision is perhaps more crucial than in synovigenic species in which females can produce eggs throughout life.

Oviposition decisions of parasitoids vary as some species are attracted to larger hosts (Charnov et al. 1981; Waage 1982; Godfray 1994), some to smaller or intermediate hosts, or are indiscriminate of host body size (Jones 1982; Hare & Luck 1991; Bernal et al. 1998). The parasitoid should attack the best quality hosts and avoid those in which successful larval development is doubtful. *D rapae* is a koinobiont parasitoid (Zhang & Hassan 2003) where host paralysis is transient and sometimes absent, allowing hosts to continue development after parasitism. Thus, the resources available for the developing koinobiont parasitoid are not fixed. Previous studies indicate that female *D. rapae* select older (larger) cabbage aphid nymphs for oviposition (Kant et al. 2008) but the reasons behind that behaviour has not been tested.

The purpose of the present study is to examine the effects of *D. rapae* oviposition preferences by comparing the fitness parameters of adults emerged from four different age groups of cabbage aphid nymphs. The results of this study will be useful in developing biological control strategies for cabbage aphid using *D. rapae*, to overcome the low population of *D. rapae* in the early spring in New Zealand (Kant et al. 2010).

Materials and methods

Parasitised and unparasitised cabbage aphids were collected from a commercial cauliflower field in Palmerston North, New Zealand. The colonies of the host and the parasitoid were established and maintained in laboratory conditions at 22±2°C, 60-70% RH and 16 h (L) photoperiod. The aphids were reared on cabbage seedlings (cultivar "Autumn Pride") in ventilated plexiglass cages (30×30×30 cm). Parasitised aphid mummies were removed from plants and were kept individually in 2ml microcentrifuge tubes until adult emergence. The emerged *D. rapae* were sexed under a stereomicroscope (Leica MZ12), paired for mating in a glass vial and fed 10% honey solution.

In the first experiment, mated females (n=15) were given a choice of different age 1, 3, 5 and 7-day cabbage aphid nymphs. The nymphs of these ages were selected as representatives of different sizes. Age of the cabbage aphid nymphs was positively correlated to size of the nymph. Fifty adult aphids were transferred to a fresh plant and allowed to produce nymphs for 24 h before adults were removed. The nymphs were allowed to develop until they reached the required age for the experiments. Ten aphids each of age 1 day-old and 5 day-old were transferred to a cabbage seedling and ten nymphs each of age 3 day-old and 7 day-old aphids were transferred to another cabbage seedling. The aphids were allowed to settle on the plants and both plants were placed in an oviposition arena, a similar cage as described above. In each case the older nymphs were readily distinguished from the younger ones. A mated female was released into the oviposition arena for 24 h, then transferred to a new oviposition arena and released into another oviposition arena with the same configuration of aphids, again for 24 h. This process was repeated until the death of the female.

After parasitism, each age group of aphid nymphs was allowed to develop on individual cabbage seedlings in order to distinguish different age groups. The more easily handled older nymphs were transferred to separate cabbage seedlings, leaving the younger nymphs. Once the parasitised aphids were mummified, they were transferred to individual 2 ml microfuge tubes and kept until adult emergence. The emerged *D. rapae* were sexed and their body length was measured. To measure the body length, the insects were dissected, and head, thorax and abdomen were placed on a glass slide in saline solution. The length of each tagma was measured as maximal length in dorsal aspect using calibrated eyepiece micrometer. The measurements were then added together. The potential fecundity of the newly emerged females from nymphs of different age groups was estimated by dissecting their ovaries and counting the number of eggs. A subset of adults was fed with 10% honey solution and their longevity was observed.

In the second set of experiments the 1, 3, 5 and 7-day old aphids were dissected 4 days after being parasitised and the growth of the developing parasitoid larvae was estimated by measuring their head width. Those dissected aphid samples contain more than one parasitoid larva were not used for measuring the larval size.

Statistical analyses

Data on immature development time, adult body size, adult longevity and egg load of *D. rapae* were normally distributed (goodness-of-fit test) and were analysed using analysis of variance (ANOVA). The means were separated using a Tukey's studentised range (HSD) test. The percentage data of adult emergence from hosts of different ages were arcsine transformed prior to ANOVA. Regression analysis was used to identify the relationship between host age and the size of parasitoid larvae, and between host age and the size of adult parasitoids. The sex ratio of the offspring were analysed by Chi square test. All analyses were carried out at $\alpha=0.05$ level of significance.

Results

Oviposition by Diaeretiella rapae female in hosts of different ages

D. rapae emerged from all parasitised cabbage aphid nymphs regardless of their age group. A greater number of 7-day old cabbage aphid nymphs were parasitised by *D. rapae* compared to the younger nymphs. About 70% of all aphid mummies collected resulted from parasitism of the older hosts (5 and 7 days old) ($F_{3, 56} = 9.33$, $P < 0.001$). The number of mummified aphids collected from 1-day and 3-day hosts did not differ significantly (Fig. 1).

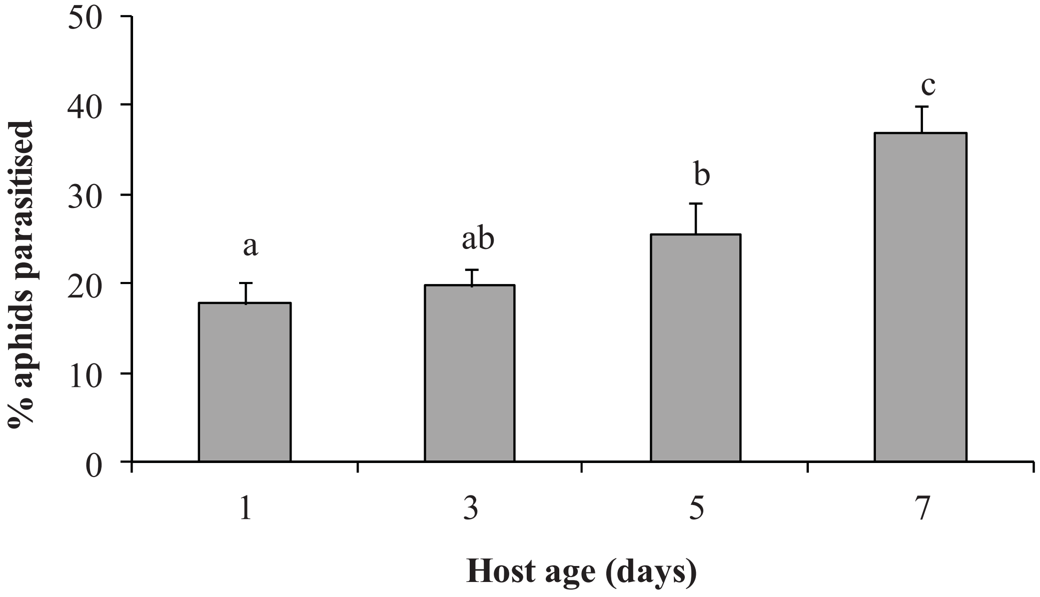

Figure 1: Percentage of cabbage aphids parasitised by *Diaeretiella rapae* when offered nymphs of different ages in a choice test. Means with the same letters are not significantly different (Tukey's HSD P<0.05), error bars represent the standard error.

Immature development time in different hosts

Development time, from egg to adult, of male and female parasitoids varied with the age of the host in which they developed. In younger hosts, females took significantly longer to develop than males ($F_{7,\ 186}$ =17.94, P<0.001). However, in older hosts the development time of males and females was not different (Fig. 2). Females developed significantly quicker in older hosts, while males took about the same time to develop into adults regardless of host age, although development time in older hosts was lower (Fig. 2).

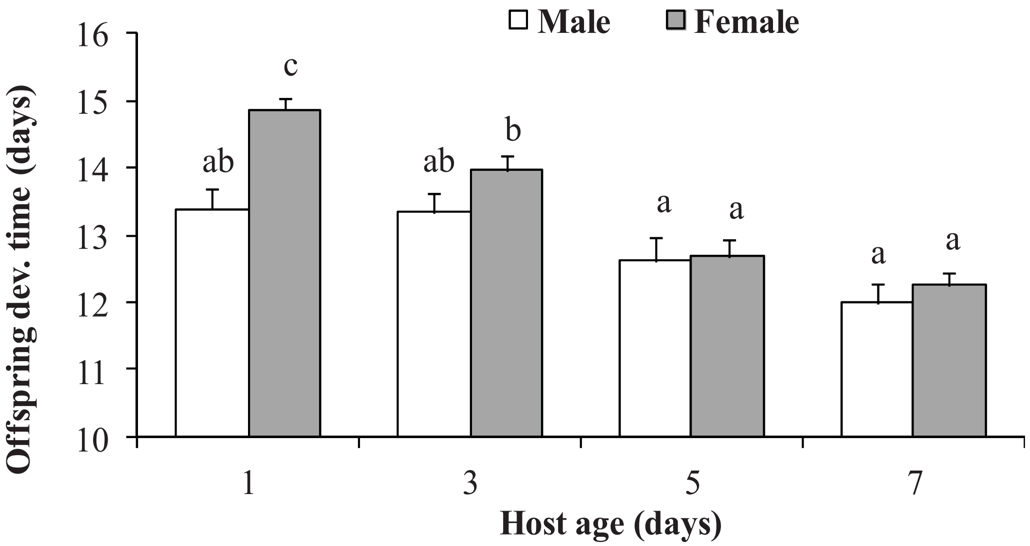

Figure 2: Development time, from oviposition to adult emergence, of male and female *Diaeretiella rapae* offspring in cabbage aphid nymphs parasitised at four different age. Means with the same letters are not significantly different (Tukey's HSD P<0.05), error bars represent the standard error.

Variation in the growth of parasitoid larvae developing in different age hosts appeared after four days of oviposition. A positive correlation was observed between larval size and host age at the time of oviposition (R^2=0.57, $F_{1,\ 59}$ =59.91, P<0.0001). The larvae developing in 7-day old hosts were larger than the larvae developing in other hosts ($F_{3,\ 44}$ =20.63, P<0.001; Fig. 3).

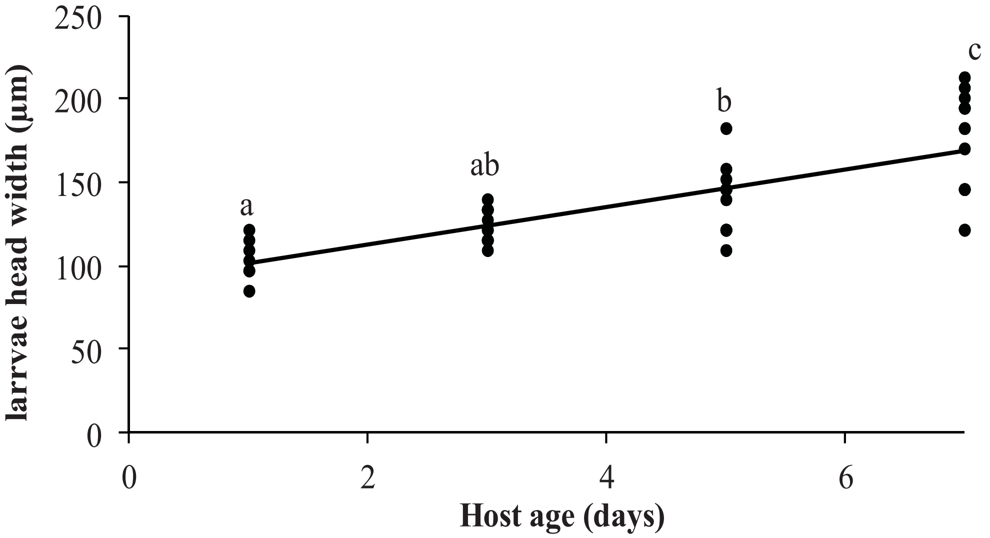

Figure 3: Relationship between the head width of developing *Diaeretiella rapae* larvae after 4 days of parasitism and the age of host at the time of parasitism. Means with the same letters are not significantly different (Tukey's HSD P<0.05).

Sex ratio of Diaeretiella rapae offspring emerged from hosts of different ages

Significantly more females than males emerged from aphids parasitised at 5 and 7-days old hosts (P<0.01). The numbers of males and females emerged from hosts parasitized at 1 and 3 days old were not significantly different (P>0.05). Overall female-biased progeny emerged from all age groups of hosts (Table 1).

Table 1: Female/male sex ratio of the *Diaeretiella rapae* offspring emerged from hosts of different ages. Columns with the same letters are not significantly different (P>0.05).

Offspring	1 day old	3 day old	5 day old	7 day old	Combined
Female	29 a	24 a	40 b	46 b	139
Male	21 a	18 a	24 a	17 a	80
Sex ratio	1.38	1.33	1.67	2.7	1.74
χ^2 value at α=0.05	1.28	0.8	4	13.35	15.89

Longevity of Diaeretiella rapae emerged from hosts of different ages

Parasitoids emerged from older hosts lived significantly longer than those emerged from younger hosts. The effect was most apparent in females that emerged from 7-day old hosts ($F_{3,\ 90}$ =6.12, P<0.001) (Fig. 4). Females from the 5-day old hosts also lived longer than those emerged from 1-day hosts. However, no significant difference was found in the longevity of females emerged from 1-day versus 3-day or 3-day versus 5-day hosts (Fig. 4). Male longevity was similar irrespective of the host age, except the males emerged from 7-day old hosts lived longer than the males emerged from 3-day old hosts (P=0.01) (Fig. 4).

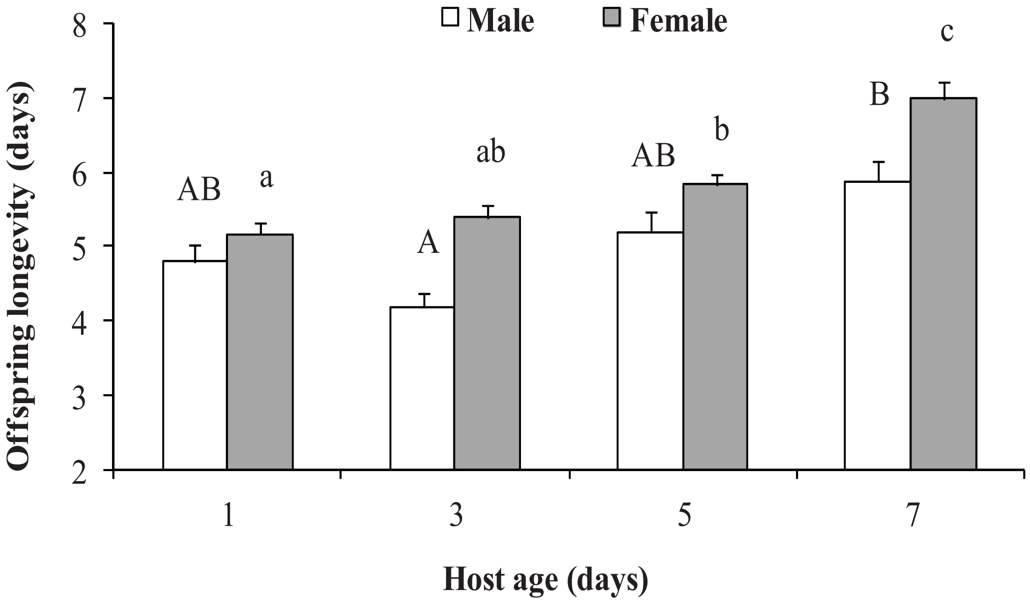

Figure 4: Longevity (mean ± SE) of male and female *Diaeretiella rapae* emerged from cabbage aphid parasitised at different age. Means with the same letters are not significantly different (Tukey's HSD P<0.05).

Body size of the offspring emerged from hosts of different ages

The body size of adult *D. rapae* was positively correlated with the age of their host. Variation in body size was most apparent in female offspring when all host age groups were analysed ($F_{3,\ 34}$ =15.49, P<0.001). Females that emerged from 3-day old hosts were larger than those emerged from 1-day old hosts, however, the size difference in females that had emerged from 5-day old and 7-day old hosts was not significant (Fig. 5). The size of males emerged from different age hosts was not significantly different (Fig. 5)

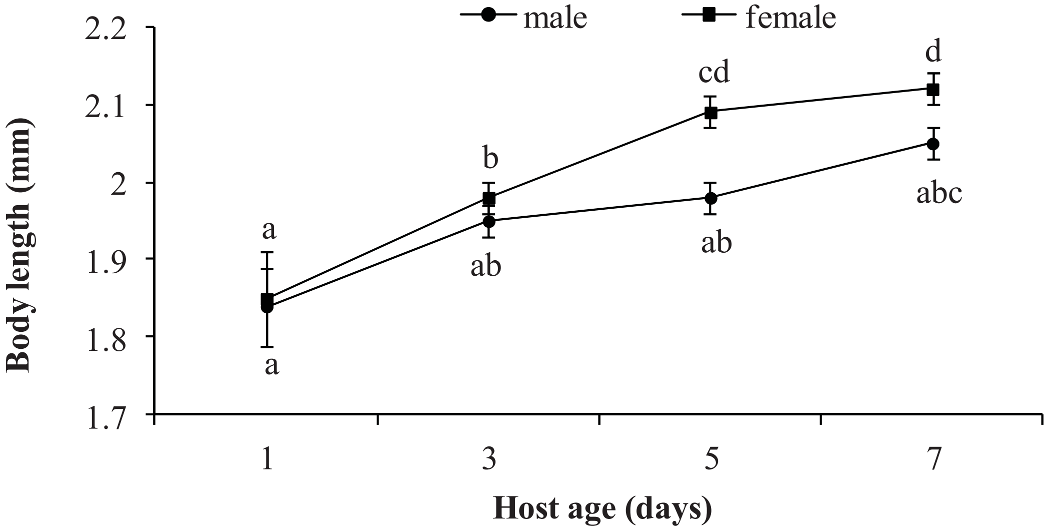

Figure 5: Body length (mean ± SE) of male and female *Diaeretiella rapae* emerged from cabbage aphid parasitised at different age. Means with the same letters are not significantly different (Tukey's HSD P<0.05).

Egg-load of female offspring emerged from hosts of different ages

The mean number of eggs found in the ovaries of females newly emerged from 7-day old hosts was significantly greater than in females emerged from 1-day and 3-day old hosts (Fig. 6). There was no significant difference in the egg load of females emerged from 1-day, 3-day and 5-day old hosts (Fig. 6).

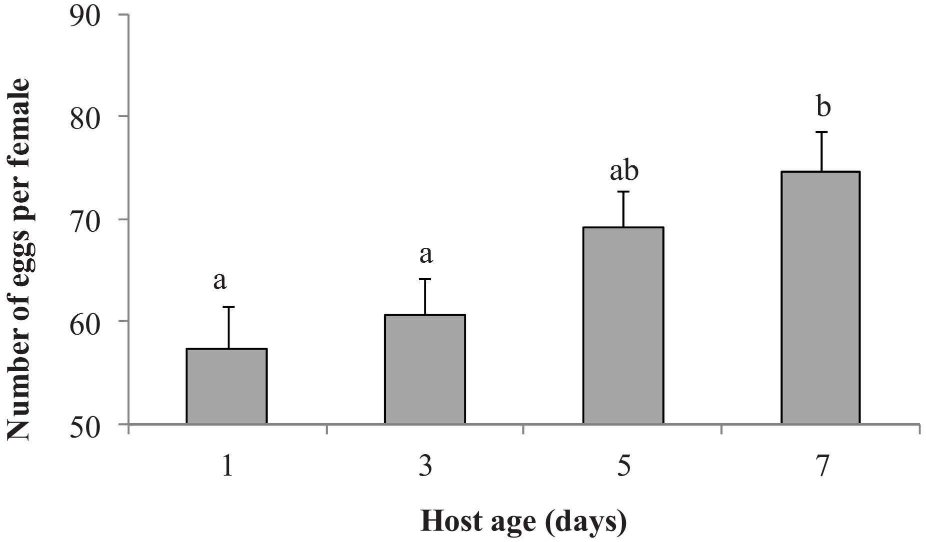

Figure 6: Egg load (mean ± SE) of female *Diaeretiella rapae* emerged from cabbage aphid hosts parasitised at different age. Means with the same letters are not significantly different (Tukey's HSD P<0.05).

Discussion

Unlike predators, which often feed on many prey individuals before achieving maturity, parasitoids are dependent on nutrition available in a single host for their complete development. Therefore, selection of a high quality host is very important. *Diaeretiella rapae* left the majority of younger and thus smaller hosts, unparasitised. Most of the parasitoids emerged from the older, larger hosts. This preference for larger hosts is consistent with observations of other parasitoid species (Elzinga et al. 2003; Harvey et al. 2004; Sandanayaka et al. 2009). This relationship makes sense if host size provides an indication of the amount of resources available to the developing larvae (Charnov 1982; Islam & Copland 1997; Bennett & Hoffmann 1998), and the quality of food available to the immature stages has a strong influence on the physical and physiological traits of the adult (Vinson & Iwantsch 1980; Jervis et al. 2008), and is consistent with the theory of optimal host acceptance (Stephens & Krebs 1986).

In contrast to our observations, the parasitoid *Monoctonus paulensis* (Ashmead) selects younger, smaller pea aphid nymphs among those available (Chau & Mackauer 2000). It has been proposed that defensive behaviour of hosts that correlates with their age /size also influences host selection (Chau & Mackauer 2000), resulting in a trade-off between quality and defensive behaviour of a host (Godfray 1994). He and Wang (2006) suggested that the pea aphid parasitoid *Aphidius ervi* (Haliday) selected medium size hosts over smaller and larger ones to optimise the trade-off between lower nutrient levels in smaller hosts, and a stronger defensive behaviour of larger hosts. Defensive behaviours such as kicking, body shaking and walking away have been observed in older cabbage aphid nymphs when *D. rapae* attacks them (R. Kant, unpublished data) but this appears to be insufficient to prevent more frequent attacks on larger cabbage aphid nymphs, and commonly, deposition of more than one egg per individual (Kant et al. 2008).

The benefits of oviposition in larger hosts are readily apparent in the growth and development of immature *D. rapae*. The first instar larvae in older hosts were significantly larger in size, and female adults developed quicker than those in younger hosts, suggesting more nutrients were available in larger host and that nutritional quality of nymphs increases with their age. However, the developmental time of males did not differ between larger and smaller hosts, probably because the quality and quantity of nutrients available in smaller hosts were sufficient for the development of male *D. rapae*, which are smaller than the females. Sequeira and Mackauer (1992) found that nutritional requirements of males and females vary in *A. ervi*. Similarly, Jarosik et al. (2003) found that females of *Aphidius colemani* (Viereck), another aphid parasitoid, grew bigger than males in the same size hosts.

Parasitoids can increase their reproductive fitness by reducing development time (Harvey & Strand 2002). Rapid development increases the fitness of *D. rapae* males by increasing their chances of finding virgin females for mating (Kant & Sandanayaka 2009). In some species, males wait near the female mummies for their emergence to mate at the earliest opportunity (Werren 1980). Females also benefit from early mating, as this allows them to have more of their eggs fertilised. Thus, early reproduction in *D. rapae* would increase the fitness of this species. Males of *D. rapae* mate several times in their life, while females mate once (Kant 2009), therefore, male *D. rapae* increase their fitness by having multiple matings if they develop faster. Further, we found that *D. rapae* larvae developing in 7-day old aphid nymphs grew faster than in 5-day old nymphs, but the body size of emerging adults was not different. This could be because the instant resources available to the parasitoid larvae were greater in 7-day than in 5-day old hosts, the quantity of resources was sufficient in both host ages.

Longer life of parasitoids emerged from larger hosts indicates another potential fitness gain in *D. rapae*. Longevity of *D. rapae* adults showed a positive correlation with their size. By living longer, females may encounter and parasitise more hosts. In contrast, males who can inseminate up to six females a day (Kant 2009) may become sperm-depleted quickly. This suggests male *D. rapae* have evolved to maximise early mating opportunities, rather than having a protracted mating period. However, it has also been found that female *D. rapae* become sperm-depleted if their male mate had previously mated three or more times; multiply-mated males appear to be sperm-depleted (R. Kant unpublished data). Although males gain fitness by maximising mating frequency, females may lose its fitness if mated with a sperm depleted male.

Host age significantly affected the sex allocation decision of *D. rapae*. Females manipulated the sex ratio of offspring by allocating the majority of unfertilised eggs (producing males) to younger hosts, and fertilised eggs (producing females) to the older ones. The increased proportion of female offspring emerging from older hosts supports the host-quality-dependent sex allocation model in parasitic hymenopterans (Charnov et al. 1981). By allocating male eggs to larger hosts, female *D. rapae* underutilise the resources, whilst allocating female eggs to smaller hosts reduces fitness by yielding smaller females, that live significantly shorter time, and have significantly fewer eggs. There is also increase in mortality of females developing in smaller hosts (Jarosik et al. 2003), which would contribute to the shift in sex ratio toward males. Thus, foraging females maximise resource use and thus the quality of offspring by regulating the release of sperm from their spermatheca on the basis of the quality of host they encounter at the time of oviposition.

Parasitism of older hosts also increases the chances of survival and successful emergence of *D. rapae* adults. Both idiobiont and koinobiont parasitoid females may gain fitness by parasitising the hosts that allow quick development of the offspring. The longer the parasitised host is active, the greater is the chance of host, and thus developing parasitoid, mortality by predators. Similarly, developing parasitoid larvae are vulnerable to multiple- and hyperparasitism. The chances of parasitoid larval mortality may therefore be significantly higher in younger/smaller hosts. Thus, there is a significant impact of host selection decision on the likelihood of offspring mortality via predation, hyperparasitism and pesticide use. By ovipositing fertilised eggs in larger hosts, *D. rapae* reduces the chances of mortality of its offspring, because the offspring takes less time to develop.

The study suggests that by parasitising older hosts, *D. rapae* increase their fitness by increasing the chances of successful emergence of offspring from the parasitised hosts and producing more female offspring. For the purpose of biological control, production of more, larger and longer-lived females is beneficial because it is the females that are directly responsible for controlling the pest population. Similarly, the sex ratio has important effect on bio-control potential of parasitoids (Ode & Hardy 2008). The results suggest that production of more females can be achieved by proper selection of host aphids during their mass production. As cabbage aphid populations naturally increase faster than *D. rapae* populations in spring (Kant et al. 2010), inundation with *D. rapae* during spring may help in suppressing the initial cabbage aphid population in the glasshouse or in the field.

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Chapter Eleven

Body size and fitness relation in male and female *Diaeretiella rapae* (Hymenoptera: Aphidiidae)

Abstract

A strong relationship exists between the body size and fitness in parasitoids. However, it is unclear whether the relationship is symmetric or asymmetric in males and females. The present study investigated the body size and fitness relationship in *Diaeretiella rapae* emerged from small and large nymphs of cabbage aphid *Brevicoryne brassicae*. A positive relationship existed between the size of aphid host and the growth of parasitoid larva developing in it. The fitness gain in males and females was not proportionate to their body size gain. Females mated with larger males produced 10% more female offspring than females mated with smaller males. However, females that developed in large hosts produced 62% more offspring (total male and female) than the females emerged from smaller hosts. The findings suggest that the total number of offspring and the progeny sex ratio were affected by the body size of both male and female *D. rapae*.

Keywords: *Diaeretiella rapae*, body size, sex ratio, resource allocation, fitness

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The results of previous study found that large size host brought fitness to female *Diaeretiella rapae*. This chapter compared body size and fitness relationship in male and female *D. rapae.*

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Introduction

Body size in insects is an important trait that affects fitness of individuals (Henry et al. 2009). The quantity of resources consumed by individuals during ontogenesis is directly related to their final body size (Godfray 1994). Size varies continuously due to the effects of natural selection on resource acquisition (Chown & Gaston 2010). Thus, body size and fitness in organisms influence their efficiency in acquiring resources (Van Alphen & Visser 1990). However, the scope of resource acquisition by an individual is finite in some insects, while others can acquire as much as they can subject to availability of the hosts. For example, predators can consume varying number of prey for their growth and development, while resources available to developing parasitoids are fixed by their solitary host. Idiobiont parasitoids completely paralyse their hosts during oviposition which stops the host's growth and development. Thus, the developing parasitoid larvae have a strictly fixed resource. However, koinobiont parasitoids allow their hosts to feed and grow, therefore, their host resources could be dynamic in nature (Sequeira & Mackauer 1992; Wyckhuys et al. 2008) if the host continues to feed.

In parasitoids, both the quality and quantity of resources available to larvae are important for their overall growth and development (Sequeira & Mackauer 1992; Harvey & Strand 2002). Larger hosts are generally considered better than smaller ones, because they provide more resources to the developing larvae (Mackauer 1986). Resources gained by parasitoid larvae are the main (capital) resources that determine the future reproductive efficiency of parasitoids during their adult life (Jervis & Kidd 1986a).

The quantity of resources immediately available to a newly hatched parasitoid larva (instant resources) depends on the size of the host in which oviposition occurred. Therefore, adult parasitoids might consider the size of potential hosts during oviposition, irrespective of their foraging habit as idiobiont or koinobiont (Godfray 1994). Furthermore, host selection should favour the strategy that utilises limited resources more efficiently. Another critical question is whether an ovipositing female should be selective in allocating the sex of its offspring. Studies have found that in parasitoid species where sex of offspring can be facultatively controlled by the mother before oviposition, females allocate fertilised eggs (female offspring) to good quality hosts and unfertilised (male) eggs to lower quality hosts (Charnov et al. 1981; Wyckhuys et al. 2008; Kant et al. 2012a). This is evident when the female has a choice of different quality hosts, but how does the female behave if she encounters hosts of uniform quality - either all large (high quality) or all small hosts?

Optimal foraging and game theories (Stephens & Krebs 1986; Hammerstein & Selten 1994; Perry & Pianka 1997) predict that natural selection will favour parasitoid behaviour that maximises their fitness. If a foraging female shows a preference for allocating fertilised eggs to hosts of a certain size, it indicates that there are fitness consequences (Charnov 1982). Behavioural preferences of females in terms of reproductive fitness gain can be tested experimentally. This study examines a comparative reproductive fitness gain in male and female *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *D. rapae* is an endoparasitoid of cabbage aphid *Brevicoryne brassicae* (Homoptera: Aphidae) and several other aphids attacking cruciferous and non-cruciferous plants (Pike et al. 1999). Female *D. rapae* emerge with matured eggs (Bernal & González 1997) and prefer to oviposit after mating (Kant et al. 2012c). The mated female *D. rapae* prefer to oviposit into larger (older) aphid nymphs, despite oviposition in the larger hosts requiring longer host-handling time and more energy (higher number of probes per host) (Kant et al. 2008). The benefit of time- and energy-consuming oviposition in larger hosts is the quicker development and higher longevity of adults emerged from these hosts (Kant et al. 2012a). Differences in body size of male and female *D. rapae* have been found in an uncontrolled open system (Kant et al. 2012b) as well as in a controlled laboratory condition (Kant et al. 2012a). In the wild population, 79% of the mating male *D. rapae* were smaller than their female partner (Kant et al. 2012b). However, it is still unclear whether the relationship between parasitoid body size and fitness is similar in male and female *D. rapae*. Morris and Fellowes (2002) suggested that the relationship between fitness and size is stronger in female parasitoids.

The current study estimated the size-fitness relationship in *D. rapae* by considering the following aspects: (1) larval growth and body size of male and female *D. rapae* developed in small and large cabbage aphid nymphs, (2) the relationship between host size and resource allocation in parasitoid larvae, (3) the effect of body size of mating pairs on offspring number and sex ratio.

Material and methods

Insect colonies

Colonies of cabbage aphid, *B. brassicae* and its parasitoid *D. rapae* were established from a commercial cauliflower field near Palmerston North, Manawatu, New Zealand. Insects were reared in laboratory on cabbage seedlings (cultivar: 'Autumn pride') in 30×30×30 cm Plexiglas cages The colonies were maintained at 22 ± 2°C, 60-70% RH and 16 h light photoperiod.

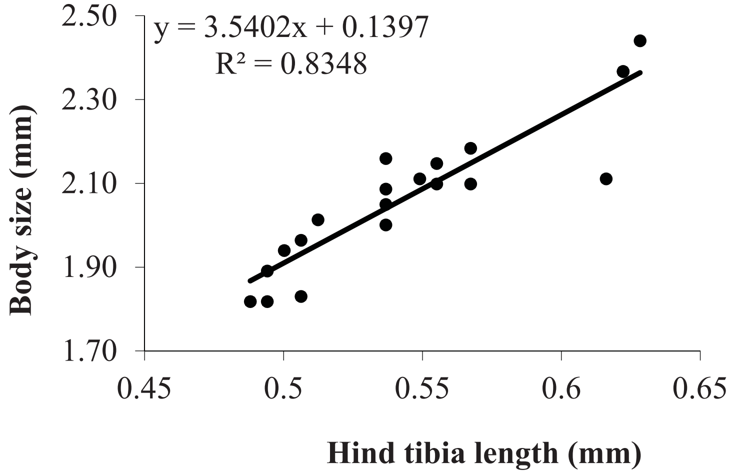
Oviposition and sex allocation by Diaeretiella rapae in a no-choice test

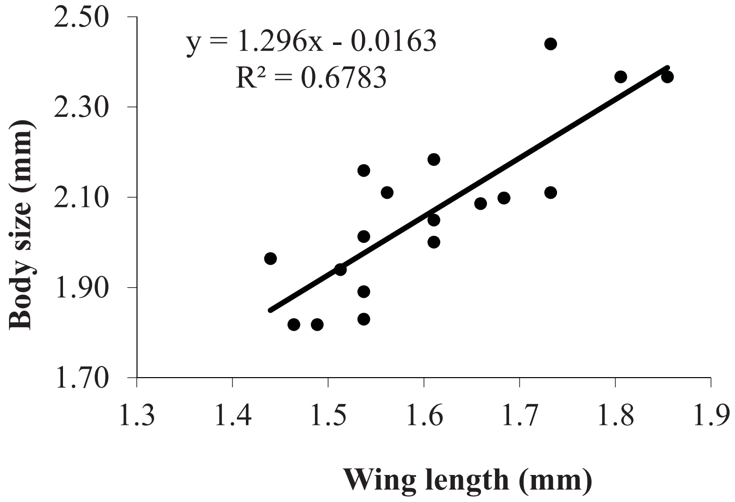
Cabbage aphid nymphs of two age groups, small 1-2 day old and large 6-7 day old, were offered separately to mated, 1-2 day old, 10% honey-fed *D. rapae* females. Size of cabbage aphid nymphs is positively correlated with their age (Kant et al. 2008b). Each female was offered 30 nymphs of a particular size for 24 h for four consecutive days. The parasitised aphids were transferred to cabbage leaves for their development and were observed daily for mummification. Each mummified aphid was transferred to an individual microfuge tube until emergence of adult parasitoid. Male and female *D. rapae* emerged from small and large aphids were measured.

Measurement of Diaeretiella rapae body length

The adults were first killed by putting them in the -20°C freezer and then stored in 70% ethanol. To measure the body length, the insects were dissected, and head, thorax and abdomen were placed on a glass slide in saline solution. The length of each tagma was measured as maximal length in dorsal aspect using a calibrated eyepiece micrometer. The measurements were then added together. To simplify this process, and to find the adequate substitute for body size measurement, the body size of *D. rapae* adults was regressed against their hind tibia length, wing length and head width.

The hind tibia length, wing length and head width were positively correlated to the total body length of *D. rapae* (Fig. 1). The hind tibia length was highly correlated to the total body length of *D. rapae* adults (body length = 3.54x + 0.139, $R^2 = 0.83$, where x = hind tibia length). Therefore, hind tibia length was used to estimate the size of adults in the rest of the experiments. Ten females were tested in each group.





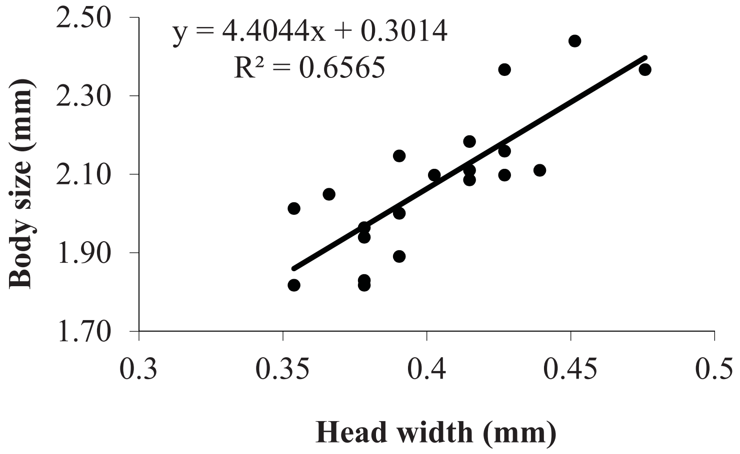


Figure 1: Relationship between body parts, hind tibia length, wing length and head width with the total body size (combined head, thorax and abdomen lengths) in *Diaeretiella rapae*.

Resource acquisition by Diaeretiella rapae larvae in small and large hosts

A separate experiment was used to assess the growth of larvae in small and large hosts. Parasitised aphids were transferred to cabbage leaves and after 4 days of parasitism the aphids were dissected under a microscope. The size of each larva was quantified by measuring the width of its head capsule. Twenty larvae were dissected from each small and large size hosts.

We developed a resource allocation index (RAI) to compare the amount of host resources (nutrients) allocated to the *D. rapae* larvae after 4-day of parasitism until *D. rapae* larvae undergo pupation in the hosts. Therefore, RAI indicates the growth of larvae during the period from 4-day old larvae to adult, while developing in either large or small host.

$$RAI= (HTL - MHW)/HTL$$

As larval size and adult size could not be measured on the same *D. rapae* individual, the average size of 4 day old larvae in small and large hosts (quantified as mean head width, MHW) was substituted for actual larval measurements. The size of emerging adults was measured as hind tibia length (HTL). The index was calculated individually for all males and females that emerged from large and small hosts. Mean RAI was compared for males and females emerged from large and small hosts.

Reproductive fitness of large and small Diaeretiella rapae

The parasitoids obtained from the previous experiments were used to determine their reproductive gain. Adults emerged from 1-2 day old aphid nymphs were considered “small” and those emerged from 5-6 day old aphids were considered “large”. Four mating combinations were tested: 1) small male x small female; 2) small male x large female; 3) large male x small female, and 4) large male x large female. In each case a male and a female were paired in a small vial (7.5 cm height x 2.5 cm diameter) for mating. Each pair was offered 10% honey solution and left in the glass vial for 24 h. Each mated female was offered 30 cabbage aphids (5-6 days old nymphs) on a cabbage seedling in a cage for 24 h every day until the death of the female. For each mating combination, 10 pairs were tested.

The parasitised aphids were transferred to a new cabbage plant for development. Once the aphids were mummified, they were transferred to individual vials until the adult parasitoids emerged. The number of mummies and offspring resulted from each mating were counted. The total number of offspring (adults emerged) was used as a fitness criterion for females; the number of female offspring was used as a fitness criterion for males.

Body size gain and reproductive gain in male and female Diaeretiella rapae

Body size gain in male and female *D. rapae* was calculated as percentage increase in their body size when they developed in large hosts (5-6 day old) compared to small hosts (1-2 day old). Reproductive gain of female *D. rapae* is the percentage increase in the number of offspring (combined male and female offspring) produced by females emerged from large hosts compared to small hosts. Reproductive gain in male *D. rapae* is the percentage increase in the number of female offspring produced by females mating with large males compared to offspring produced by females mating with small males.

Statistical analysis

Analysis of variance (PROC GLM, SAS 9.2) was used to analyse differences in the means, including the number of adults and number of females emerged from small and large size hosts, body size mating combinations, hind tibia length of male and female, and differences in the RAI in large and small hosts. When the overall results were significant, the differences among treatments were compared using Tukey's studentised (HSD) test. Ordinary least squares regression was used to examine the relationship between hind tibia length and body size of adult parasitoid. Sex ratios were analysed using binary logistic regression (PROC LOGISTIC, SAS 9.2). All analyses were carried out at $\alpha = 0.05$ level of significance.

Results

Oviposition and sex allocation by Diaeretiella rapae in a no-choice test

In a no-choice test, the number of aphids parasitised by female *D. rapae* per day during the four day oviposition period did not differ whether the females were offered small or large aphid nymphs ($F_{1, 18} = 0.57$; $P = 0.46$) (Fig. 2). A significantly higher proportion of females (74.5%) emerged from larger hosts (Logistic regression: $\chi^2 = 32.86$, $P < 0.001$), while the proportion of males and females emerged from smaller hosts (Logistic regression: $\chi^2 = 1.47$, $P = 0.22$).

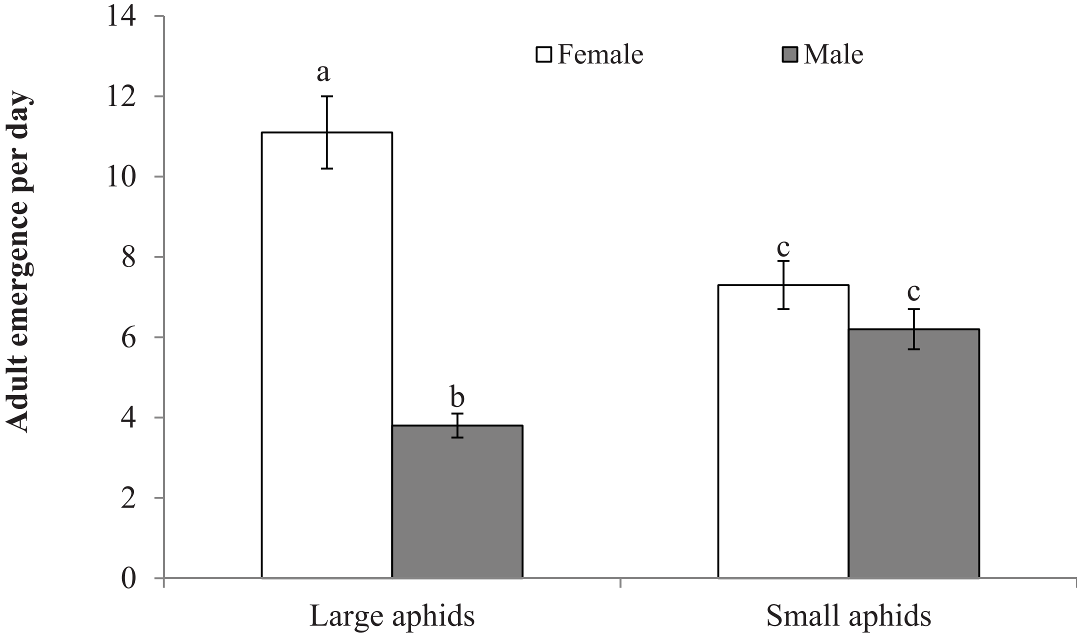


Figure 2: Mean (±SE) number of male and female *Diaeretiella rapae* emerged per day from large (5-6 day old) and small (1-2 day old) *Brevicoryne brassicae* nymphs. Means with the same letters are not significantly different at α=0.05 (Tukey's HSD).

Body size gain in Diaeretiella rapae larvae and adults

The size of *D. rapae* larvae found in the dissected cabbage aphid nymphs was positively correlated to the size of the nymph [$y = 0.106x + 11.22$, where y = larval head width (µm) and x = aphid body length (µm)] ($R^2 = 52.1$, $P = 0.002$). The mean (±SE) head width of the *D. rapae* larvae at 4 days after oviposition was larger in large hosts (156.9±4.8µm) than in small hosts (117.7 ± 5.6 µm) ($F_{1, 46} = 28.31$, $P < 0.001$).

In general, the adults emerged from the large hosts were larger in size than those emerged from small hosts ($F_{1, 73} = 44.80$, $P < 0.001$) and the females were larger than the males ($F_{1, 73} = 11.34$, $P < 0.01$). Body size dimorphism was found in males and females emerged from large hosts, but not in those emerged from small hosts (Fig. 3). Asymmetric increase in body size was found in male and female *D. rapae* emerged from small and large hosts. Females emerged from large hosts were on average 13% larger than females from small hosts ($F_{1, 39} = 34.26$; $P < 0.001$) while males from large hosts were 5% larger than males from small hosts ($F_{1, 35} = 12.43$, $P < 0.01$).

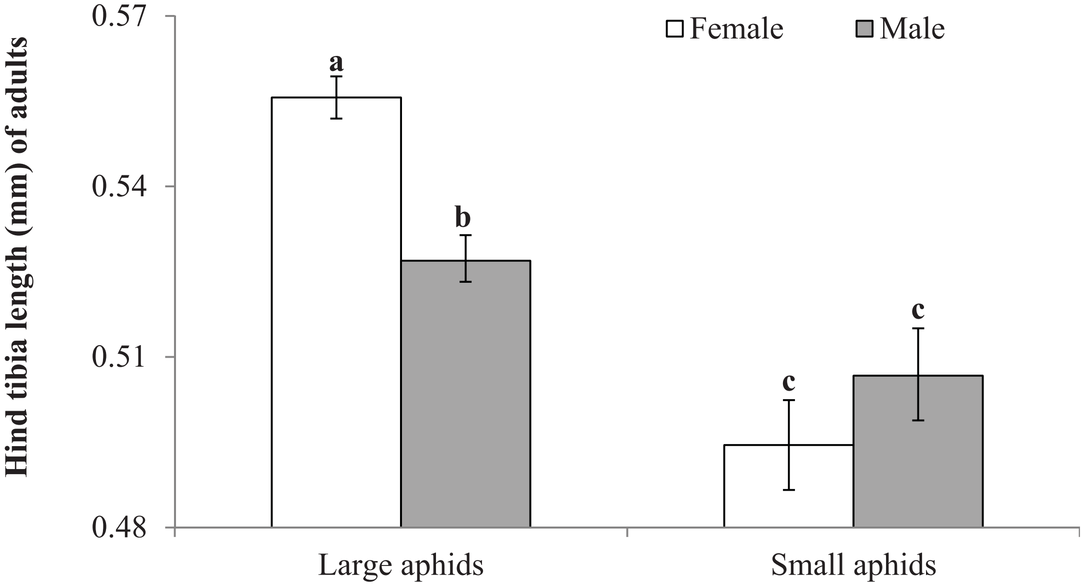


Figure 3: Mean (±SE) body size (hind tibia length) of male and female *Diaeretiella rapae* emerged from large (5-6 day old) and small (1-2 day old) *Brevicoryne brassicae* nymphs. Means with the same letters are not significantly different at $\alpha=0.05$ (Tukey's HSD).

Resource/nutrient acquisition by male and female Diaeretiella rapae

RAI depended on the size of the host and the sex of the parasitoid developing in host ($F_{3, 64} = 83.23$, $P < 0.001$). The mean RAI was greater in smaller hosts than in larger hosts (Fig. 3), indicating that in small hosts a larger proportion of the host's resources is allocated to developing parasitoid. There was no significant difference in RAI of males and females developing in smaller hosts. RAI of females developing in larger hosts was greater than RAI of males developing in those hosts (Fig. 4).

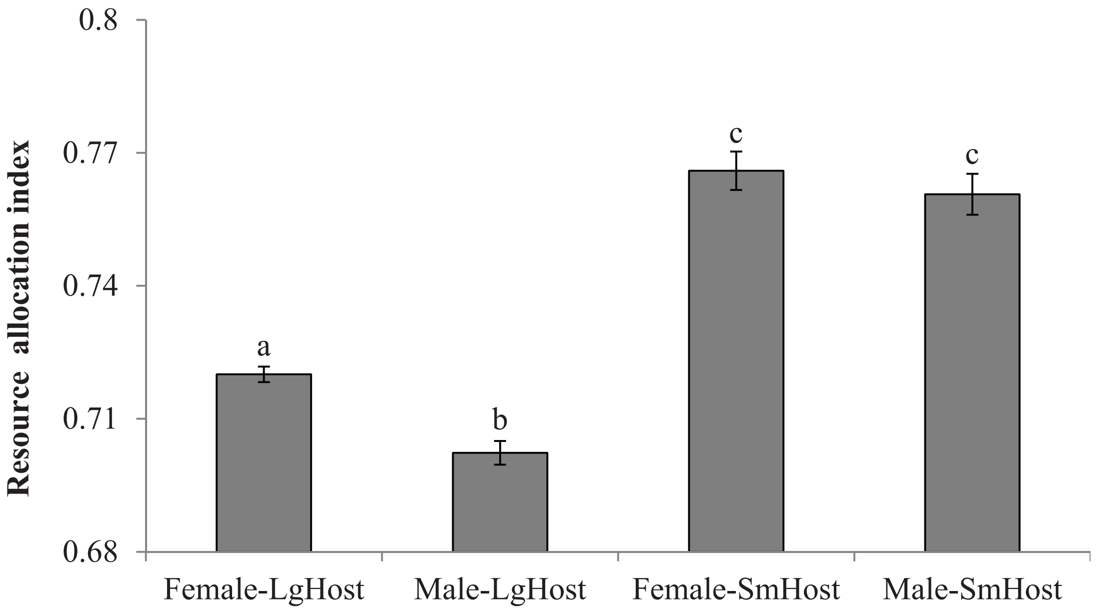


Figure 4: Mean (±SE) values of Resource allocation index (RAI) for male and female *Diaeretiella rapae* developed in large (5-6 day old) and small (1-2 day old) *Brevicoryne brassicae* nymphs. Means with the same letters are not significantly different at α=0.05 (Tukey's HSD).

Reproductive gain in large and small Diaeretiella rapae

The number of offspring produced by *D. rapae* depended on the body size of male and female ($F_{3,\ 51} = 14.37$, $P < 0.001$). The mean (±SE) number of offspring produced by larger females (63.7 ± 2.3) was significantly greater than the number of offspring produced by smaller females (43.9 ± 2.4) (Table 1). The number of offspring produced by a large female mated with a large male was significant greater than the number of offspring produced by a large female mated with a small male. However, the number of offspring produced by small females was similar whether they mated with large or small males (Table 1). Mating combination "large male x large female" produced the highest number of females (Table 1). When the sex ratios of the offspring produced by small and large males were compared, larger males produced a higher proportion of females (Logistic regression: $\chi^2 = 12.25$, $P < 0.001$).

Table 1: Mean (±SE) total offspring and female offspring produced from different mating combinations of "large" and "small" male and female *Diaeretiella rapae*. Adults emerged from 5-6 day old parasitised *Brevicoryne brassicae* nymphs were considered "large", while adults emerged from 1-2 day old parasitised nymphs were considered "small". Means with the same letters are not significantly different, α=0.05 (Tukey's HSD).

Mating combination	Total no. of offspring	Female offspring	Sex ratio
Large Male x Large Female	66.9±6.4 a	46.4±2.4 a	2.19 a
Large Male x Small Female	48.1±2.7 b	36.7±2.3 b	3.36 b
Small Male x Large Female	60±4.2 a	35.4±2.4 b	1.4 c
Small Male x Small Female	38.7±3.6 c	25.3±2.5 c	1.78 a

Body size gain vs. reproductive fitness gain

An asymmetry was observed between body size gain and reproductive fitness gain in male and female *D. rapae*. Although both male and female *D. rapae* gained body size by developing in larger hosts, the females gained disproportionately more fitness than males by developing in larger hosts (Fig. 5).

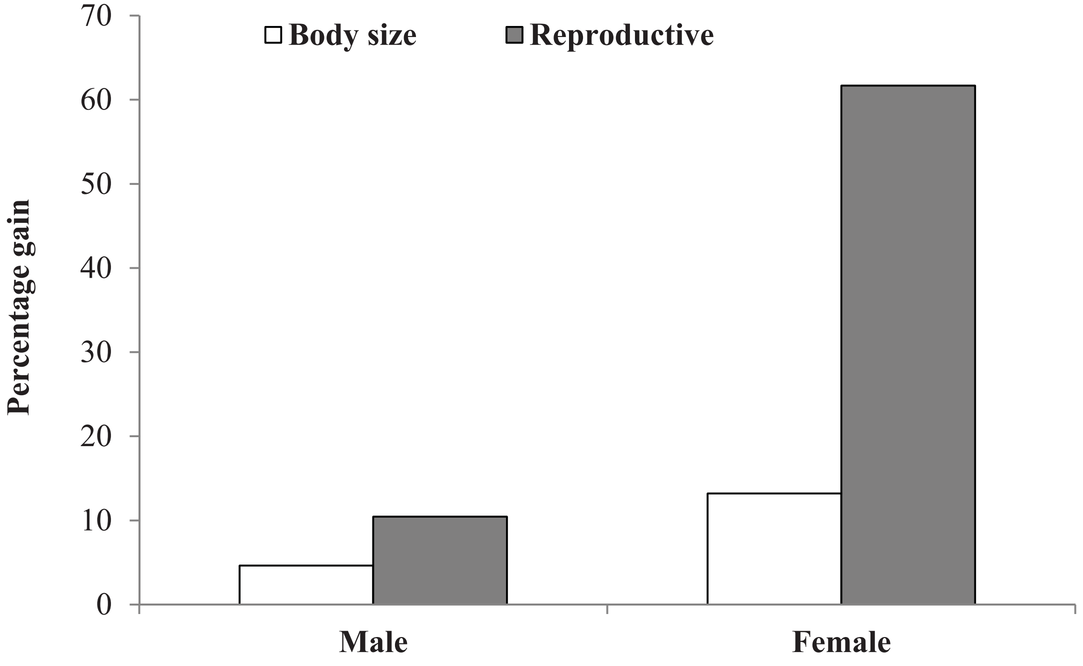


Figure 5: Body size gain and reproductive fitness gain in male and female *Diaeretiella rapae* developed in large (5-6 day old) *Brevicoryne brassicae* nymphs, as % increase relative to individuals developed in small (1-2 day old) aphid nymphs. Fitness gain in males was calculated in terms of increase in female progeny while fitness gain in females was calculated in terms of total (combined male and female) progeny.

Discussion

Growth of *D. rapae* larvae developing inside the aphid nymphs was affected by the size of nymph in which oviposition occurred. The parasitoid larvae have finite resources for completing their development, limited to whatever is available in a single host (Godfray 1994). The large body size of larvae developing in the larger hosts suggests that large hosts contained more resources that were immediately available to the newly hatched larvae, compared to the smaller hosts. Studies of superparasitism in *D. rapae* suggested that more resources are available in larger hosts because 2-5 parasitoid larvae can develop simultaneously without compromising their growth, although only one individual completes its development (Kant et al. 2011; Kant et al. 2012b). The smaller cabbage aphid nymphs did not sustain development of more than two larvae per host, and higher host mortality was found in small superparasitised aphids (Kant et al. 2011).

Emergence of larger adults from larger hosts further suggests that greater amount of resources are available in larger hosts. Larger hosts also provide more space to the developing parasitoid larva while larvae developing in small hosts have limited space for growth before host mummification. The size of males and females that developed in small hosts was similar, but a clear sexual dimorphism was found between those developed in large hosts, with females significantly larger than males. Females emerged from larger hosts were significantly larger than those emerged from small hosts, while the increase in body size of males was not significant. This suggests that resources available in small hosts are sufficient for males to attain a maximum body size, but might be limiting for females.

Sexual dimorphism in wild population of *D. rapae* has also been found (Kant et al. 2012b). The evolutionary consequences in fitness gain is expressed as foraging behaviour of *D. rapae* females, which prefer to allocate fertilised (female) eggs to larger hosts (Charnov et al. 1981). Solitary parasitoids developing in larger hosts will benefit in terms of increased adult size which is predicted to be positively correlated with life-history and demographic characters such as fecundity and survival (Mackauer & Sequeira 1993; Kant et al. 2012a).

D. rapae is a koinobiont species, which allows its host to develop and reproduce (Zhang & Hassan 2003). Some of the resources acquired by the parasitised aphid hosts for their growth and reproduction are sequestered for growth of parasitoid larvae. Couchman and King (1979) found that the feeding rate of parasitised cabbage aphids varied in relation to the growth and development of *D. rapae* larvae in aphid body. The resources available in hosts (especially small hosts) at the time of oviposition are not sufficient for the parasitoids to complete their development; rather, their development depends on the host continuing to feed and grow (Sequeira & Mackauer 1992). In smaller hosts, due to lower feeding efficiency, the parasitoid might lack resources and as a result consume the aphid nymphs developing simultaneously with the parasitoid in the parasitised host. In this study, when large-size parasitized aphids were dissected after 4 days of parasitism they contained parasitoid larvae and underdeveloped aphid nymphs, whereas only parasitoid larvae were found inside the body of small-size parasitised aphids. Larger hosts might have enough resources at the time of oviposition for parasitoids to complete their development, although hosts have to survive long enough (Godfray 1994).

The total number of offspring and the progeny sex ratio of *D. rapae* were affected by the body size of both parents, indicating that both sexes contribute to the fitness of *D. rapae*. Larger *D. rapae* females emerge with higher egg load (Kant et al. 2012a) and produce more offspring than smaller females (Cloutier et al. 2000; Chown & Gaston 2010). Females mated with larger males produced significantly more female offspring, suggesting that the size of *D. rapae* male has strong influence on the progeny sex ratio. This could be due to higher sperm stock of males emerged from larger hosts (Lacoume et al. 2006). The progeny sex ratio was not significantly different between (large male x large female) and (small male x small female) combinations, suggesting that females can manipulate the number and proportion of female progeny to maintain the male/female balance in the population.

A large number of reproductive behaviours of parasitoids are influenced by their body size, which affects their fitness (Stearns 1992; Sagarra et al. 2001). Greater longevity of large female *D. rapae* allows them to reproduce longer (Kant et al. 2012a). Large *D. rapae* males may be able to inseminate more females, as has been shown for larger males of *Dinarmus basalis* (Lacoume et al. 2006). Furthermore, large males and females benefit during intersexual mate selection (Visser 1994; Joyce et al. 2009). Larger males have been found to win the male-male combats during mating, which are common in hymenopterans (Abe et al. 2005; Joyce et al. 2009). Female *D. rapae* might gain fitness by producing larger males in the presence of conspecific competition, where larger males have edge on smaller ones in male-male combat.

The results indicate that larger hosts immediately provide more resources to the developing parasitoid larvae. Resources gained by *D. rapae* larvae affected their body size and their reproductive fitness. Larger males and females produced higher numbers of progeny as well as higher numbers of females. The increase in body size and the gain in reproductive fitness were not symmetrical in male and female *D. rapae*. Females received disproportionately more benefit than males by developing in larger hosts.

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Chapter Twelve

Host selection for self-superparasitism by *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae)

Abstract

Superparasitism (repeated oviposition in same host) indicates either a fitness benefit for the parasitoid, or an inability of ovipositing females to discriminate parasitised versus unparasitised hosts. Superparasitism in *Diaeretiella rapae,* the solitary endoparasitoid of cabbage aphid *Brevicoryne brassicae*, was studied in the laboratory. When given a choice of parasitised and unparasitised aphids, 70% of females made their attack on unparasitised hosts first, even though already-parasitised aphids were less defensive to parasitoid attack and ovipositing females had shorter host-handling time. The female superparasitised hosts during repeated attack and the large hosts were superparasitised more often than the small ones. Although superparasitised hosts carried two to five parasitoid larvae, only one larva developed into an adult. The body size of parasitoid larvae, as measured 4 days after oviposition, was affected by the total number of larvae in the host. Superparasitism strategies of *D. rapae* are discussed in the context of reproductive fitness.

Keywords: superparasitism, host defence, *Brevicoryne brassicae*, *Diaeretiella rapae.*

The results of previous chapter showed that *Diaeretiella rapae* choose to parasitise older parasitoid nymphs and gains reproductive fitness. This chapter explores the host selection and discrimination for superparasitism by *D. rapae*.

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Introduction

Reproductive fitness of female parasitoids depends on their ability to assess the suitability of hosts as resources for successful development of their larvae. Superparasitism is a common phenomenon (Van Alphen & Visser 1990; Jaramillo et al. 2006) in which female parasitoids lay egg(s) in already-parasitised hosts, and the larvae developing inside the host compete for the limited resources (Bai & Mackauer 1992). Self-superparasitism (oviposition in a host previously parasitised by the individual) could reflect an opportunity for fitness gain to the parasitoid or indicate that the ovipositing female is unable to discriminate between already-parasitised and unparasitised hosts (Van Lenteren et al. 1978).

In gregarious parasitoids, where more than one individual emerges from a single host, superparasitism could increase fitness by increasing the total number of emergences per host (Silva-Torres et al. 2009). In solitary parasitoids, however, only one individual per host will emerge, irrespective of the number of eggs laid or the number of larvae developing in a host. Ovipositing more than one egg per host by solitary parasitoids means death of all but one offspring, which means the self-superparasitising females lose the extra egg(s) laid (Volkl & Mackauer 1990; Tracy Reynolds & Hardy 2004). Nevertheless, superparasitism could be advantageous when unparasitised hosts are not readily available (Van Alphen & Visser 1990). Superparasitism may also increase the chance of survival of at least one larva, as two or more larvae probably depress the host defence system more effectively than one (Mackauer & Chau 2001).

This study looked at the superparasitism strategy of *Diaeretiella rapae*, a solitary endoparasitoid of cabbage aphid *Brevicoryne brassicae* and several other aphids of cruciferous and non-cruciferous plants (Pike et al. 1999). There is little information on superparasitism in this cosmopolitan wasp, although Lester and Holtzer (2002) suggested superparasitism in *D. rapae* occurs at low host density. Previous studies on *D. rapae* showed that females prefer larger hosts for oviposition, when given a choice of different sizes of hosts (Kant et al. 2008). It is important to understand the superparasitism behaviour in terms of fitness consequences for the parasitoid, as this may improve biological control programmes at the level of mass rearing and/or during augmentative release (Hamelin et al. 2007). The following questions were addressed in the present study to understand the superparasitism strategy of *D. rapae*: (1) Is the female able to distinguish between unparasitised and parasitised hosts? (2) Does the female show any preference for host size during superparasitism? (3) Does defensive behaviour of already-parasitised and unparasitised aphids differ during parasitoid attack? (4) What are the effects of superparasitism on growth of parasitoid larvae?

Materials and methods

Insect culture

Cultures of *D. rapae* and its host, *B. brassicae,* were established from individuals collected in a cabbage field in Palmerston North (Manawatu, New Zealand). Aphids were maintained on 8-weeks old cabbage *Brassica oleracea var. capitata* (cv. Summer Globe Hybrid) at 20±2°C under 65% RH and 16 h light regime. *Diaeretiella rapae* were reared on large (5-7 days old) cabbage aphid nymphs.

Discrimination between parasitised and unparasitised host

In order to get parasitised aphids, 1-day old mated *D. rapae* females were each offered five cabbage aphid nymphs in a Petri dish. There were two groups of females (n=35 in each group), and these were offered either small (1-2 days old) or large (6-7 day old) nymphs. Two sizes of aphids were used in order to make easy visual reference of unparasitised versus already-parasitised aphids when recording behaviour. Parasitism was observed and the parasitised aphids were transferred to cabbage leaves for 1 hour for later use in the host discrimination test; the unparasitised aphids were discarded.

The discrimination ability of *D. rapae* females was examined in a two-part experiment. In the first part, each of the 35 females previously used to parasitise 1-2 day old aphids was offered a small (1-2 day old) self-parasitised aphid nymph and a large (6-7 day old) unparasitised nymph in a Petri dish. In the second part of the experiment, the other 35 females (those used to parasitise 6-7 day old nymphs) were each offered a large self-parasitised aphid and a small unparasitised aphid. The first encounter of the female (with unparasitised or already-parasitised host) and defensive behaviour of aphids were recorded. After parasitism or superparasitism, the aphids were transferred to cabbage seedlings for their development, for 4 days, and subsequently dissected under a stereomicroscope (Olympus SZX12). The number of larvae found in each host was recorded and the larval body lengths were measured using Motic Imaging software (version 2.0).

Host size preference for superparasitism

The host size preference for superparasitism was tested by offering four aphid nymphs: 1, 3, 5 and 7-day old (age of aphid nymphs is directly correlated to their size (Kant et al. 2008) to a 1-day old mated *D. rapae* female in a small Petri dish. The female was allowed to oviposit for 20 min and the behavioural responses of the female and the hosts were recorded for that period. Twenty-five females were tested in this experiment. After parasitism, the aphids were dissected and parasitoid larvae were counted and body lengths were measured as in the previous experiment.

Parasitoid attack and host defence behaviour

During parasitism and superparasitism, duration of the host-parasitoid encounter and the number of times each female *D. rapae* inserted her ovipositor into the host during the encounter were recorded. Defensive behaviours (kicking, abdomen shaking and walk-away from oviposition site) of unparasitised and already-parasitised aphid nymphs were recorded and quantified.

Data analysis

Logistic regression (PROC LOGISTIC, SAS 9.2) was used to analyse the first attack and oviposition by *D. rapae* in the unparasitised versus already-parasitised host choice test, walk-away by host during the attack, and for analysis of superparasitism incidences in different size hosts. Chi-square tests were used to compare incidence between different size hosts. Linear regression was used to express the relationship between the size of host and the size of developing larvae. Analysis of variance (PROC GLM, SAS 9.2) was used to analyse the mean number of eggs found in the different size hosts, time spent and the number of ovipositor probing activities performed by *D. rapae* during host attack and the abdomen shaking and kicks by *B. brassicae* nymphs during parasitoid attack. A T-test was used to compare the difference in the size of larvae resulting from parasitism and superparasitism.

Results

Discrimination between parasitised and unparasitised host

When given a choice of parasitised and unparasitised aphids, female *D. rapae* were able to recognise the parasitised aphids, and 70% attacked the unparasitised ones first ($P<0.002$) (Table 1). The previously demonstrated preference for large host size contributed significantly to the female choice, as the females attacked the already-parasitised larger aphids significantly more often than smaller aphids ($P<0.001$). The unparasitised small and already-parasitised large aphids were equally preferred for attack ($P=0.06$).

When females made their first attack on unparasitised hosts, larger hosts were attacked more frequently than smaller hosts ($P=0.031$) and 90% of the attacks on large hosts resulted in oviposition, compared to 71% of attacks on small hosts ($P=0.098$) (Table 1). When females first attacked already-parasitised hosts, the attacks resulted in oviposition significantly more often in large hosts than in small hosts ($P<0.04$). The females superparasitised large hosts more often than small hosts ($P<0.001$). The least preferred for oviposition was small already-parasitised hosts.

Table 1: First attack and oviposition of female *Diaeretiella rapae* in two choice tests: (1) Small unparasitised versus Large already-parasitised and (2) Large unparasitised versus Small already-parasitised *Brevicoryne brassicae* nymphs. Percentage value in oviposition column indicates percentage of attacks that resulted in parasitism.

	First attack		Oviposition	
Choice experiments	Unparasitised	Already-parasitised	Unparasitised	Already-parasitised
Small unparasitised vs Large already-parasitised	21	14	15 (71%)	11 (80%)
Large Unparasitised vs Small already-parasitised	29	6	26 (90%)	3 (50%)

Host size preference for superparasitism

When given a choice of different sizes (ages) of cabbage aphid nymphs, larger aphids were more often superparasitised than the smaller ones (P<0.03; Fig 1).

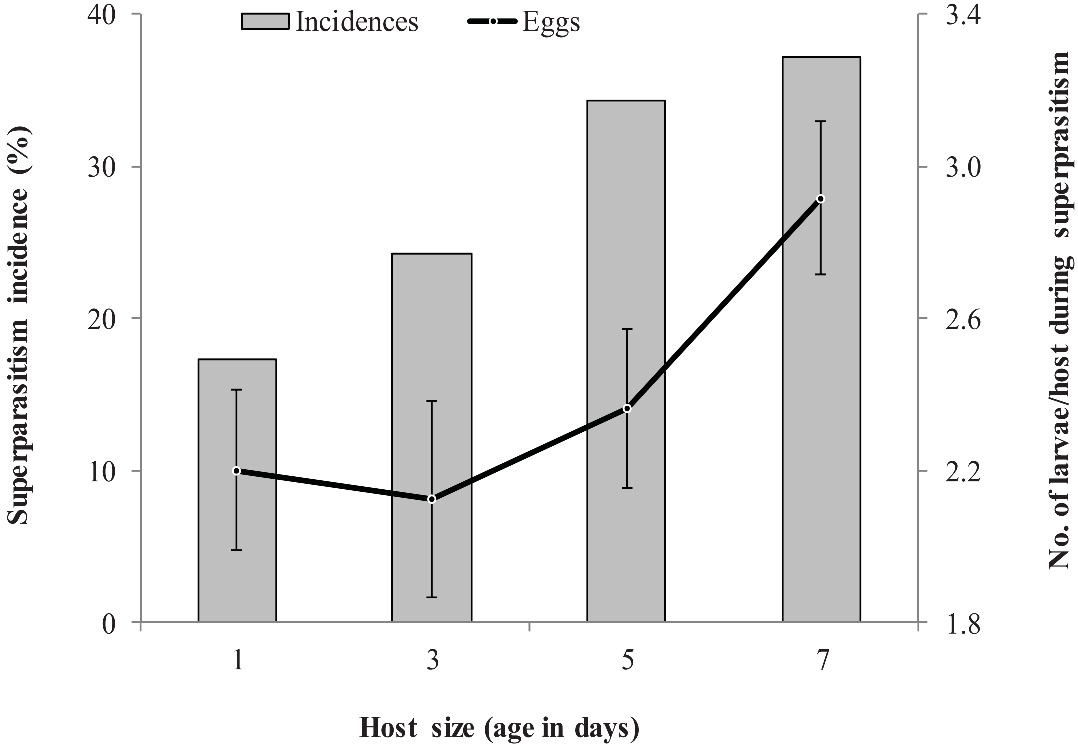

Figure 1: Incidence of superparasitism by *Diaeretiella rapae* among cabbage aphid hosts of differing size (age) and mean number of larvae per host after 4 days of parasitism. The error bars represent the standard error.

Overall, the mean number of eggs laid in different size hosts was not significantly different (P=0.07). However, when smaller (1 and 3 days old) hosts and larger (5 and 7 days old) hosts were grouped together, the difference in the mean number of eggs laid in larger and smaller hosts was significant (P<0.03). The number of parasitoid larvae found in superparasitised hosts varied from two to five per aphid.

The size of *D. rapae* larvae found in the dissected aphids 4 days after oviposition, was positively correlated with the size of aphid in which they developed (P<0.0001) (Figure 2).

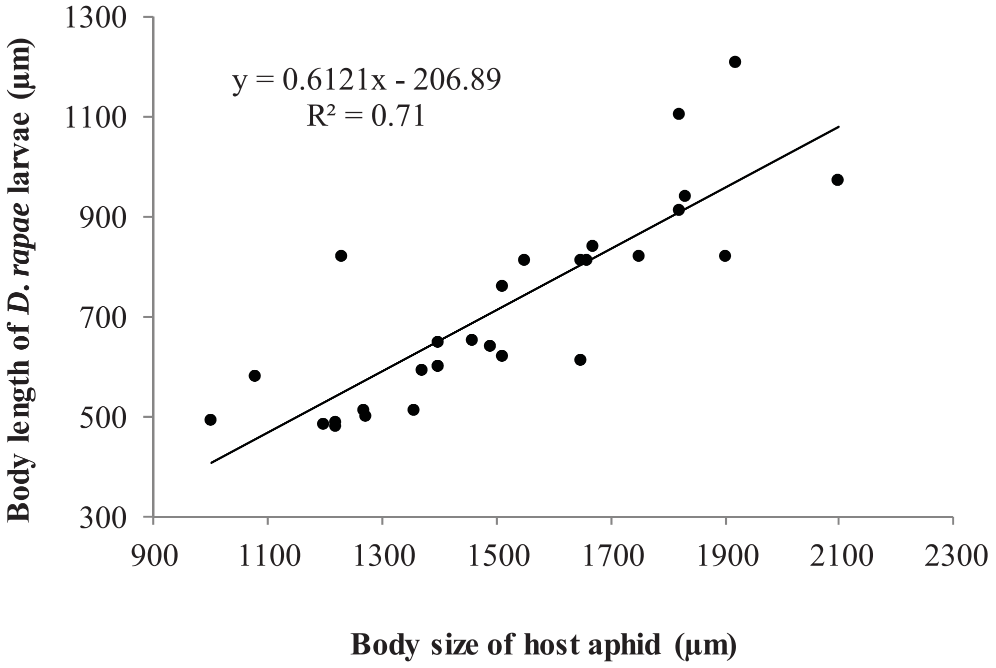

Figure 2: Relationship between the size of cabbage aphid hosts and the size of single *Diaeretiella rapae* larvae developing in them.

The size of the parasitoid larvae was also dependent on whether they developed as a single larva or as multiple larvae in superparasitised hosts ($P<0.001$). In larger hosts, the larvae were significantly larger when developed as a single larva than when developed as multiple larvae ($P<0.0001$) (Table 2). However, the size of larvae developed in smaller hosts did not differ significantly between single larva and multiple larvae ($P=0.25$) (Table 2).

Table 2: Size of *Diaeretiella rapae* larvae developing in *Brevicoryne brassicae* when single-larva and multiple-larvae were present per host. Same letter within a column indicates no significant difference (α=0.05).

Development condition of the *D. rapae* larvae	Larval size (µm)	
	Larger host	Smaller host
Single larva	841±56 a	581±41 a
Multiple larvae	549±19 b	505±46 a

The intensity of superparasitism (number of larvae ≥ 2 per host) did not influence the size of developing larvae significantly (P=0.71). The major difference in the size of larvae (10%) was found between the larvae developing in pairs (594.2±41.2 µm) and the larvae developing at five per host (539.7±34.1 µm).

Parasitoid attack and host defence behaviour

Ovipositing females of *D. rapae* had shorter host-handling time (P<0.001) and were less aggressive to the hosts (P<0.003) during superparasitism than first parasitism. Already-parasitised and unparasitised aphids defended themselves from the parasitoid attack, although defensive behaviour involved significantly more kicking in unparasitised aphids than in the already-parasitised ones (P<0.01) (Table 3). The number of times unparasitised and already-parasitised aphids shook their abdomen during the parasitoid attack did not differ significantly (P>0.06). Aphids also defended themselves by walking away from the parasitoid attack, and this behaviour was displayed by 83% of unparasitised aphids and by 51% of already-parasitised ones during the *D. rapae* attack (P<0.005).

Table 3: Comparison of behavioural responses in parasitoid-host encounters during first parasitism and superparasitism. Parasitoid (*Diaeretiella rapae*) expenditure per host was measured as time spent and the number of probes (insertions of the ovipositor) administered by the female. Host (*Brevicoryne brassicae*) defence behaviour per encounter was measured as the number of abdomen shakings and the number of kicks.

	Parasitoid expenditure		Host defence	
Oviposition	Time Spent (s)	Probes	Abdomen shaking	Kicks
First parasitism	29.7±3.9 a	7.2±0.6 a	3.1± 0.6 a	3.4±0.6 a
Superparasitism	8.8±1.1 b	4.7±0.5 b	1.9±0.3 a	1.4±0.1 b
F-value	26.35	9.91	3.67	6.74

Discussion

Females of *D. rapae* were able to discriminate the unparasitised hosts from the already-parasitised ones, and showed clear preference for laying eggs in unparasitised hosts when making oviposition decisions. In solitary parasitoids, after intrinsic competition among the larvae, only one adult individual emerges per host, irrespective of the number of eggs laid in that host (Godfray 1994c). Therefore, the ability of a female to distinguish unparasitised hosts from already-parasitised ones would allow her to save eggs from direct loss through superparasitism, and would probably gain reproductive success by increasing the number of parasitised hosts.

Females of *D. rapae* self-superparasitised hosts when they had repeated encounters with the same host. Repeated encounters would be expected when the number of hosts is limited. When the number of aphids available to a parasitoid is far less than the ovipositing capacity of the female, it might be advantageous to the female to lay more than one egg per host (Godfray 1994c; Zhang et al. 2010). Females of *D. rapae* used in this research were young, naïve and mated, and *D. rapae* females emerge with developed eggs (R. Kant, unpublished data). Therefore, egg loads of these females could have encouraged them to perform repeated oviposition in the available hosts, as high egg-loads in parasitoids encourage superparasitism (Keasar et al. 2006; Silva-Torres et al. 2009). Laying multiple eggs in one host increases the chance of survival and successful emergence of at least one individual (Rosenheim & Hongkham 1996; Zhang et al. 2010).

The number of eggs laid by *D. rapae* females in a host was far fewer than the number of times the females probed with their ovipositor. This suggests that some of these ovipositor probing activities of a female lead to oviposition and the rest may use for assessing the quality of the host before oviposition and injection of venom to paralyse host appendages to facilitate oviposition. Host paralysis in parasitoids is common. Idiobiont parasitoids permanently paralyse and stop growth and development of their hosts, while koinobiont parasitoids, including *D. rapae*, temporarily paralyse their hosts (Godfray 1994c) and allow them to grow, develop and reproduce (Zhang & Hassan 2003a). Earlier studies suggest that during attack, parasitoids not only target the main body but also the appendages (Phillips 2002; Kant et al. 2008a). The amount of time *D. rapae* females spent per encounter and the number of ovipositor probings they administered in already-parasitised hosts were fewer compared to unparasitised hosts. This weaker defence in already-parasitised aphids during superparasitism could be due to the temporary paralysis caused when these aphids were first attacked. Since the second parasitism was done just about an hour after the first parasitism, it is possible that the effects of paralysis from the first parasitism persisted in the aphids.

Growth and development of parasitoid larvae were influenced by both the size of hosts and the number of larvae developing per host. A direct effect of the host size on development of larvae is expected, because more resources are available in larger hosts compared to smaller ones (Godfray 1994c). The offspring of *D. rapae* developed in older, and also larger, *B. brassicae* are of relatively large size (Kant et al. 2009). This suggests that larger cabbage aphids offer more resources to developing parasitoid larvae. Since the host resources for developing parasitoid larvae are fixed, the more eggs laid in a host, the smaller share an individual larva gets during growth (Bai & Mackauer 1992).

The size of hosts *D. rapae* females encountered significantly affected their superparasitism decisions. The females preferred to superparasitise larger hosts. Some of the superparasitised small aphids died and those that survived had no more than two larvae in them. In contrast, up to five larvae were found in living larger hosts. Larger (older) hosts have stronger immune defence than the younger or smaller ones (Jervis et al. 2005). Therefore, laying more than one egg in a host could be an adaptive strategy of *D. rapae* females to weaken the defence system of the large host.

In addition, self-superparasitism may provide extra nutrition to the surviving parasitoid larva under low host density conditions. In aphid nymphs superparasitised by *D. rapae*, only one larva successfully develops into an adult after winning the intrinsic competition, and the winning larva consumes all the resources including the siblings (cannibalism) (Goubault et al. 2003) and thereby gains more nutrients. This study shows that when more than two larvae (up to five) of *D. rapae* developed in a host, they all gained equal mean body size, which suggests that the larvae utilised the available resources faster when the number of larvae in a host increased. When the resources in superparasitised host deplete quicker, the host may need more resources for its own survival and could eventually increase feeding. As *D. rapae* is a koinobiont parasitoid, which allows the parasitised host to live and develop, the multiple larvae developing in a superparasitised host may enhance the feeding efficiency of the host.

This study suggests that females of *D. rapae* maximise parasitism by avoiding superparasitism. However, superparasitism occurs at a low host density (Lester & Holtzer 2002b), which may be a strategy to enhance provisioning to the surviving offspring. Furthermore the superparasitism could also be a strategy to utilise the host resources more efficiently, which could maximise the quality (if not quantity) of the progeny. Further research is underway to examine the benefits of superparasitism in *D. rapae*.

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Chapter Thirteen

Manipulation of reproductive strategies by *Diaeretiella rapae* (Hymenoptera: Aphidiidae) while competing for host

Abstract

Parasitoid fitness depends on the ability to manipulate reproductive strategies when in competition. The present study investigated the parasitism and sex allocation strategies of the cabbage aphid parasitoid *Diaeretiella rapae* at a range of host and conspecific densities. The number of hosts parasitised by female *D. rapae* increased with the number of available hosts when they were foraging alone. However, the proportion of hosts parasitised by a foraging female decreased as host density increased. The proportion of female offspring produced decreased with elevated host density. Increase in the number of *D. rapae* females ovpositing together increased the total amount of parasitism, but the relative contribution of each female (i.e. number of nymphs parasitised) decreased. A smaller proportion of offspring were females when *D. rapae* mothers were competing for the same hosts. The decrease in the proportion of female offspring was mainly due to reduced emergence of females, while the number of males produced remained stable. Oviposition experience affected the sex allocation strategies of female *D. rapae*. Females foraging in the presence of one or more conspecifics increased their parasitm rate in their second oviposition bout, and also produced relatively more females in their second oviposition bout. Competing females had shorter lives. The study suggests that both host and foundress densities have significant effects on progeny production and longevity of foraging females.

Keywords: reproductive fitness, oviposition, foundress density, host density, longevity

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The earlier chapter showed that female *Diaeretiella rapae* prefer larger host for allocating fertilised eggs. In this chapter explores female's oviposition strategies in terms of conspecific competition for host.

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Introduction

The reproductive strategy of a parasitoid, in terms of oviposition rate and sex allocation, affects their fitness as well as the dynamics of host-parasitoid interaction (Hassell, 2000; Roitberg et al., 2001). The number of ovipositions and sex allocation choices of a female vary among species and depend on several factors, including competing for hosts at different host and parasitoid densities (Fellowes et al., 2005; Godfray, 1994).

In the field, the distribution of hosts varies spatially and temporally. While foraging, female parasitoids encounter hosts and competing conspecifics at varying densities. The foraging females can maximise their oviposition when hosts are abundant (Godfray, 1994). Dense populations of hosts also provide more opportunity for females to be host-selective during oviposition. When host density is limiting a female might need to make decisions that bring immediate benefits. For example, in a host-constrained situation, females might parasitise the available hosts irrespective of their quality, while abundant hosts' situation is expected to lead females to choose the best quality hosts for oviposition.

When the density of females exploiting a host patch together it results into intraspecific competition and interferes with oviposition (Outreman et al., 2005). In competitive situations, some species might increase parasitism rate to maximise their chance to produce progeny, while others might leave the patch and search for hosts elsewhere, or might temporarily postpone oviposition (Le Lann et al., 2011). Since parasitic wasps can control the offspring sex, and thus sex ratio of their progeny, it is important to know how females manipulate oviposition in host-constrained or in competitive situations. The sex allocation strategy of parasitoid females are influenced by intraspecific competition (Charnov, 1982; Fellowes et al., 2005; King, 1989) and is also likely to affect the host-parasitoid dynamics (Godfray, 1994; Ode & Hardy, 2008; West et al., 2002). Producing female offspring is important, but can be more costly than producing males as it involves fertilization of eggs before oviposition, so the oviposition decision of a female could bring long term consequences for the parasitoid population. Hamilton (1967) proposed the Local Mate Competition theory (LMC) which suggests that when females forage in the presence of conspecific females they tend to produce more males. Large number of males bring fitness to the mothers because their sons will compete for mates with non-sibling males (Somjee et al., 2011). Competing with conspecifics can also affect the physiological condition of the foraging females and the resulting offspring (Morag et al., 2011). The pro-ovigenic or weakly synovigenic species have a limited number of eggs, and become exhaustive quickly when they compete with other females which could also affect their longevity.

Unlike predators that directly consume their prey, host suppression efficiency of a parasitoid is directly linked to its reproductive fitness. An ability to manipulate reproductive strategies in adverse and favourable conditions can be an important attribute of parasitoids used in biological control programmes (Godfray, 1994; Roitberg, et al., 2001). Therefore, for use in biological control programmes, it is important to identify the response of the parasitoid to different host densities (Mills & Lacan, 2004). Various life history models have been developed to understand the effect of host and parasitoid densities on the population dynamics of the parasitoids (Hassell & May, 1973; Murdoch, 1975). In general, the models suggest trade-offs between fitness components such as progeny production, sex ratio, superparasitism, and resources available to the parasitoid (Bell, 1980; Reznick, 1985; Rosenheim, 1999).

The chances of encountering variable densities of hosts and conspecific parasitoids are higher when the host is sedentary and its reproductive cycle is short, like in aphids. In this research, we examined the relationship between reproductive success and host and female density in the cabbage aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *Diaeretiella rapae* is a cosmopolitan parasitoid attacking more than 50 different species of aphids in a range of agricultural and horticultural crops (Pike et al., 1999). Female *D. rapae* appear to manipulate their oviposition strategies when presented different densities of Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Bernal et al., 1994), and *D. rapae* reduces host handling time when *D. noxia* density is higher (Lester & Holtzer, 2002). However, it is not clear how females manipulate the offspring sex ratio in relation to host density or foundress density. Here, we investigated the following aspects of *D. rapae*: (1) in what way does the reproductive strategy (parasitism rate and offspring sex allocation) of *D. rapae* change in response to different host densities?, (2) how do females alter their reproductive strategies in competitive and non-competitive conditions?, (3) does oviposition experience affect progeny production and sex allocation behaviour of foraging females?, and (4) is longevity of foraging females affected by competitive conditions and availability of hosts?

Materials and methods

Parasitoid and host cultures

Cabbage aphid *Brevicoryne brassicae* and its parasitoid *D. rapae* were originally collected from a cauliflower field in Palmerston North, New Zealand. The insect cultures were maintained in an environmental chamber at 20 ^{o}C, 65% RH and 16 h light, on cabbage seedling (cv. Summer Globe Hybrid); all experiments were conducted in the same environmental conditions. Parasitoids used in this research emerged from 5-6 day old parasitised aphids, and were offered hosts of the most preferred size (5-6 day old) for oviposition (Kant et al., 2012a). The female *D. rapae* used in the experiments were 1 day old, mated and fed with 10% honey solution.

Host density and reproductive strategies of Diaeretiella rapae

Reproductive strategies of *D. rapae* (parasitism rate and offspring sex allocation) were observed at six host densities: 5, 10, 15, 20, 30 and 50 cabbage aphid nymphs. At each host density, aphid nymphs (5-6 day old) were offered to a single parasitoid female in a Petri dish for 2h. Each host density was replicated 12 times. After oviposition, the aphids were transferred to cabbage leaves for growth and development, and were observed until mummification. Each mummified aphid was transferred to a 2ml microfuge tube and regularly observed until the parasitoid emerged. The number and sex of the emerged adults were recorded.

Foundress density and reproductive strategies of Diaeretiella rapae

Reproductive strategies of *D. rapae* in a non-competitive (female foraging alone) and in competitive conditions at three foundress densities (2, 3 and 5 females) were studied at a constant host density of 30 aphid nymphs. In this experiment, pre-oviposition time (time between introduction of females in oviposition arena and the first oviposition), offspring number and offspring sex ratio were recorded. Parasitism rate, number of hosts parasitised per female, was compared among foundress densities. Females used in foundress density experiments were kept together for 30 min before they were moved into the oviposition arena together. At each foundress density, 5-6 day old nymphs were offered to the female(s) in a Petri dish for 2h. Each foundress density was replicated 10 times.

Oviposition experience and reproductive strategies of Diaeretiella rapae

The effect of oviposition experience on parasitism and sex allocation was studied at different host and parasitoid densities. Number and sex ratio of the offspring emerged from high (30 aphids per day) and low (5 aphids per day) host densities were examined. The oviposition of the females in those categories was recorded for three consecutive days (first, second and third day). In each category 12 females were tested. Similarly, the effect of oviposition experience on reproductive success was examined at different foundress densities (2, 3, and 5 females together). Each foundress group was offered 30 nymphs for two consecutive days, regarded as first and secon oviposition. Each foundress density was replicated 10 times. The number of offspring and the proportion of females produced were compared in each category. Pre- oviposition time (duration between female introduced in oviposition arena and first oviposition) was recorded

Effects of host and parasitoid densities on longevity of Diaeretiella rapae

Longevity of female *D. rapae* foraging at different host densities, and females foraging in the presence of conspecifics, was examined. To test the former, single females were offered 5, 10, or 30 hosts per day until they died. For each host density 12 females were tested. Similarly, to test the effect of foundress density, each female group (2, 3, and 5 females together) was offered 10 hosts per day for their entire life and their longevity was recorded. Each foundress density was replicated 10 times.

Statistical analysis

Parasitism rate, adult emergence, offspring sex ratio in host and parasitoid density experiments were analysed by generalized liner model (glm) function with family = poisson or family = binomial using R2.15.1. Analysis of variance (PROC GLM, SAS 9.2) was used to analyse differences in the means including, mean pre-oviposition time, mean number of offspring produced in oviposition experiment and mean longevity of females during host and foundress density experiments. Means were compared using a Tukey's studentised (HSD) test. Linear regression was used to analyse the relationship between host density and parasitism rate. Curve estimation (regression analysis) of adult emergence and sex ratio (female %) with relation to host density was done using the S-function of PASW Statistics 18.

Results

Host density and oviposition strategies of Diaeretiella rapae

The number of *D. rapae* adults emerged after parasitism of cabbage aphid nymphs increased with the density of hosts offered to females [sequential analysis of deviance: G = 138.37, d.f. = 66, P < 0.0001] (Figure 1). However, parasitoid emergence did not differ between host densities of 30 and 50 (P = 0.91). Similarly, the number of male and female also increased with host densities

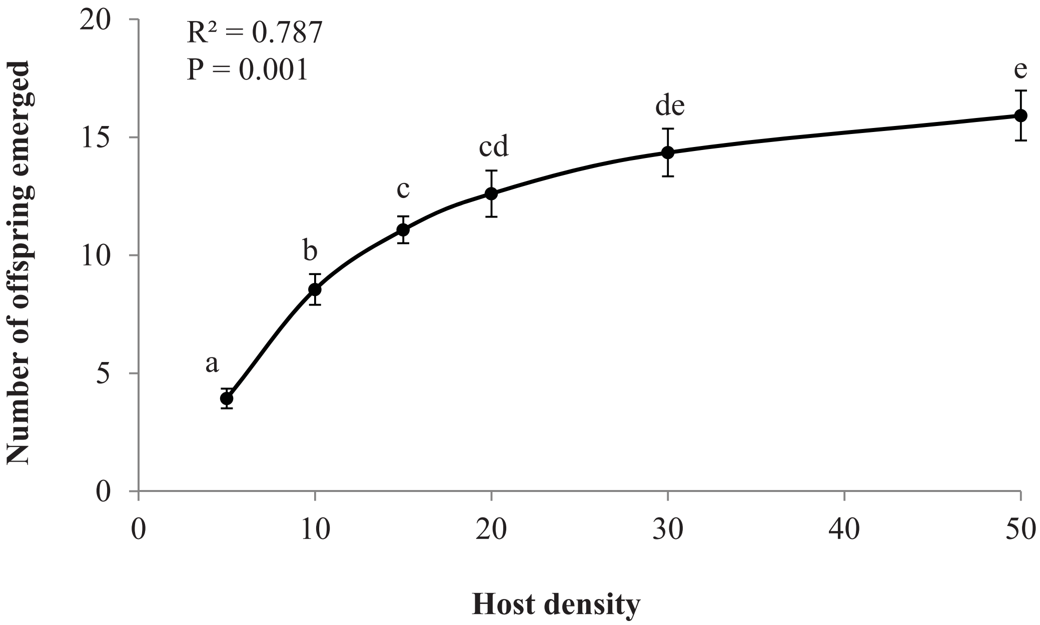

Figure 1: Relationship between the mean (±SE) numbers of *Diaeretiella rapae* offspring produced and the density of *Brevicoryne brassicae* nymphs offered, y = number of offspring produced, x = host density. Regression analysis was applied using S-function of PASW Statistics). Means with the same letters are not significantly different at α = 0.05 (Tukey's HSD).

Overall, the rate of parasitism (percentage of cabbage aphid nymphs parasitised) by *D. rapae* decreased with an increase in the host density ($F_{5, 66}$=21.16; P<0.001). However, the change in parasitism rate was not significant between 10, 15 and 20 host densities (Figure 2).

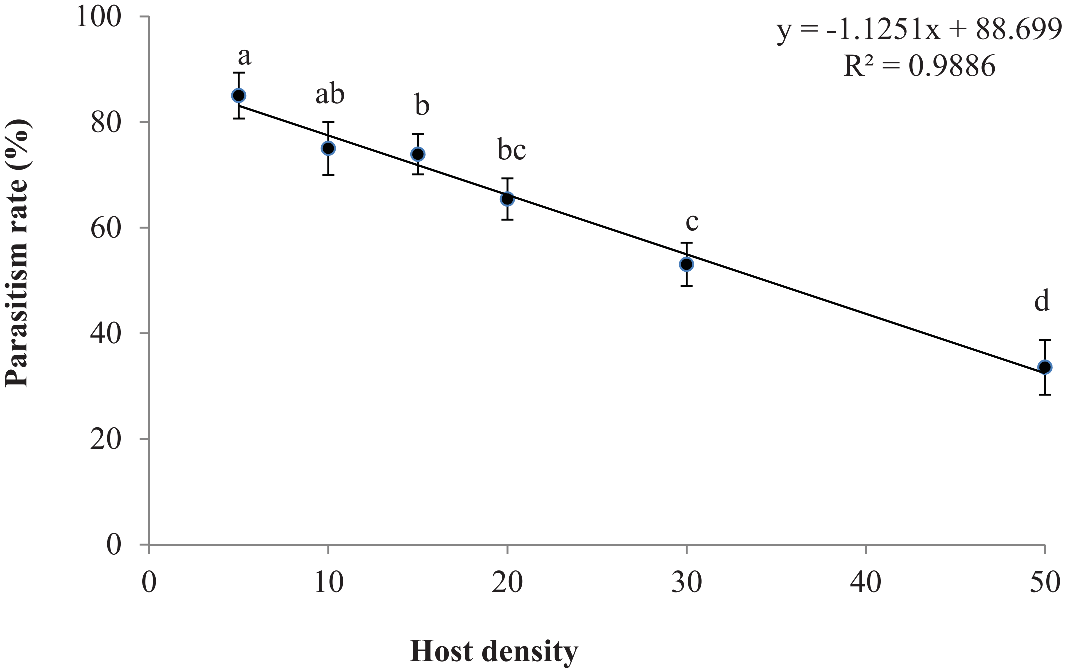

Figure 2: Parasitism rates (percentage hosts parasitised) of *Diaeretiella rapae* when offered *Brevicoryne brassicae* nymphs at different densities. A linear regression analysis was applied to examine the relationship. Means with the same letters are not significantly different (Tukey's HSD $P<0.05$), error bars represent the standard error.

The sex ratio (%female) of *D. rapae* offspring emerged from parasitism of cabbage aphids varied with the number of hosts offered ($F_{1,\,71} = 23.8$; $P < 0.001$) (Figure 3). The proportion of females initially decreased as host density increased from 5 to 10 to 15, but then plateaued at host densities of 20, 30 and 50 (Figure 3). The decrease in the proportion of female offspring was attributed to the increase in the number of male offspring, while the number of females emerged at different host densities was stable.

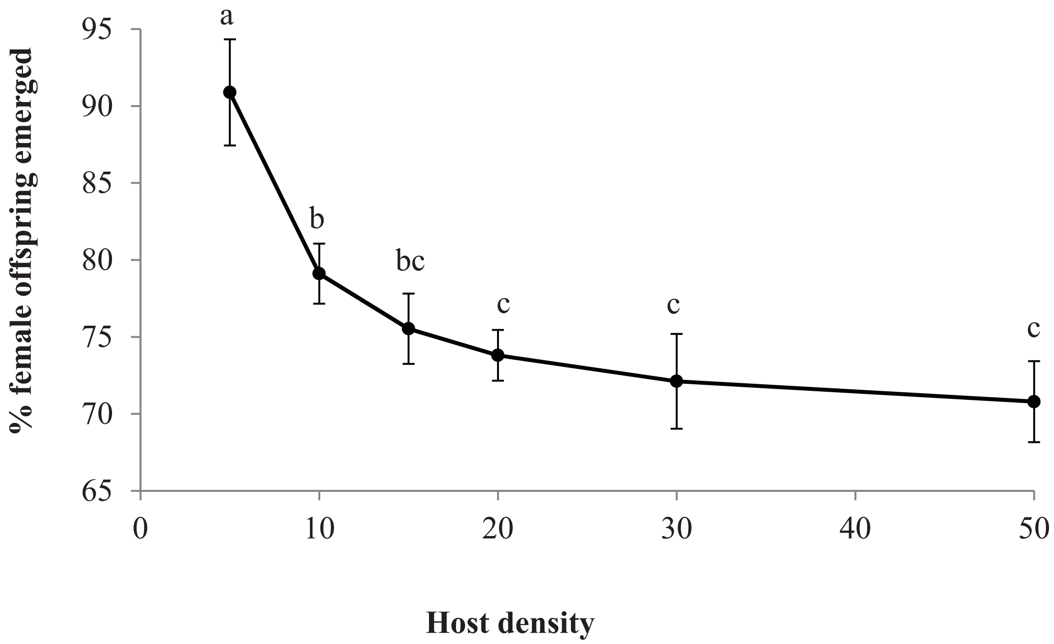

Figure 3: Relationship between percentage female *Diaeretiella rapae* produced (Sex ratio) and the density of *Brevicoryne brassicae* nymphs offered, y = female offspring percentage, x = host density. Regression analysis was applied using S-function of PASW Statistics. Means with the same letters are not significantly different (Tukey's HSD $P<0.05$), error bars represent the standard error.

Foundress density and oviposition strategies of Diaeretiella rapae

The total number of cabbage aphid nymphs parasitised by *D. rapae* increased with female density ($F_{3,\,44} = 3.43$; P = 0.024). However, the increase in the number of parasitised hosts was not in proportion to the increase in the number of females. There was no significant difference in the number of hosts parasitised whether one or two females foraging together (Figure 4).

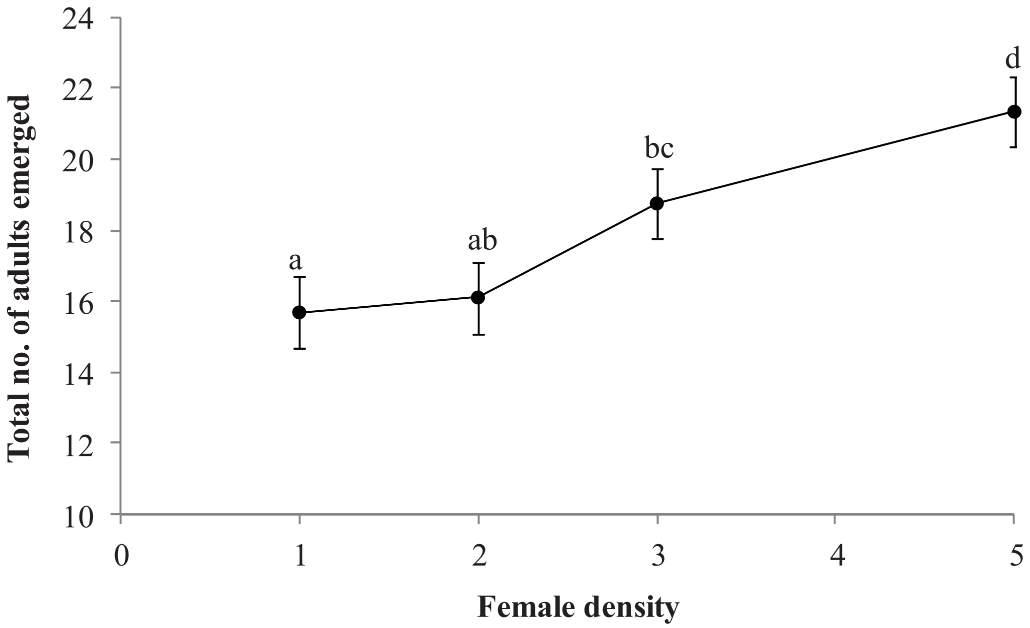

Figure 4: Mean (±SE) number of *Diaeretiella rapae* adults produced when *Brevicoryne brassicae* nymphs (30 individuals per day) were offered to different densities of mated female *D. rapae* (foundress density). Means with the same letters are not significantly different at α = 0.05 (Tukey's HSD).

The parasitism efficiency (number of nymphs parasitised per female) decreased in competitive conditions. The mean number of female offspring emerged per female decreased significantly with increasing foundress density ($F_{3, 44} = 49.05$; $P < 0.001$) (Figure 5). However, the number of males did not change significantly when two or more females were ovipositing together ($P = 0.26$).

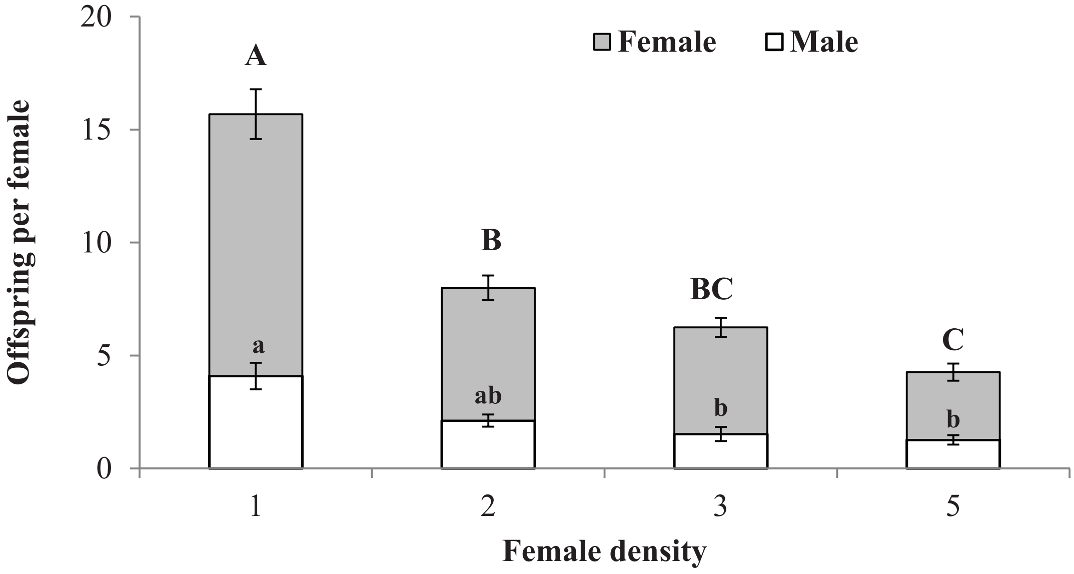

Figure 5: Mean (±SE) number of male and female offspring emerged per foundress *Diaeretiella rapae* from *Brevicoryne brassicae* parasitised at different foundress densities (number of females ovipositing together). Means with the same letters (capital letters for females, small letters for males) are not significantly different at α = 0.05 (Tukey's HSD).

Oviposition experience on reproductive strategies of Diaeretiella rapae

The parasitism rate and sex allocation strategies of females changed with their foraging experience. At high host density (30 hosts), the mean number of hosts parasitised on the first and second day was significantly greater than the number of hosts parasitised on the third day ($F_{2, 33}$ = 20.04; P = 0.001) (Table 1). However, the number of offspring emerged from first and second day at high host density parasitism was not significant (Table 1). The sex ratio (% female) remained same in all the three oviposition days ($F_{2, 33}$ = 0.62; P = 0.542)

In the case of low host density (5 nymphs), the mean number of hosts parasitised on the first, second and the third day was not significantly different ($F_{2, 33}$ = 2.31; P = 0.115). The pattern of daily sex allocation at low host density differed from sex allocation at high host. The proportion of female offspring emerged from third day oviposition decreased from the first day oviposition at low host density ($F_{2, 33}$ = 3.98; P = 0.028) (Table 1).

Table 1: Number and sex ratio (% female) of the offspring produced by mated female *Diaeretiella rapae* ovipositing alone at low and high host *Brevicoryne brassicae* densities. Within columns, same letters indicate no significant difference ($\alpha = 0.05$, Tukey's HSD test).

Oviposition	High host density (30 nymphs)		Low host density (5 nymphs)	
	Total offspring	% female	Total offspring	% female
First day	15.9 ± 0.9 a	72.1 ± 3.1 a	4.2 ± 0.2 a	90.4 ± 3.4 a
Second day	18.6 ± 0.9 a	74.8 ± 4.4 a	4.2 ± 0.2 a	80.4 ± 4.7 ab
Third day	10.3 ± 0.9 b	77.5 ± 2.3 a	3.6 ± 0.2 a	71.1 ± 5.9 b

Similarly, oviposition experience influenced the reproductive strategies of *D. rapae* when they were foraging in presence of other conspecifics. The number hosts parasitised by female *D. rapae* depends on the number of females foraging together ($F_{2, 66} = 4.31$; $P = 0.017$) and the oviposition experience of the females ($F_{1, 66} = 21.54$; $P < 0.001$) (Table 2). The mean number of female offspring produced by female *D. rapae* increased during second oviposition (17.6 ± 0.8) than in the first oviposition (13.7 ± 0.8) ($F_{1, 66} = 13.43$; $P < 0.001$).

Table 2: Effects of oviposition experience (first vs. second oviposition) on mean (±SE) number of *Diaeretiella rapae* offspring emerged after parasitism of *Brevicoryne brassicae* at various foundress densities. All foundress density treatments were offered same number (30) of cabbage aphid nymphs. Within columns, same letters indicate no significant difference ($\alpha = 0.05$, Tukey's HSD test).

Foundress density	First Oviposition		Second Oviposition	
	Male	Female	Male	Female
2	4.2 ± 0.4 a	11.7 ± 1.1 a	3.1 ± 0.5 a	16.1±1.5 a
3	4.6 ± 0.5 a	14.1 ± 1.2 b	2.9 ± 0.4 a	17.7±1.2 ab
5	6.3 ± 0.5 b	15.0 ± 1.4 b	3.8 ± 0.4 a	18.9±1.4 b

Effects of host and parasitoid densities on longevity of female Diaeretiella rapae

Longevity of female *D. rapae* was affected by both the number of females ovipositing together and the number of hosts offered to the female when she was foraging alone. Females offered fewer hosts per day lived longer than those offered hosts at higher density every day ($F_{2, 33} = 7.12$; $P = 0.003$) (Figure 6).

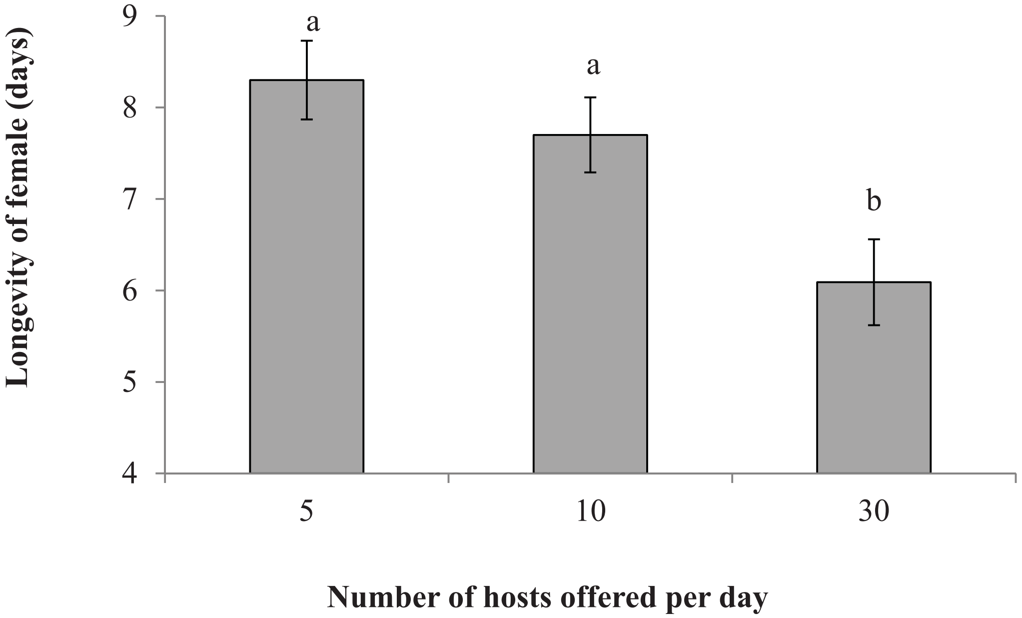

Figure 6: Mean (±SE) longevity of female *Diaeretiella rapae* offered different densities of *Brevicoryne brassicae* nymphs per day. Means with the same letters are not significantly different (Tukey's HSD P<0.05).

The female *D. rapae* lived relatively shorter lives when they oviposited in the presence of other females ($F_{3, 43} = 11.15$; $P < 0.001$) than when they were alone (Figure 7).

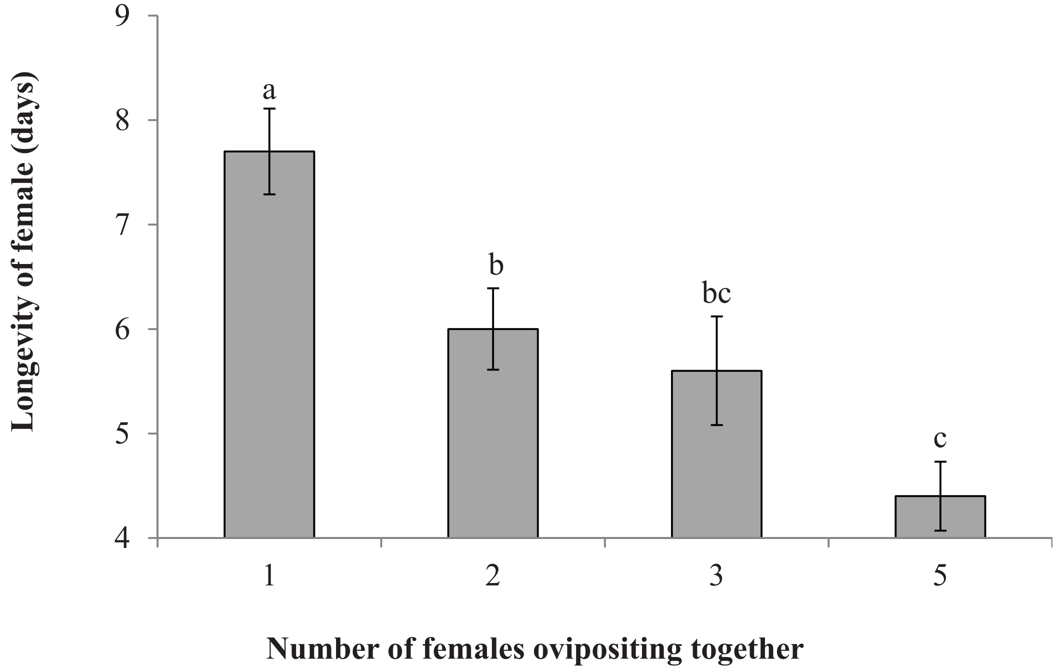

Figure 7: Mean (±SE) longevity of female *Diaeretiella rapae* when offered *Brevicoryne brassicae* nymphs (10 individuals per day) at different foundress densities. Means with the same letters are not significantly different (Tukey's HSD P<0.05).

Discussion

The effects of competition on *D. rapae* oviposition strategies were clearly observed in this study. Female *D. rapae* preferred to allocate more fertilised eggs (female offspring) when hosts were limited, but produced more males when foraging in the presence of conspecifics. This confirms that female *D. rapae* can facultatively manipulate their oviposition strategies to maximise reproductive fitness gain.

The number of cabbage aphid nymphs parasitised by *D. rapae* increased as the density of nymphs increased. At high host density, the rate of host-parasitoid encounters is greater, resulting in more chances for parasitoid oviposition. The oviposition behaviour of *D. rapae* is consistent with the classic type II functional response, which suggests that with increasing host density the total number of hosts parasitised by a parasitoid increases, but the proportion of hosts parasitised by each female decreases (Bernal, et al., 1994; Holling, 1959). Parasitoids respond to kairomones (chemicals present in the host) that induce oviposition, and hosts at higher densities are more attractive to parasitoids (Battaglia et al., 2000). Elevated densities of *B. brassicae* probably stimulated *D. rapae* more than when fewer aphids were present, resulting in increased interaction with hosts. Honeydew produced by *B. brassicae* provides foraging female *D. rapae* with information about quantity and quality of hosts (Shaltiel & Ayal, 1998). However, *D. rapae* foraging *D. noxia* did not responded quicker when the *D. noxia* density was higher (Lester & Holtzer, 2002). This could be due to *D. rapae* being less responsive to *D. noxia* than *B. brassicae* density. Several studies have found that *B. brassicae* is a preferred host of *D. rapae* (Bernal et al 1994; Kant et al 2012a).

A positive effect of host *B. brassicae* density on parasitism rate has also been found in *D. rapae* foraging in an open uncontrolled environment (Kant et al., 2012b). Since *D. rapae* females emerge with some mature eggs (R. Kant, unpublished data), a female increases its fitness by maximising parasitism. However, in the present study, the proportion of hosts parasitised by *D. rapae* decreased as host densities increased from 10 to 50. This could be either because the amount of time available to the female (2h) was insufficient to parasitise all available 50 hosts or females get exhausted and could not replenish their egg stock fast enough to do more oviposition. While foraging, female *D. rapae* spend more time and energy (stings and eggs) per host due to repeated encounter when they are provided low density hosts (Kant et al., 2008; Lester & Holtzer, 2002).

A greater proportion of female offspring emerged at low than high host density, from 5 through 15 hosts per female. The decrease in the proportion of female was mainly due to increase in number of males at high host density. When females get a chance to produce few offspring they generally produced female offspring this probably because the virgin female can still reproduce although produce only males (Godfray, 1994). Furthermore, female *D. rapae* also superparasitise its host to manipulate progeny sex ratio to produce female offspring at low host density (Kant et al., 2011). However, the proportion of female remained around 70% despite the host density reached 50 (Fig 3). This suggests that female *D. rapae* kept the male number minimal while manipulated their number based on host density (Abe et al., 2009; West, 2009). The initial increase in the proportion of males produced by female *D. rapae* was probably to have enough males in the population. By producing fewer males, mothers reduce competition among their own sons when the number of females is limited (Godfray, 1994; Somjee, et al., 2011). Polygynous and monandrous nature of *D. rapae* facilitate all females to mate even the proportion of females in the population is greater than proportion of males (Kant et al.

unpublished data). Furthermore, haplodiploidy nature of D. rapae allows females to reproduce even before mating. This reflects the female's ability to alter its reproductive strategy to achieve long term gain.

The number of female *D. rapae* foraging together affected the reproductive behaviour of each female. When two or more females foraged together, they attacked their hosts quicker than when foraging alone. Sometimes, a host was attacked by two females simultaneously from different sides, which probably resulted in their interfering with each other. The shorter pre-oviposition time suggests that females are more active and responsive in attacking and ovipositing their hosts in the presence of competition. Although the total number of host parasitised by *D. rapae* increased with higher foundress density, the per capita parasitism rate decreased. This may be due to the increased frequency of female-female encounters, which can cause mutual interference and eventually affect the foraging efficiency of the females (Elliott, 2003; Hassell, 2000; Kristoffersen et al., 2001; Lynch et al., 1998). If ovipositing females walk away from hosts and avoid oviposition due to female-female interference, the parasitism efficiency of each female is adversely affected. Thus, higher foundress density resulted in less time being available to each female for oviposition, and influenced oviposition strategies. A game-theory model predicts that females adjust oviposition/clutch size when they experience more female competition during progeny production (Ives, 1989).

Female *D. rapae* can discriminate between parasitised and unparasitised hosts, but superparasitise at low host density (Kant, et al., 2011). Since *D. rapae* females parasitised only about 50-75% of their hosts at high host density, the chances of superparasitising the hosts (ovipositing more than one egg per host) might be low when hosts are abundant. This allows the female to avoid competition for their developing offspring (parasitoid larvae). Competition also affected the sex allocation strategy of *D. rapae*. Fewer female offspring were produced when foundress females oviposited together. This was attributed to a decrease in the absolute number of female offspring, while the number of male offspring did not change significantly. Hamilton's model (1967) states that when two or more females are ovipositing together, the proportion of female offspring in the population decreases.

The results oviposition experiences suggest previous experience helps female *D. rapae* to manipulate their reproductive and sex allocation strategies. When females oviposited in the presence of conspecifics their parasitism rate increased after first oviposition bout. The foundress also produced more female offspring in the second oviposition bout. Furthermore, females when foraging with 4 other competitors they also reached their end of life on second oviposition that occurred on the third day. Thus, females might utilise their stored sperm for producing more female offspring during their second oviposition. However, increase in proportion of female offspring on second oviposition also occurred when females were competing with just one or two females and they were in their middle of life. We can speculate that the foundress produced more females in order to compensate the population sex ratio to increase the overall productivity of the patch (Abe & Kamimura, 2012).

Competition for hosts reduced the longevity of female *D. rapae*. The presence of conspecifics increased the activity of females and frequency of female-female interactions, exhausting females during foraging. Physiological changes may occur in females when they are constrained and competing for limited hosts, and this reduced longevity has also been recorded in the parasitic wasp *Copidosoma koehleri* when reared in the presence of other conspecifics (Morag, et al., 2011). Female *D. rapae* also had shorter lives at high host density probably, because more resources were utilised for prasitising a large number of available hosts. This suggests that resources available to the female for reproduction and metabolic maintenance are not separate, and can be interchanged as required (Ellers et al., 2000; Francisco, 2001).

Reproductive strategies play an important role in the effectiveness of insect parasitoids as biological control agents. The results of this study indicate that female *D. rapae* adjust their reproductive strategies with changes in host densities and in the number of females foraging together. Females increase parasitism rate by allocating their resources for reproduction at high host density, while at low host density the same resources are diverted towards body maintenance for enhancing longevity and extending the oviposition period. Similarly, when females start ovipositing in a competitive situation, they reduce the oviposition rate and allocation of fertilised eggs. This might be a strategy to save the sperm and eggs for future oviposition; otherwise, these eggs and sperm (when ovipositing fertilised eggs) may be lost through superparasitism.

Acknowledgements

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General Discussion and Conclusions

Each chapter of this thesis has its own discussion. The purpose of this section is to integrate the components of the thesis.

Reproductive fitness of *D. rapae* depends on its behaviour during mating and oviposition. The two activities are not mutually exclusive, but were found to complement each other for maximising fitness of *D. rapae*. Reproduction in parasitoids occurs at the cost of its host (Godfray 1994). Therefore, there is a direct relevance of understanding the cause-effect relationship between reproductive behaviour and efficiency of parasitoid in biological control programmes.

Study on parasitism strategies of *D. rapae* in an uncontrolled/open environment showed a low reproductive output of *D. rapae* at low temperature in early season [Chapter 1]. However, the parasitism rate increased in early summer compared to early spring. Results on the performance of this species from other corners of the world also identified low output at the beginning of the season (Liu 1989b; Zhang & Hassan 2003; Shukla et al. 2008). This suggests a host-parasitoid asynchrony between *D. rapae* and its host population in early season in New Zealand. The proportion of females in the *D. rapae* population also increased significantly when the temperature became more favourable to parasitoid development in early summer. This indicates that the increase in the proportion of females during early months of the season directly favoured parasitism rate in *D. rapae,* because females are directly responsible for controlling the host population. Males are essential, but their number probably is not required to be on par with females. Various factors that affect the sex allocation in parasitoids are discussed below.

Application of sex allocation theories to *Diaeretiella rapae*

Although the theory of natural selection favours the equal production of male and female offspring (Fisher 1930), sex ratio in parasitoids is usually divergent from the equality (Godfray 1994). Sex allocation in haplodiploids is predicted by two main theories, Local Mate Competition (Hamilton 1967) and Conditional Sex Allocation (Charnov 1979). The former suggests that the proportion of female offspring (daughters) produced by a parasitoid is influenced by the number of females (foundress density) foraging together. The latter suggests that good quality hosts often attract parasitoids to deposit fertilised eggs (female offspring), while lower quality host receives unfertilised eggs (male offspring).

As per the prediction of Hamilton's theory, the oviposition behaviour of *D. rapae* changed when two or more females were foraging together [Chapter 13]. In the presence of conspecific competition, the proportion of female offspring decreased in *D. rapae*. This was mainly due to a decrease in the number of female offspring produced per female in the presence of conspecific competition, compared to when there was no competition during oviposition. There was no significant difference in the number of male offspring produced per female, whether the oviposition occurred in the presence or absence of competition. The females probably treat a host patch as being of poor quality when more than one female is present on it (Visser et al. 1992). Parasitoids often prefer to oviposit unfertlised eggs on a poor quality patch, while looking for good quality patch to oviposit fertilised eggs to produce female offspring (Schreiber et al. 2002). In insect parasitoids, the hosts are not consumed immediately after attack, but remain in the environment after being exploited, and are vulnerable to attack by competitors or other predators. Thus, it can be inferred that competition for hosts can negatively affect the reproductive behaviour of a female parasitoid.

The validity of Conditional Sex Allocation theory was tested in *D. rapae* based on the argument that an ovipositing female would invest a large share of its resources where she will get maximum benefit (Trivers & Willard 1973). Therefore, foraging females should choose high quality hosts for allocating fertilised eggs while using smaller hosts for unfertilised eggs. Female *D. rapae* preferred to lay their fertilised eggs in older/larger host in a host-choice test, even after spending more energy and time to overcome the defensive behaviour of these aphids [Chapters 9 and 10]. However, when *D. rapae* females were offered large and small hosts in a no-choice test, the overall sex ratio of offspring produced by female *D. rapae* was not significantly affected by the size of host, whether offered only small or large hosts. This shows that the relative quality of a host plays an important role in oviposition decisions of *D. rapae*.

The sex ratio of offspring was further affected by mating delay in *D. rapae* females [Chapter 8], and mating with a sperm-depleted male [Chapter 5]. Since female *D. rapae* mates once in her life time, if she mates with a multiple-mated male she may suffer sperm shortage, and eventually produce fewer females and more males than the female mated with a virgin male. Similarly, female *D. rapae* whose mating was delayed for four or more days produced more male offspring than the females mated immediately after emergence [Chapter 8].

The proportion of females and males in a population is very important during oviposition, and is a crucial factor in the mating success and pest suppression ability of a parasitoid (Ode & Hardy 2008). The increase in proportion of male offspring resulting from foundress competition suggests that *D. rapae* might not produce the offspring sex-ratio desired in mass production for biological control. However, the problem of low female/male sex ratios in mass production could be reduced by releasing already mated females, enabling the females to produce more female offspring than those spending time in locating mates. The research also suggests that use of virgin and young males during mating might help in producing more female offspring.

Preference-Performance Hypothesis

Preference-performance hypotheses have mainly been used to understand the consequence of a preference by analysing the performance in insects. The hypothesis predicts that oviposition (preference) should correspond to host suitability for offspring development, and female parasitoids are thus assumed to maximize their fitness by ovipositing in high quality hosts (Jaenike 1978). It has been applied in behavioural testing of insects including preference for different host size, host age, host-insect species, and host-plant species. This hypothesis is analysed in this study for understanding different aspects of reproductive behaviour of *D. rapae*.

The preference-performance hypothesis was first examined in terms of host-age preference in *D. rapae,* and was analysed in terms of reproductive performance of offspring emerged from younger and older hosts. Unlike predators, which often consume several prey individuals to complete their development, development of parasitoids depends on the resources available in a single host. This drives parasitoid evolve to be more selective for hosts (Vinson & Iwantsch 1980; Godfray 1994; Strand & Casas 2008). Host searching and oviposition behaviour of *D. rapae* clearly showed that females preferred to attack older/larger hosts over younger/smaller hosts [Chapter 9]. Female *D. rapae* spent much more time (host handling time) and energy (in terms of number of probes per host) on older than younger hosts. The preference for larger hosts returned a fitness gain to female *D. rapae* [Chapter 10]. The adults emerged from older (larger) hosts were larger in body size, lived longer and the females had higher fecundity than females emerged from younger (smaller) hosts [Chapter 11]. Females emerged from large hosts produced about 60% more offspring than the females emerged from smaller hosts, and the proportion of female offspring was also higher in the former. The number of female offspring resulting from larger males was 16% more than the number of offspring resulting from small males. This suggests that it is beneficial for female *D. rapae* to oviposit fertilised eggs in larger hosts. The size of a parasitoid is usually directly proportional to host size/age, and a positive correlation between paraitoid size and its fitness has been found in other studies (Charnov 1979; Charnov 1982; Cloutier et al. 2000). Therefore, the preference of females to deposit fertilised eggs in larger hosts for producing larger female offspring validates the hypothesis of larger hosts providing more resources to the developing parasitoid larvae compared to smaller hosts (Stephens & Krebs 1986).

The haplodiploid nature of reproduction in *D. rapae* gives females an option to choose between mating and oviposition. However, only sons are produced by the unmated female. Therefore, oviposition before mating could lead to overproduction of males in the parasitoid population (Godfray 1994). Male *D. rapae* emerge before females (protandry) and the majority of males also emerge within a few hours after the onset of daylight, while females emerge late and their emergence occurs throughout the light period [Chapter 2]. Late emergence of females further strengthens their choice between mating and oviposition during foraging. Therefore, it is an important to test the preference between mating and oviposition in *D. rapae* female, and how their choice affects their performance (reproductive fitness).

When the choice between mating and oviposition was tested in laboratory conditions it was found that about two-third of female *D. rapae* mated before oviposition, even when they had much greater chances of encountering hosts than potential mates [Chapter 7]. The other one-thirds of females oviposited first before mating, but they produced only 10% of their total oviposition before mating. This clearly indicates the preference of *D. rapae* for mating before oviposition. Female *D. rapae* also oviposited the majority of their eggs only after fertilising them to produce female offspring, which could only be achieved after mating in haplodiploids. Mating in *D. rapae* was very quick and successful in the presence of host aphids. The females who oviposited before mating also superparasitised hosts (oviposited in the already parasitised host) if there insufficient unparasitised hosts present. This probably caused loss of some eggs (fecundity) in the females who oviposited before mating. In another experiment, when effect of mating delay was tested in *D. rapae* [Chapter 8], it was been found that females loose fitness by overproducing males. This shows that choosing to mate before oviposition brings fitness gains in *D. rapae* female. Therefore, it can be inferred that in the presence of mates and hosts, a virgin female should prefer to mate first before ovipositing to increase its reproductive fitness.

This study clearly identified preferences of *D. rapae* adults during host-size selection and mating/oviposition choice. These preferences are directly linked to fitness gain in *D. rapae*. The understanding of these preferences in *D. rapae* can be directly applied in a biological control programme. For example, female parasitoids can be offered large hosts to produce more efficient adults in mass production. The study also suggests that a release of mated females may increase the proportion of females in the population and provide quicker suppression of pest population.

Effect of intrasexual and intersexual competition in *Diaeretiella rapae*

Parasitoids often compete for hosts and mates in the field situation (Godfray 1994), and the presence of competitors and degree of competition changes the foraging behaviour of parasitoid (Field 1998).

Female *D. rapae* also displayed a change in their oviposition behaviour in the presence of conspecific competitors during foraging. Female *D. rapae* did not avoid oviposition in the presence of competition, but actively parasitised the hosts [Chapter 13]. Incidents were recorded when two females attacked the same host at the same time. Although female *D. rapae* were able to discriminate between the parasitised and unparasitised hosts, when competing for hosts, *D. rapae* females laid up to nine eggs in a host despite only one adult emerging per host (superparasitism) [Chapter 12]. Superparasitism is the general means of competition in parasitoids (Haccou & van Alphen 2008). The proportion of female offspring that emerged from hosts parasitised under competitive conditions was less than that emerged from hosts parasitised without competition. This shows that although females remained active during oviposition despite the competition, they altered their reproductive strategies. Females also lived for shorter periods when they foraged in the presence of other females, which was probably due to intense intrasexual competition for hosts.

Superparasitism possibly exhausted the ovipositing females, suggesting that the females invested more energy in reproduction under competitive conditions. Food provisioning to adult *D. rapae* had positive effects on their longevity and reproductive potential [Chapter 6]. Therefore, it is suggested that females could perform better in the availability of an assured supply of food. However, neither female nor male *D. rapae* required food immediately after emergence in order to mature their eggs or sperm.

Foraging behaviour of *D. rapae* also changed with host density. The rate of host-parasitoid encounters increased with increasing host density. The higher host-parasitoid encounter rates increased chances of females to parasitise their hosts. The oviposition behaviour of *D. rapae* is consistent with the classic type II functional response which suggests that increase in host density increases the total number of parasitism, but proportion of parasitism decreases with density (Holling 1959). Superparasitism was observed in *D. rapae* when host numbers were limited [Chapter 12]. This was probably because females spent more time per hosts, resulting in repeated encounters with the same host [Chapter 9]. In field conditions, kairomones present in the host induce oviposition in parasitoid species (Battaglia et al. 2000). Earlier studies on *D. rapae* found that honeydew produced by *B. brassicae* provides qualitative as well as quantitative information to the foraging female (Shaltiel & Ayal 1998).

Presence of inter- and intrasexual competition was found in *D. rapae* during mating. When male and female *D. rapae* were paired for mating, males were more active courting for mating, while females showed “coy” behaviour [Chapter 3]. However, the mating decision was generally made by the females. Sexual selection comes to play when one sex becomes more selective, and generally it is females who choose the mate (Andersson 1994; Moynihan & Shuker 2011). Sexual selection is an important part of the theory of natural selection described by Darwin in “On The Origin of Species” (1859) and “The Descent of Man and Selection in Relation to Sex” (1871). Sexual selection influences individual fitness in two different ways - by making themselves attractive to the opposite sex (intersexual interaction) and by deterring same-sex rivals (intrasexual interaction). Intersexual selection occurs before copulation (pre-copulatory selection) through various morphological/external traits or through behavioural display including courtship [Chapter 3], and after copulation (post-copulatory selection) through sperm competition. Since female *D.*

rapae mates once in its life [Chapter 5], like some other hymenopterans, therefore, there is no opportunity for sperm selection by female *D. rapae*. Thus, it is presumed that the pre-copulatory mate selection in *D. rapae* must be intensive.

When more than one male was competing for a female, severe intrasexual contest came into play which changed the behaviour of males [Chapter 4]. The initial female-male contest often changed to a female-male-male contest. The arrival of an additional male interfered with male-female mating and ultimately became a male-male contest. The mating female eventually escaped mating [Chapter 4]. A similar phenomenon was found in an open/uncontrolled environment where about half of the mating pairs observed were male-male [Chapter 1]. Male *D. rapae* become sexually receptive immediately after emergence, more actively participate in mating than females [Chapter 3], and fight for mates if the operational sex ratio is male-biased or when more than one male is present during mating.

Male *D. rapae* emerged before females and mated several times in their life, but mating in female *D. rapae* occurred only once [Chapter 3]. This indicates that despite a large number of female *D. rapae* physically present in the population, the average number of females who can participate in mating is less than the number of males, creating a male-biased called Operational Sex Ratio (OSR) (Emlen 1976). Therefore, males have to compete for females in a male-biased OSR and pass through strong intrasexual conflicts before they secure virgin females. Mating systems in species with skewed OSRs have intense sexual selection, and male-male competition for mates becomes severe (Emlen & Oring 1977). The high concentration of male *D. rapae* could cause mating interference and consequently mating delay [Chapters 1 and 4]. Mating delay adversely affected the fitness of both male and female *D. rapae* [Chapter 8]. Due to intrasexual contests among males, females looking for mates might not find the appropriate mate, compromising with mate selection. When a female *D. rapae* mate with a multiple-mated male, it can become sperm-depleted and produce more male offspring [Chapter 5]. Therefore, it can be inferred that the competition severely affects the parasitoid's reproductive behaviour and the reproductive efficiency of the species, and host-parasitoid dynamics are affected due to interference and aggression of males in biological control (Haccou & van Alphen 2008).

There is an increasing worldwide pressure for using biological control and integrated pest management (IPM) in order to minimise chemical pesticide use in agriculture. Demands for organic products (or products without spray residues) have been growing due to the awareness of the harmful effects of pesticides on humans and the natural environment. Moreover, increasing incidences of pesticide resistance have led to sudden pest outbreak. Insect outbreaks are predicted to increase with global warming and climate change through shifts in insect populations and through disruption of community interactions (Stireman et al. 2005; Gerard et al. 2010). Thus, the problem of insect pests is likely to intensify in the future. In such a scenario it is important to find alternative solutions for pest management. Biological control is one strategy for response to the problem of pest infestation and insecticide resistance arising due to climate change and overuse of chemical pesticides. To design effective biological control it is important to understand the interaction between the pest and biocontrol agent. This study provides comprehensive information on interactions between cabbage aphid and *D. rapae*, a system of horticultural significance. The results of this study will contribute to the development of effective biological control programmes for cabbage aphid and other aphid species using *D. rapae* or other parasitoids in New Zealand and elsewhere.

A lack of synchrony between cabbage aphid and its parasitoid *D. rapae* has been reported in this and other studies. Since the reproductive efficiency of a parasitoid is directly linked to its parasitism efficiency the finding of a low reproductive output of *D. rapae* in early season is likely to be the main reason this asynchrony. Better synchronisation could be achieved by inundative early season field release of lab-reared adult *D. rapae*, especially in organically-managed crops. Release of females only or highly female-biased *D. rapae* population would be most effective and quickest in suppressing aphid population. In glass houses, it is recommended that the sex-ratio of adults be monitored closely, with addition females being released to maintain the sex ratio at an effective level for maximum suppression by *D. rapae*. The proportion of females in glasshouse biocontrol is even more important than in open field situations. The study suggests that one male per 3-4 females could be an ideal sex ratio of *D. rapae* for the management of aphids. Releasing mated, 1-2 day old and honey fed females could further increase the suppression of aphid populations using this parasitoid.

The preference for large hosts (5-7 day old) shown be *D. rapae* can be used for mass production of females in insectaries. Females foraging under condition with less intensive competition produce female-biased broods. This behaviour could also be utilised for producing more females in insectaries. Light triggers mating and oviposition in *D. rapae*; therefore, it is important to maintain proper light in insectaries and glasshouse to improve the efficiency of parasitoid mass production and biological control, respectively. Releasing parasitoids early in the morning and on sunny days is likely to provide better aphid suppression. Female *D. rapae* also superparasitise at low host density and are capable of discriminating among potential hosts based on their quality. Therefore, a ratio of one female per 10-15 aphid nymphs during field release could minimise superparasitism and achieve better suppression of the aphid population.

In this thesis, I have reported and discussed the mating and oviposition behaviour of *D. rapae,* and a range of factors, mostly biotic, that affect its reproductive fitness. The research has provided vital information on the host parasite interaction in this aphid-parasitoid system. The study suggests that strong intrasexual competition and intersexual selection exists during mating and oviposition in *D. rapae*. Female *D. rapae* manipulate their mating and oviposition strategies to maximise fitness gain. This study identified a need for inundative release of the parasitoid, especially in early spring when the population of *D. rapae* is not on par with the aphid population. Sex ratio plays an important role in a parasitoid's ability to suppress a pest population. Overproduction of males, a well-known problem in mass production, can now be minimised by manipulating the oviposition behaviour of the parasitoid by offering hosts of a specific age or size during oviposition. Findings on the importance of adult nutrition for reproductive potential and longevity of *D. rapae* suggest that provisioning artificial food such as honey solution in glass houses/insectaries, or growing nectar-producing plants in the field, could further increase the efficiency of *D. rapae* in biological control programmes.

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Appendix 1: Phylogenetic analysis of *D. rapae*

Phylogeny of *Diaeretiella rapae* (Hymenoptera: Aphidiidae): comparing Australia and New Zealand Population using mitochondrial DNA.

Introduction

Studies have shown that parasitism behaviour and efficiency of *Diaeretiella rapae* in biological control depends on the population of the parasitoid (Vaughn et al. 1996). Genetic studies on *D. rapae* have also revealed marked variation among individuals from different locations (Baker et al. 2003; Baer et al. 2004). This study compares Australasian *D. rapae* individuals collected from Melbourne (Australia) and Palmerston North (New Zealand) with previously published haplotypes from different regions of the world based on mtDNA analysis. Sequence of mitochondrial DNA (mtDNA) located on gene CO-I was obtained from representative individuals of these two populations, and used in phylogenetic analysis with published data.

Material and methods

Parasitised mummies of cabbage aphid *Brevicoryne brassicae* on cabbage plants were collected in Palmerston North, New Zealand and in Melbourne, Australia. The mummies were kept in laboratory conditions until adult emergence. The adult *Diaeretiella rapae*, identified according to Powell (1982), emerged from the mummies and were killed by freezing them at -20^{o}C. The samples were later stored in 95% ethanol.

Male and female *D. rapae* samples stored in ethanol were first air-dried before DNA extraction. Whole adults were used for each extraction with Qiagen DNeasy tissue kit. Extraction followed manufacturer’s protocol except for final DNA elution in 30 µl elution buffer. DNA was quantified using a NanoDrop Spectrophotometer at absorbance 260/280 and 260/230. The DNA was diluted based upon the DNA concentration in each samples.

Polymerase chain reaction (PCR) was carried out to target mitochondrial Cytochrome oxidase subunit I (COI) gene using primers COI. P1 and COI 1R used in earlier studies (Baer et al. 2004) (Table 1).

Table 1: The primers and their sequence used for PCR of the DNA fragment of a particular area of gene CO-I.

Primer	Sequence (5`- 3`)	Position
COI.P1	TTGATTTTTTGGTCA(TC)CC(TA)GAAGT	2492
COI.1R	AGCTCCTATTGATAAAACATAATG	2942

PCR amplifications were carried out in 10µl reactions using standard conditions. PCR products were qualified and quantified using 1% TAE agarose gel electrophoresis with SYBRsafe stain and Invitrogen 1kb plus DNA ladder (Life Technologies Corp.). The PCR products were cleaned up using SAP/EXOI (USB Corporation) digestion to eliminate unincorporated primers. Purified DNA fragments were sequenced using BigDye 3-1 chemistry and visualised with an ABI 3730 (ABI) automated sequencer.

Published sequences were downloaded from GenBank including representatives of *D. rapae* haplotypes identified by Baer et al. (2004). The DNA sequences were checked and edited using Sequencher 4.9 (Gene Codes Corporation) and aligned using Se-Al (Rambaut 1996). Phylogenetic analysis (distance matrix and phylogenetic tree) was carried out using Geneious Pro. 5.3.4 (Drummond et al. 2009).

Results and Discussion

COI sequences were obtained from four *D. rapae* individuals, comprising a male and female each from Melbourne and Palmerston North. These were aligned with published sequences representing haplotype variation detected in the north hemisphere (Baer et al. 2004). The total alignment was trimmed to the length of our shorter sequences resulting in a data set 642bp in length. Reducing the length of the alignment in this way reduced the number of different haplotypes, such that several published haplotypes were found to be synonymous with others (Table 2). Of 43 haplotypes reported by Baer et al. (2004), only 15 (including the new Austsralasian variant) remained in the shorter COI fragment. We noted that the largest synonymy was of 16 variants that were identical at this size (haplotype BB, Table 2). Thus, BB has been recorded at most locations in the northern hemisphere that have been surveyed. Haplotypes nucleotide sequences differed by less than 1% (Table 3). Translation of the mtDNA coding sequence resulted in a protein alignment of 213 amino acids. A single AA substitution was evident in one of the 15 sequences. Haplotype O differed from the others by replacement of Phenylalanine with Leucine.

All four *D. rapae* from Australian and New Zealand populations had the same mtDNA COI haplotype (ANZ), which was distinct from all haplotypes previously reported for this species (Table 2). Phylogenetic analysis indicates that the ANZ was most similar to KK (pairwise distance 0.002, Table 3), a haplotype recorded from USA, UK, France, Czech Republic and Georgia (Fig. 1, Table 4).

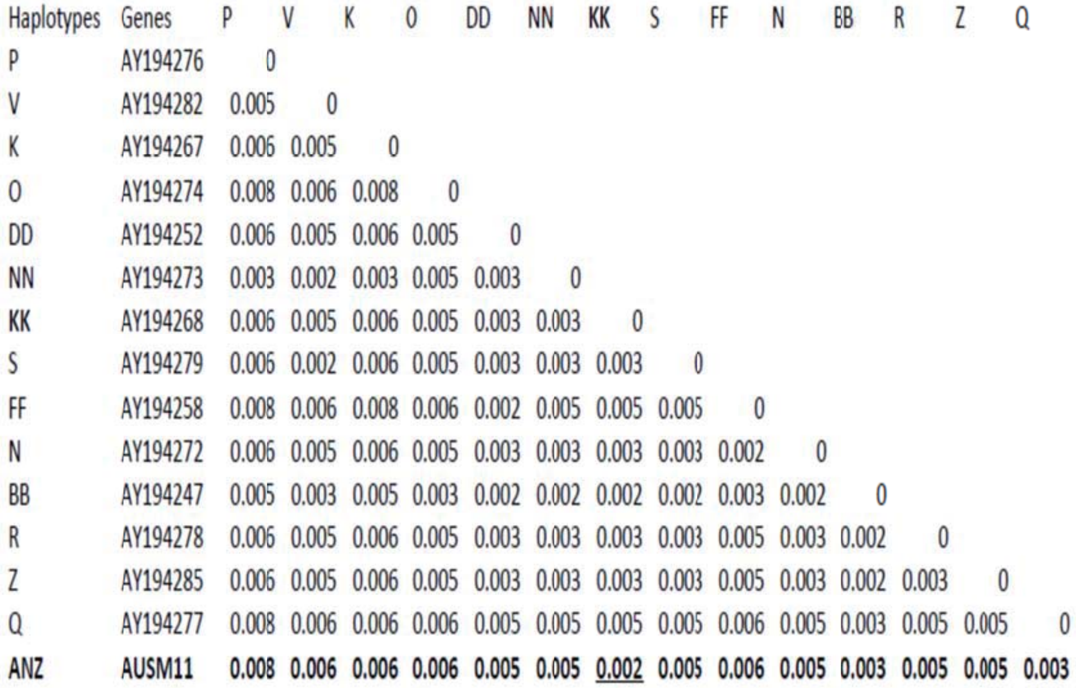
The scale of sampling employed in a previous study of *D. rapae* using mtDNA (Baer et al. 2004), implies that, although ANZ is genetically similar to haplotype KK and others, it probably did not originate directly from Europe or North America, or northern Asia. *Diaeretiella rapae* is globally distributed and well recognised in Africa, southern Asia and South America (reviewed in Chapter Introduction). Any of these places could have been the source of *D. rapae* in Australasia, and it has previously been proposed that Sri Lanka was the origin of the Australian population (Carver & Stary 1974a). As *D. rapae* was self-introduced to New Zealand, it is most likely that this occurred from Australia. Further study is required to locate the most likely original source of this parasitoid, so that behavioural, ecological and reproductive comparisons can be made.

Table 2: Haplotypes of *Diaeretiella rapae* and their collection locations. Haplotypes and location codes were kept same as given by Baer et al. (2004). Haplotypes in the bracket share same DNA sequence as the first bold haplotype code, resulting from alignment of the 642 bp COI fragment analysed here. Hosts were cabbage aphids (CA), or Russian wheat aphids (RA).

Haplotypes	Genes	Host	Location code*
P	AY194276	CA	B1C
NN (M)	AY194273	RA	B1C, CW, CB
K (L)	AY194267	RA	B1C, W1W
V (U, W)	AY194282	CA, RA	CW, CA, SB
S (FFF, T)	AY194279	RA	CW
Q	AY194277	CA	SB
KK (JJ, LL)	AY194268	CA	B1C, SB, CB, HY, UK
DD (OO)	AY194252	RA	KD
FF (GG, HH)	AY194258	RA	B1C, KD, KI
N	AY194272	CA	SB
Z (GGG, X)	AY194285	CA, RA	B1C, KD, PP, UT, HY
O	AY194274	CA	B1C
R	AY194278	CA, RA	B1C
BB (EEE, I, B, A, BBB, CC, E, F, EE, C, CCC, J, DDD, G, AAA)	AY194247	CA, RA	CA, CT, CW, KD, PP, IE, JD, SQ, SB, UK, SG, MM, BIC, W1W
ANZ	**ANZMF**	**CA**	**ME, PN**

*Locations codes have been pooled to include overlapping sites on global scale: B1C includes FWS, ARW, B1W, B2W, FWN, and FC; W1W includes W2W and W3C; MM included MS; SQ included ST; SB included SB2, SB4, SB5; IE includes IF.

Table 3: Pair-wise genetic distances among *Diaeretiella rapae* haplotypes for this and previous study calculated using HKY model of DNA evolution. Haplotypes codes include representatives of each variable sequence identified from comparison of 642bp fragments obtained for the Australasian samples.



Haplotypes	Genes	P	V	K	O	DD	NN	**KK**	S	FF	N	BB	R	Z	Q
P	AY194276	0													
V	AY194282	0.005	0												
K	AY194267	0.006	0.005	0											
O	AY194274	0.008	0.006	0.008	0										
DD	AY194252	0.006	0.005	0.006	0.005	0									
NN	AY194273	0.003	0.002	0.003	0.005	0.003	0								
KK	AY194268	0.006	0.005	0.006	0.005	0.003	0.003	0							
S	AY194279	0.006	0.002	0.006	0.005	0.003	0.003	0.003	0						
FF	AY194258	0.008	0.006	0.008	0.006	0.002	0.005	0.005	0.005	0					
N	AY194272	0.006	0.005	0.006	0.005	0.003	0.003	0.003	0.003	0.002	0				
BB	AY194247	0.005	0.003	0.005	0.003	0.002	0.002	0.002	0.002	0.003	0.002	0			
R	AY194278	0.006	0.005	0.006	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.002	0		
Z	AY194285	0.006	0.005	0.006	0.005	0.003	0.003	0.003	0.003	0.005	0.003	0.002	0.003	0	
Q	AY194277	0.008	0.006	0.006	0.006	0.005	0.005	0.005	0.005	0.006	0.005	0.003	0.005	0.005	0
ANZ	**AUSM11**	**0.008**	**0.006**	**0.006**	**0.006**	**0.005**	**0.005**	**0.002**	**0.005**	**0.006**	**0.005**	**0.003**	**0.005**	**0.005**	**0.003**

Table 4: Distribution of *D. rapae* haplotypes reported from this and previous study The codes are as reported by Baer et al. (2004). Haplotype codes include representatives of each variable sequence identified from comparison of 642bp fragments obtained for the Australasian samples. X indicates presence of haplotype at given location.

Code	**Location**	**Country**	**BB**	**KK**	**Z**	**FF**	**K**	**V**	**NN**	**R**	**Q**	**N**	**P**	**S**	**DD**	**O**	**ANZ**
B1C	Boulder3KL	USA	**X**	**X**					**X**	**X**			**X**			**X**	
FWS	Fruita CO RWA	USA	**X**			**X**	**X**										
ARW	Fort Collins	USA	**X**							**X**							
B1W	Boulder5KL	USA	**X**														
B2W	Boulder3KE	USA	**X**		**X**												
FWN	Fruita CO R	USA	**X**			**X**											
FC	Fruita CO C	USA	**X**														
W1W	Prosser WA	USA	**X**														
W2W	Yakima WA	USA	**X**				**X**										
W3C	Prosser WA CA	USA	**X**				**X**										
CW	Wuqia	China	**X**					**X**	**X**					**X**			
CA	Altai	China	**X**					**X**									
CT	Tacheng	China	**X**														
SB2	Moravia	Czech Rep	**X**								**X**						
SB4	Ceske Budejovice	Czech Rep	**X**	**X**				**X**				**X**					
SB5	Techobuz	Czech Rep	**X**														
CB	Caucasus	Georgia		**X**					**X**								
KD	Dmitrievka	Kazhakhstan	**X**		**X**	**X**									**X**		
KI	Iachmen	Kazhakhstan				**X**											
PP	Parachinar	Pakistan	**X**		**X**												
UT	Tashkent	Uzbekistan			**X**												
IE	Ehglid	Iran	**X**														
IF	Fars	Iran	**X**														
JD	Disi	Jordan	**X**														
SQ	Qatara	Syria	**X**														
ST	Tel. Hadya	Syria	**X**														
HY	Hyeres	France		**X**	**X**												
UK	Rothamnsted	UK	**X**	**X**													
SG	Grenada	Spain	**X**														
MM	Marrakech	Morocco	**X**														
MS	Settat	Morocco	**X**														
ME	**Melbourne**	**Australia**															**X**
PN	**Palmerston N**	**New Zealand**															**X**

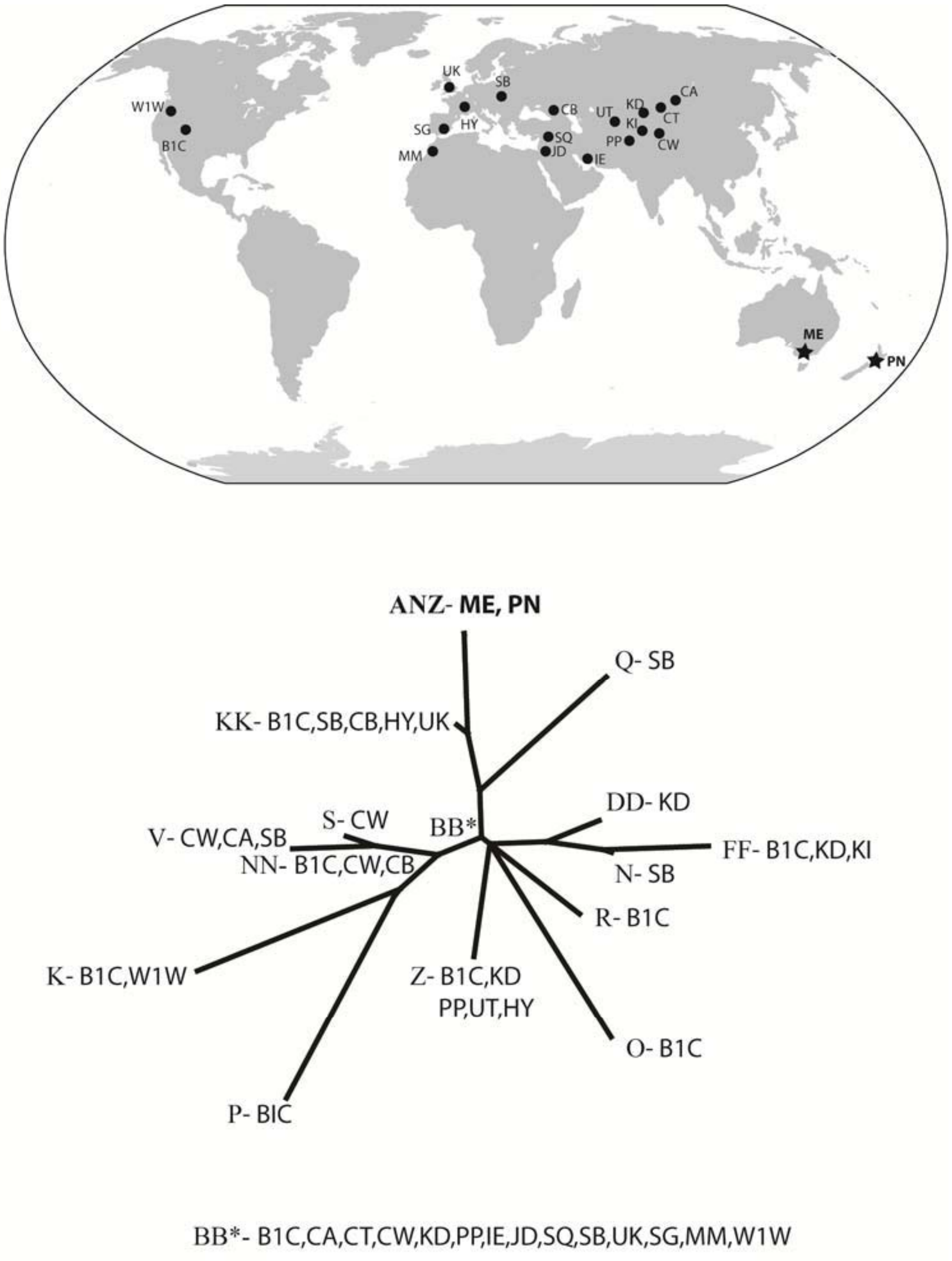


Figure 1: Unrooted phylogenetic tree of *Diaeretiella rapae* based on analysis of 642 bp of mtDNA COI, using the HKY model and NJ criterion as implemented in Geneious Pro. 5.3.4 (Biomatters Ltd.). The first letter indicates haplotype code and those following indicate location codes, as mapped.

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Appendix 2: Supplementary data on superparasitism

Reproductive fitness analysis of cabbage aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) during superparasitism

Introduction

This is a part of superparasitism studies on *Diaeretiella rapae*. The data presented here could not be included in the Chapter 12 (published paper). The study looked into superparasitism strategies of *D. rapae* at different host and foundress densities. Development time and body size of the offspring emerged from parasitised vs. superparasitised conditions were examined.

Materials and methods

Parasitism and superparasitism by *D. rapae* were investigated at four host densities (5, 10, 20 and 30), and at four foundress densities (1, 2 3 and 5) separately. In host density experiment, a mated, honey-fed, 1-day old female was offered different host densities for 1 h. Similarly in foundress density experiment, females of different densities were offered 5 hosts for 1 h. The parasitised aphids were transferred to cabbage seedling for feeding and development; the aphids were dissected after 4 days. The number of larvae found in each host and the average number of eggs oviposited per female were compared at different host and foundress densities. The mean number of eggs oviposited per females was calculated by dividing the number of females foraging together by the total number of larvae (cumulative) found in all the aphids offered to the female(s). Some of the parasitised and superparasitised aphids were allowed to mummify and reach adult emergence. Development time and body size of adults developed in parasitised (n=25) and superparasitised (n=25) conditions were compared. Body size of adults was measured using hind tibia length as done in Chapter 10.

Effects of host density on parasitism and superparasitism were analysed by binary logistic regression (PROC LOGISTIC, SAS 9.2). Analysis of variance (PROC GLM, SAS 9.2) was used to analyse the mean number of eggs found per host at different parasitoid densities, number of eggs oviposited per female, development time and body size of adults emerged from parasitised and superparasitised conditions.

Results and discussion

Host and parasitoid densities affected parasitism and superparasitism strategies of *D. rapae*. The proportion of parasitised hosts was greater at low host density than at high density (Logistic regression: χ^2 =26.02; P<0.001) (Fig. 1). Similarly, superparasitism occurred only at low host density, where a greater proportion of hosts was superparasitised at 5 hosts than at 10 and 20 hosts density (Logistic regression: χ^2 =10.53; P<0.01) (Fig. 1). The chances of a female to encounter the same host increase with decreasing host density. Repeated encounters cause females to superparasitise their hosts (see Chapter 12).

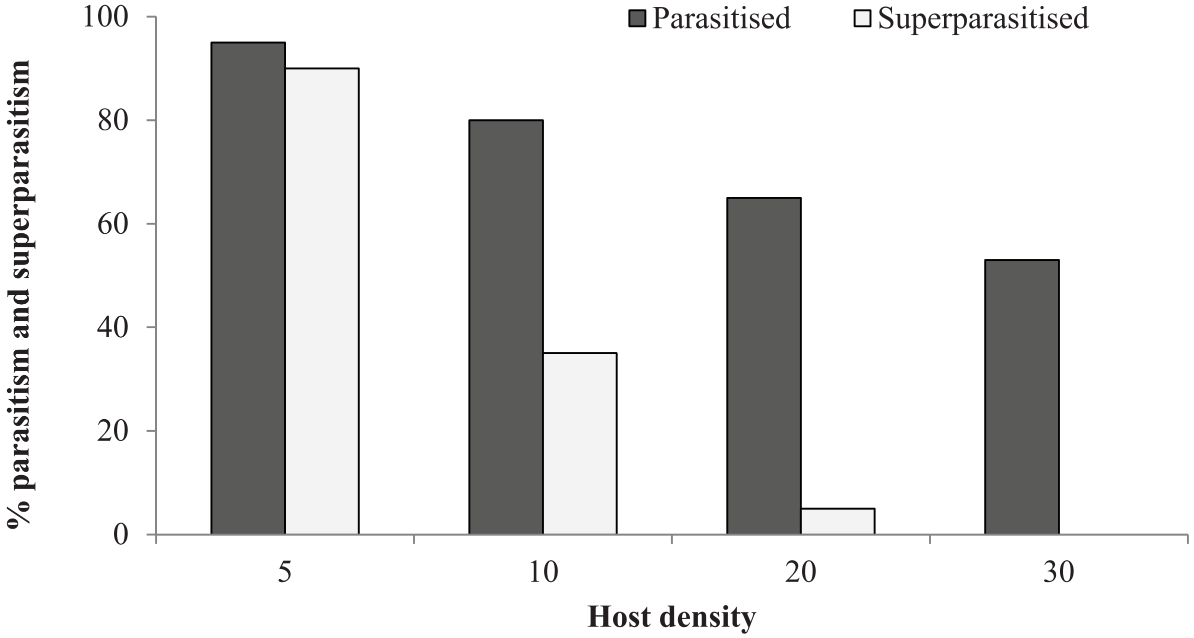


Figure 1: Percentage parasitism (one egg laid per host) and percentage superparasitism (more than one egg laid per host) by *Diaeretiella rapae* when different densities of cabbage aphid nymphs *Brevicoryne brassicae* were offered to them.

The number of eggs oviposited in a host increased with increase in the number of female foraging together ($F_{3, 53}$ = 10.37; P<0.001) (Fig. 2). The number of eggs oviposited per female reduced when more than one female were ovipositing together ($F_{3,53} = 6.62$; $P<0.001$) (Fig. 2). However, the degree of competition did not increase the per capita oviposition ($F_{2,39} = 1.2$; $P>0.73$). Results indicate that females continued to oviposit in a host despite the fact that only one adult emerges per host. However, reduced oviposition when more than one female were ovipositing together (high foundress density) indicates that the females manipulate their oviposition strategies to save their eggs for future oviposition because female *D. rapae* emerge with developed eggs (see Chapter 6).

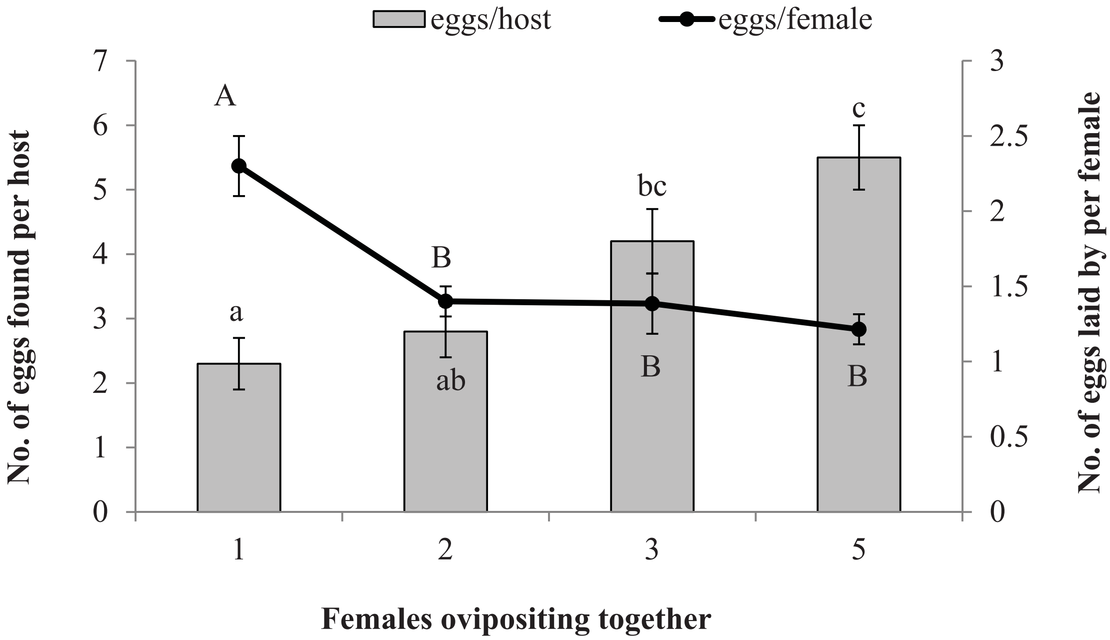


Figure 2: The mean number (±SE) of eggs found per *Brevicoryne brassicae* nymphs (bar diagram) and number of eggs oviposited per *Diaeretiella rapae* female (line diagram) when different number of females were parasitising together. Means with the same letter are not significantly different (α=0.05).

Development of *D. rapae* larvae was slow when more than one larva (superparasitism) was found per host (Chapter 12). However, the overall development time of adults was slightly shorter when they developed in superparasitised (11.68±0.33 days) than in parasitised (12.63±0.42 days) condition ($F_{1,36} = 3.12$; $P<0.08$). The body size of the adults developed in superparasitised condition was larger (2.24±0.06 mm) compared to the adults developed singly (2.03±0.03 mm) ($F_{1,20} = 6.62$; $P<0.01$). Quicker development and larger body size of the adults developed in superparasitised condition were probably due to the ‘winning’ developing larva consuming not only all the resources present in its host, but all other larvae as well. Cannibalism has been reported in *D. rapae* and several other solitary endoparasitoids.

Results of this study suggests that *D. rapae* superparasitise its host only when host density is low. However, by losing eggs through superparasitism, female gains some fitness by quicker development and larger body size of its offspring (Chapter 10).

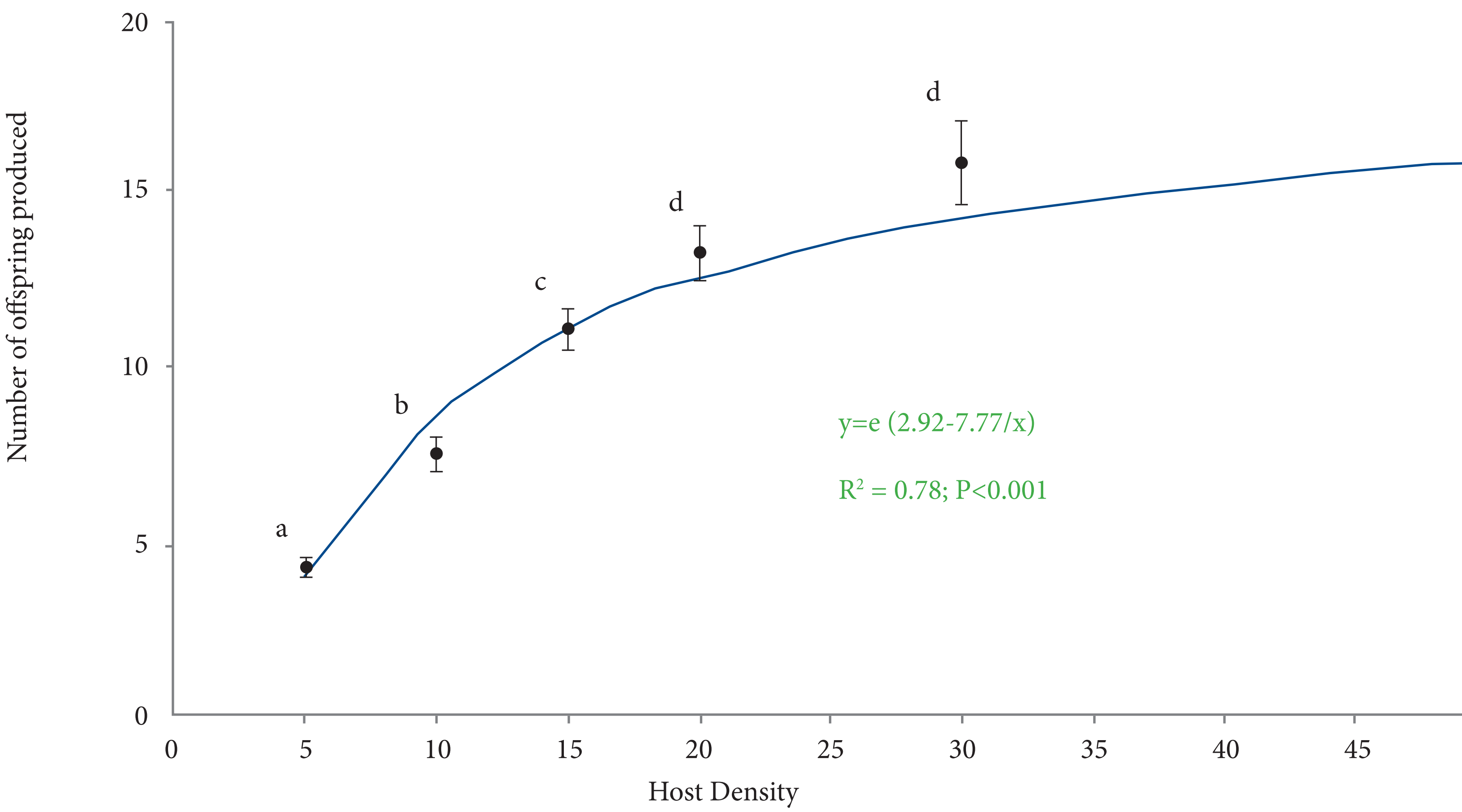
ESULTS:

e cabbage seedlings were heavily infested with aph
2±25 individuals/plant) and parasitoids (mummies 7
r plant). The parasitism rate varied between 25% and 3
g. 1).

e female/male parasitoid sex ratio was lower in the firs
eks (1.2±0.1) than in the second 5 weeks period (1.6±
<0.05; Fig. 2).

ting in *D. rapae* was observed throughout the photoperi
ly 49% of the mating pairs comprised a male mount
a female and 45% were male mounted on another ma
ikelihood ratio test showed that the male-female mat
s more prevalent during midday compared to morn
d evening (P < 0.05) (Table 1).

osts than when females foraged alone (P < 0.001) (F



igure 1: (a) Relationship between the numbers of *Diaeretiella rapae* offspring produced and the densit f *Brevicoryne brassicae* nymphs offered.