FORAGING AND REPRODUCTIVE PATTERNS F THE HYPERPARASITOID WASP, *DE OCERUS CARPENTERI* (MEGASPILIDAE)

by

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THESI JBMITTED IN PARTIAL FULFILLMENT OF QUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in the Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

April 1997

APPROVAL

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Degree: Doctor of Philosophy

Title of Thesis:

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ABSTRACT

Foragin d reproductive behaviour of *Dendrocerus carpenteri* (Hymenopter egaspilidae), a solitary hyperparasitoid of aphids, was studied in th oratory using as hosts the pea aphid (*Acyrthosiphon pisum*) and t primary hymenopterous parasitoids (*Aphidius ervi*, *Ephedrus cal icus*, and *Praon pequodorum*). Examination and oviposition ti by females of *D. carpenteri* varied with the species and the deve ental stages of the primary parasitoids within mummified a s; host choice was influenced more by the developmen ge than by the species of primary parasitoid.

Female *D. carpenteri* located single or clumped mummies on plants with e success, but the structure of the plant canopy influenced se ing behaviour and efficiency. When the density of mummies on f was increased, females responded by increasing patch time ar ood size in a density-dependent manner. Giving-up time increase th host density and the decision to leave a patch was apparently ir nced by re-encounters with self-marked hosts, but not by prior ing experience. Brood sex ratio did not vary with host density.

Female *D. carpenteri* produced non-binomial sex ratios of offspring by ting the sequence of male and female eggs laid during a sing iposition bout. Both the order and type of sequentially encountered hosts influenced the allocation of offspring sexes. The production schedule of sons and daughters was reset after a period during which no hosts were encountered. Re-encounters with self-marked hosts affected the ratio of male to female eggs laid in broods. Females appear to use simple decision rules to ensure that daughters will find mates regardless of variations in host quality and patch size.

Pea aphids containing larvae of *A. ervi*, *A. pisivorus*, *Monoctonus paulensis*, and *P. pequodorum* remained at their feeding sites. In contrast, those containing immature *E. californicus* generally dispersed

from their colonies prior to mummification; dispersal increased with density but was inhibited in the absence of light. The foraging and reproductive [illegible]erns of *D. carpenteri* may have evolved as a result of the physiolog[illegible]nd behavioural interactions among its host complex.

ACKNOWLEDGEMENTS

The co tion of this project would not have been possible without the a ance of many people. I thank my senior supervisor, Dr. M. Macka or his support, guidance and patience. I also thank both Dr. B. R rg and Dr. G. Gries for serving on my supervisory committee ar oviding helpful feedback.

Gratitu extended to Dr. H. R. MacCarthy for his encouragem nd a much appreciated 'writing tune-up' when I was misfiring anc ling.

Thanks go to Ms. A. Chau, Dr. J. P. Michaud, Dr. C. Nicol, and Mr. M. Otto f eir friendship and cooperation during my stay in the lab.

I am in ed to Mr. R. Long for his outstanding photographic work and Dr. asner for identification of specimens from my hyperparasit olonies.

Finally, nk my parents for both their love and understanding throughout n ademic trials.

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CHAPTER 1

Background to the Research

INTRODUCTION

Thompson (1994) favored the following definition for coevolution: "evolution involving a series of reciprocal changes in two or more noninterbreeding populations that have a close ecological relationship and act as agents of natural selection for each other". To be more succinct, coevolution is 'reciprocal change among interacting species'. The theme of coevolution has been the focus of many important topics in biology including: community organization, life histories, speciation, and phylogeny. The literature, reviewed in Futuyma and Slatkin (1983) and Thompson (1994), is full of studies that have examined the ways in which species specialize in their interactions with each other and how these relationships may result in reciprocal evolutionary change. Among the various interspecific associations, parasitism is the most specialized and it represents an intimate association with a negative, exploitative effect on one of the partners (Price 1980).

The imagery of an 'arms race' has often been used to describe the coevolution of exploitative interactions (Van Valen 1973, Dawkins and Krebs 1979, Weis *et al.*1989). In this scenario, one species is evolutionarily pursuing another in a race of defences and counterdefences. Janzen's (1980) definition of coevolution also implies this kind of 'tit for tat' interchange when applied to exploitation. Although Thompson (1986, 1994) has shown that continuous and symmetrical escalation may be constrained, the arms race analogy is still a valuable heuristic tool for understanding coevolutionary interactions between hosts and their parasites or parasitoids. In some parasitic associations, e.g. plant-fungus or mammal-virus, the two partners originate from taxa that are quite different (Mayr 1963). However, insect parasitoids usually come from the same taxonomic class as their hosts (Boulétreau 1986). On a coevolutionary level, significant biological and genetic similarities between insect parasitoids and their hosts makes them 'equally well-armed'. Study of the behavioural and evolutionary aspects of the relationship between insect hosts and their parasitoids may help us to better understand not

only coevolutionary processes, but also the role of parasitoids in both the regulation of host populations and the maintenance of balance between insect populations in natural and modified ecosystems. This thesis is composed of two studies that consider, from the perspective of an 'arms race', how the foraging and reproductive behaviours of a hyperparasitoid wasp may have evolved in relation to the physiological and behavioural interactions among its host complex: a group of primary parasitoids and their phytophagous host insect.

Some parasitoids develop by feeding on and eventually killing other parasitoids; this phenomenon is known as hyperparasitism and it has been extensively studied among insects. A hyperparasitic insect is any insect that will parasitize another insect that is itself parasitic on a host insect. The host insect is often phytophagous, but it can also be a predator or scavenger. Hyperparasitoids belong to seventeen families of hymenopteran parasitoids and a few families of dipteran and coleopteran parasitoids (Gordh 1981, Sullivan 1987, Gauld and Bolton 1988). These insects are often referred to as secondary parasitoids, but they can also be tertiary or even quaternary parasitoids.

Among the Hymenoptera, hyperparasitism may have evolved from primary parasitism many times and in several distinct ways. Gauld and Bolton (1988) suggested that hymenopterous hyperparasitoids may have evolved in association with the primary host, parasitoids of the primary host, or predators of the primary host. Although the host range of hymenopterous hyperparasitoids is broader at the species level than that of primary parasitoids, it appears to be mainly restricted to immature hymenopteran hosts that are natural enemies of phytophagous insects (Gordh 1981). The adult and egg stages of primary parasitoids are rarely attacked by hyperparasitoids.

Traditionally, hyperparasitism has been viewed in the context of biological control as having a detrimental impact on the ability of primary parasitoids to control pest species in agriculture and forestry (Bennett 1981). Hyperparasitoids occupy the highest trophic level in most insect host-parasitoid systems (Sullivan 1987). The conventional

belief was that hyperparasitoids possess attributes similar to those of primary parasitoids. Thus, it was assumed that hyperparasitoids would suppress populations of primary parasitoids to levels that allowed pest populations to escape regulation. However, theoretical studies have shown that hyperparasitoids may promote either stability or instability in host-parasitoid communities (Beddington and Hammond 1977, Hassell and Waage 1984). In fact, there has been speculation that hyperparasitoids play a positive role in maintaining a balance between populations of insect species in both natural and managed ecosystems (Luck *et al.* 1981).

The role and importance of hyperparasitoids in the regulation of aphid populations by aphidiid wasps have been subjects of considerable debate. Sullivan (1988) and Horn (1989) have questioned whether hyperparasitoids always reduce the potential impact of primary parasitoids on their aphid hosts. Mackauer and Völkl (1993) suggested that the overall impact of hyperparasitoids on the dynamics of aphid-aphidiid populations is limited; however, hyperparasitoids may have had an ultimate effect on the evolution of different foraging strategies by aphidiids. They also proposed that low levels of parasitoid impact on aphid populations could be explained by offspring allocation strategies to hosts and host patches of the primary parasitoids, rather than by losses due to hyperparasitism. Höller *et al.* (1993), concluded from a study of seasonal changes in aphid parasitoid and hyperparasitoid numbers in cereal crops that aphidiid females left patches of aphids before all suitable hosts were exploited if they detected the presence of hyperparasitoids. More recently, Weisser *et al.* (1995) tested the hypothesis that parasitoid searching and oviposition behaviour are directly influenced by simultaneously searching hyperparasitoids, but found no evidence to support it in a laboratory system consisting of several species of primary and secondary parasitoids. A major deficiency in the understanding of the interaction between aphidiid species and their hyperparasitoids is the lack of information about the foraging behaviour of the latter.

Few hosts of parasitoids are distributed randomly and they often occur in discrete aggregations within the environment. However, the temporal and spatial distribution of hosts may vary considerably and influence the foraging success of parasitoids. This could be especially true for hyperparasitoids, which are often generalists associated with a variety of hosts in different habitats. A focus of discussion in the literature has been strategies that primary parasitoids of insects may employ to decrease the risk of hyperparasitism among their progeny (Fritz 1982). In particular, there has been considerable interest in aphidiid wasps (Hymenoptera: Aphidiidae), which are common parasitoids of aphids (Homoptera: Aphididae), because some species may alter the behaviour of their hosts (Brodeur and McNeil 1989, 1990, 1992) or distribute their progeny (Mackauer and Völkl 1993) in ways that decrease or spread the risk of hyperparasitism. Models of the latter assume that parasitized aphids have clumped distributions and a direct correlation exists between the size of host aggregations and the probability of discovery and attack by hyperparasitoids (Ayal and Green 1993; Weisser *et al.* 1994). Aphidiid wasps typically parasitize relatively few individuals in aphid colonies; as a result, mummified aphids tend to be loosely aggregated (Mackauer and Völkl 1993). Physiological and behavioural interactions between immature aphidiids and their host aphids could also affect the distribution of mummies in the habitat (Brodeur and McNeil 1992).

The literature reports several different strategies that aphidiid wasps may use to reduce hyperparasitism among their immature progeny. These strategies mostly use spatial avoidance of hyperparasitoids. In the situation of an immature parasitoid, confined within the body of the aphid host, its location in the habitat will depend upon the aphid host. For the parasitoid to influence its location, it must actively modify the behaviour of the host. One possible strategy requires immature aphidiid wasps to modify the behaviour of host aphids so that parasitized individuals will leave their colonies and host plants prior to mummification (Brodeur and McNeil 1989). Three important assumptions of this strategy are: (1) hyperparasitoids primarily forage on plants already colonized by aphids, (2) association

with other aphids increases the risk of hyperparasitism before and/or after mummification, and (3) risks associated with leaving colonies and host plants are fewer than those associated with staying. Brodeur and McNeil (1989, 1990) found that *Macrosiphum euphorbiae* containing diapausing *A. nigripes* larvae showed negative phototaxis and moved off potato plants to mummify in concealed sites. Similarly, Höller (1991) found that cereal aphids containing nondiapausing immatures of *Toxares deltiger*, *Ephedrus plagiator*, or *Aphidius picipes* showed a high tendency to move off oats prior to mummification.

Yet, parasitized aphids may mummify on their host plants and even within aphid colonies if aphidiid wasps employ alternative strategies to reduce mortality. Four alternative strategies are: (1) limiting the number of aphids that females parasitize in a colony (Ayal and Green 1993), (2) modifying the behaviour of parasitized aphids so that they leave the aphid colony and mummify within parts of the plant canopy where the incidence of hyperparasitism is lowest (Brodeur and McNeil 1992), (3) exploiting aphid colonies tended and thus protected by ants (Völkl 1992), and (4) leaving areas which develop high hyperparasitoid densities (Höller *et al.* 1993). Overall, the optimal strategy of a primary parasitoid will be influenced by several selective factors which include the characteristics of the habitat, the distribution and density of hosts, and the density and foraging tactics of hyperparasitoids.

Two empirical studies are frequently cited as reporting field data which supports the hypothesis that insect parasitoids can manipulate the behaviour of the host to their own advantage by inducing the parasitized hosts to move to certain locations on the host plant. Stamp (1981) found that caterpillars of *Euphydryas phaeton* (Lepidoptera: Nymphalidae) parasitized by *Apanteles euphydryidis* (Hymenoptera: Braconidae) moved to high, exposed locations in the vegetation where they were less subject to either predation or hyperparasitism. Similar findings were reported by Brodeur and McNeil (1992) for parasitized *M. euphorbiae* containing nondiapausing *A. nigripes*. However, the conclusions from these studies were based only on mortality counts

because there were no data on the foraging behaviour of natural enemies. Although mortality data may support an adaptive hypothesis I argue that they are, on their own, insufficient for a complete understanding of how disruption of normal host behaviour is adaptive for the parasitoid. Parasitism forms an integrated host-parasite complex with each 'partner' in a conflicting situation (Thompson 1990). Modification of host behaviour could also be adaptations of hosts (Smith Trail 1980, McAllister and Roitberg 1987) or reflect biochemical or physiological damage that is selectively neutral (Minchella 1985).

An evaluation of whether movement by parasitized aphids, away from colonies or host plants, is adaptive for aphidiid species requires both an understanding of the proximate causes of this behaviour and the consequences of leaving or staying under different conditions. Some understanding of these consequences could be gained by studying the foraging and reproductive behaviour of a common aphid hyperparasitoid. As discussed earlier, a large number of studies have addressed the topic of coevolutionary interaction between primary parasitoids and hyperparasitoids; however, most were exclusively concerned with the behaviour and strategies of the primary parasitoids. Equally important to our understanding of these interactions are the behaviours and strategies of the hyperparasitoids; especially in terms of how they may have coevolved to counter those employed by their hosts. Yet, little is known about the foraging behaviour and reproductive strategies of aphid hyperparasitoids.

To gain a better understanding of the adapative significance of host behaviour modification within aphid-aphidiid-hyperparasitoid complexes, I conducted studies on an experimental system that consisted of a plant (broad bean, *Vicia faba* L. cv 'Broad Windsor'), an aphid (pea aphid, *Acyrthosiphon pisum* Harris [Homoptera: Aphididae]), five species of primary parasitoids (*Aphidius ervi* Haliday, *A. pisivorus* Smith, *Ephedrus californicus* Baker, *Monoctonus paulensis* Ashmead and *Praon pequodorum* Viereck [Hymenoptera: Aphidiidae]) and a hyperparasitoid (*Dendrocerus carpenteri* Curtis [Hymenoptera: Megaspilidae]). There were several advantages to using this complex of

insects. The pea aphid is easily reared in the laboratory on broad bean. The five aphidiid wasps are attacked by *Dendrocerus* species in the field, can be easily reared on the pea aphid in the laboratory, and preliminary observations suggested that their immature stages affect host behaviour differently. Female *D. carpenteri* forage primarily by walking and attack immature hosts which are immobile; both behaviours facilitate observation of foraging behaviour and design of experiments that test the effects of host density and distribution. *Dendrocerus carpenteri* is also one of the more extensively studied species of aphid hyperparasitoids in the literature, and there is considerable information on its biology and behaviour.

For coevolutionary studies it is important to establish that the community of organisms share an evolutionary history, but this may be difficult to prove when the organisms have a wide geographic distribution. The pea aphid is a globally common pest of legumes; however, it is believed to be of Palearctic-Oriental origin and was probably accidently introduced to the North American continent somewhat before or during the second half of the 19th century (Mackauer 1971). In North America this aphid can reach high densities on commercial alfalfa and pea crops. Important natural enemies of the pea aphid are aphidiid wasps. These species are solitary endophagous parasitoids of aphids. The Aphidiid family has been reviewed in detail by Mackauer and Stary (1967) and Stary (1970). A large and diverse parasitoid guild attacks the pea aphid in North America (Mackauer and Finlayson 1967). *Aphidius ervi* is of Palearctic origin and was imported from Europe into North America as a biological control agent of the pea aphid during the late 1950's and early 1960's (Clausen 1978, Gonzalez *et al.* 1978). *Aphidius pisivorus, E. californicus, M. paulensis,* and *P. pequodorum* have been reported as parasitoids of the pea aphid in alfalfa fields of the Pacific Northwest region of the US (Halfhill *et al.* 1972). These four aphidiid species seem to be indigenous to, or have been established in, North America for a long time, but their origins are subjects of debate. Mackauer and Kambhampati (1986) suggested that *A. pisivorus* and *P. pequodorum* appear to be specific to the pea aphid and were almost certainly introduced, together with their host, from

Europe into North America. *Monoctonus paulensis* and *E. californicus* are believed to be native to the Nearctic region (Mackauer and Stary 1967), but their phylogeny is not clear and they may not be distinct from similar species which are indigenous to Europe (M. Mackauer, personal communication). The available evidence suggests that all five primary parasitoids have shared a lengthy association with the pea aphid and related aphid species.

Dendrocerus carpenteri is a common, apparently cosmopolitan, hyperparasitoid of aphidiid wasps over a broad range of aphids and host plants, but it is probably of Palearctic origin (Dessart 1972, Takada 1973, Stary 1977). It is possible that *D. carpenteri* was introduced into North America from Europe with the pea aphid. In Europe, *D. carpenteri* has been recorded as a hyperparasitoid of *A. ervi* and other aphidiid wasps associated with the pea aphid (Stary 1977). Female wasps of this species attack the last larval instar and pupal stages of primary parasitoids inside the hardened skin of dead, mummified aphids. This hyperparasitoid species is ectoparasitic, because a female deposits her egg on the surface of the immature primary parasitoid and then the hyperparasitoid larva feeds externally on the primary host within the mummy (Sullivan 1988). In North America, *D. carpenteri* is one of the most abundant species of hyperparasitoid among parasitoid complexes of pea aphids on alfalfa (Mertins 1985a), but there is a lack of information on its hyperparasitism rates among different aphidiid species.

Höller *et al.* (1993) examined hyperparasitism of *Ephedrus*, *Aphidius*, and *Praon* mummies by *Dendrocerus* species on wheat and found that it was twice as high among *Ephedrus* as among the other two aphidiid species. Similarly, Sullivan and van den Bosch (1971) studied hyperparasitism of the primary parasitoid complex of the potato aphid, *Macrosiphum euphorbiae*, on *Iris germanica* L. by *Dendrocerus* species and found that it was approximately six times as high among *E. californicus* as among *A. nigripes* and an unidentified species of *Monoctonous*. Generalist hyperparasitoids are often perceived as 'opportunists' that do reasonably well in a variety of hosts

and habitats. However, the habitat, physiological state, behaviour, density, and distribution of the aphid, primary parasitoid, and hyperparasitoid may influence the incidence of hyperparasitism. There is a need to evaluate the relative importance of these factors on the susceptibility of immature aphidiid wasps to hyperparasitism by *Dendrocerus* species. The following studies were conducted to gain a better understanding of hyperparasitoid behaviour and host-parasitoid interactions.

I first investigated various hypotheses concerning the influence of parasitism on aphid behaviour. A study is presented in Chapter 2 in which I examined the effects of the immature stages of different aphidiid species, the density of aphids, and the absence of light on the behaviour of parasitized pea aphids. I found that the behaviour of parasitized aphids varied with the species of aphidiid wasp and influenced the distribution of mummified aphids in the habitat. Mummification of parasitized aphids, containing immature *E. californicus*, away from colonies or plants, apparently, was not due to a specific 'decision' but, rather, was a consequence of random disturbances. The interactions observed between immature *E. californicus* and their hosts were consistent with predictions from the pathology hypothesis which suggests that modified host behaviour is simply a result of the trauma of parasitism (Thompson 1983, Beckage 1985). Dispersal by parasitized aphids, containing immature *E. californicus*, appears to be primarily the consequence of pathology induced by parasitism; however, it may still have adaptive value for the primary parasitoid if it reduces the incidence of hyperparasitism. The results of this study suggest that the spatial distribution of hosts is potentially variable and uncertain for *D. carpenteri* and other hyperparasitoids that attack immature aphidiids within mummies.

Physical or physiological traits of immature stages of particular aphidiid species could also account for differential hyperparasitism in the field. Experiments are presented in Chapter 3 in which I compared influences of the species, development stage, and handling time of the primary parasitoid on host acceptance by *D. carpenteri*. Host

acceptance of *D. carpenteri* was influenced most by the developmental stage its host, but preference for *E. californicus* was consistent with results from field studies.

I also studied the foraging and reproductive behaviours of *D. carpenteri* to determine if they were adaptive, given the possible distributions of its hosts. Foraging patterns and offspring allocation by female wasps may be influenced by variables such as habitat structure or scale, host density or distribution, and brood size. In Chapter 4, the influence of host-patch variables on these behaviours were examined in the laboratory. The foraging behaviour of *D. carpenteri* did not appear to be particularly efficient under many of the conditions examined, but it may be adaptive when host patches are rare and dispersed throughout the habitat.

Recent theories on patch definition for parasitoids have promoted the concept of patches being discrete units of the environment that have distinct boundaries or borders defined by the behaviour of foraging females (Hassell and Southwood 1978, Waage 1979, van Alphen and Vet 1986) or the relationship between spatial patterns of parasitism and host density (Rosenheim *et al.* 1989). Although interpretations of the elementary unit of foraging have ranged from a single leaf (Li *et al.* 1992) or fruit (Reeve 1987) to an entire plant (Ayal 1987) coupled with host-derived cues, the scale by which the patch is measured has never been questioned as being other than 'spatial'. In Chapter 5, I report studies that investigated sex allocation of *D. carpenteri* and tested predictions concerning patch perception and optimal allocation of progeny under conditions of uncertain host distribution and quality. Mated females wasps produced 'precise' sex ratios and apparently used simple decision rules to ensure that daughters will find mates, regardless of variations in host quality and patch size. Morever, the sex allocation patterns of these females suggest that the borders of a 'patch' are defined by a 'temporal' and not a 'spatial' scale. I argue that this form of patch perception may be adaptive if host-derived cues are absent after female hyperparasitoids search beyond the broadest hierarchical levels of the host habitat.

In this thesis, foraging behaviour and manipulation of host behaviour were used to construct a framework in which to compare the 'strategies' of both primary parasitoids and a common hyperparasitoid of aphids. I examined complementarities between the behaviour of parasitized hosts in several aphidiid-aphid associations and the behaviour of *D. carpenteri* females. My results suggest that the foraging and reproductive behaviour of *D. carpenteri* may be 'adaptive' in context of the possible distribution of its hosts. The immature stages of some aphidiid species alter the behaviour of parasitized aphids in a manner that influences the distribution of mummies within the habitat and, possibly, the risk of hyperparasitism. In turn, the foraging and reproductive strategies of *D. carpenteri* may have evolved in response to the defensive strategies of its hosts. I propose that the behavioural adaptations of some hyperparasitoids and primary parasitoids of aphids have resulted from an evolutionary 'arms race' between these two types of organisms.

GENERAL MATERIALS AND METHODS

All insect colonies were reared in growth chambers at 20 ± 1°C, 40-60% RH and under continuous light. Stock colonies of the pea aphid were established from individuals collected in 1984 on alfalfa at Kamloops, British Columbia. Aphid colonies were maintained on potted broad-bean plants, *Vicia faba* L. 'Broad Windsor', grown in garden-mix soil. To obtain pea aphid colonies of a known age and instar, I transferred adults onto new plants. All offspring produced during an 8-h period were reared as a synchronous cohort until used for experiments.

I established laboratory colonies of five species of Aphidiidae on the pea aphid. A colony of *M. paulensis* was started from specimens that emerged from parasitized pea aphids collected on broad bean at Burnaby, BC, in July 1991. Material for the *E. californicus* colony originated from parasitized lupine aphids, *Macrosiphum albifrons* Essig, collected in July 1983 on lupine, *Lupinus polyphyllus* Lindl., at West Vancouver, BC. Colonies of three other aphidiid species were initiated from individuals that eclosed from parasitized pea aphids collected on alfalfa, *Medicago sativa* L., in the southern interior of British Columbia. The *A. ervi* colonies were established from material collected at Kamloops in September 1989 and at Keremeos, BC, in July 1993. Colonies of *A. pisivorus* and *P. pequodorum* Viereck were started from material collected at Kamloops in July 1984 and at Sorrento, BC, in June 1989, respectively. Dr. M. Mackauer (Dept. of Biological Sciences, Simon Fraser University, Burnaby, BC) examined both male and female specimens from colonies of each aphidiid species and confirmed their identification.

To acquire pre-adult primary parasitoids of a known age within mummies of similar size, I standardized rearing procedures. Newly emerged male and female wasps were fed water-diluted honey and caged together so that they could mate. I placed 6-8 females in a plastic cup covered with a clear plastic lid, together with 200-300 second nymphal instars (72 ± 4 h old) of pea aphid; preliminary studies

had shown that pre-adult parasitoid mortality was lowest in such aphids. After 2-3 h, the parasitoids were removed and the aphids transferred to new plants for rearing. Parasitized aphids usually died and mummified in their fourth instar or adult stage. I stored the mummies in paper cups at 20°C until the pre-adult parasitoids reached the desired age. *Ephedrus californicus* and *M. paulensis* develop more slowly than the other three aphidiid species, I thus standardized the parasitoids by physiological age, as opposed to chronological age for experiments. Unless otherwise stated, I used the last larval instar just prior to pupation because preliminary studies had shown that female hyperparasitoids have high oviposition success on this host stage.

I established colonies of *D. carpenteri* with specimens that originated from mummified pea aphids collected in June 1991 on alfalfa near Creston, BC. Dr. L. Masner (Biosystematics Research Centre, Agriculture Canada, Ottawa, Ontario) examined male and female specimens from my colonies and confirmed that they were all *D. carpenteri*. To obtain same-aged hyperparasitoids, I placed 8-10 mated female hyperparasitoids together with 200-300 mummies in a paper cup covered with a clear plastic dish. I applied diluted honey to the inside of the dish and allowed the females to forage for 16 h before removing them. The mummies were stored in paper cups at 20°C and any male or female *D. carpenteri* that eclosed within 24 h of each other were kept together as a cohort so they could mate. All hyperparasitoid colonies were provided with diluted honey but not aphids or hosts. I used experimental females when they were 6-8 d old and presumably had a sufficient supply of mature eggs available. To reduce possible bias resulting from size-related variation in reproductive and foraging behaviour I selected females of approximately the same size. Unless otherwise specified, female *D. carpenteri* were tested on the same species of primary parasitoid on which they were reared. To facilitate oviposition by *D. carpenteri*, the ventral surfaces of mummies were glued to substrates with a droplet of honey.

To determine if mummies from experiments were successfully hyperparasitized, I used gelatin capsules (size 00; Parke-Davis Ltd,

Scarborough, Ontario) to store individual mummies until adult wasps eclosed, which I identified and sexed. Mummies from which no wasps eclosed were dissected and their contents identified. Unless otherwise stated, all mummies were stored in growth chambers at 20 $\pm$ 1°C.

Plastic petri-dish cages (Mackauer and Bisdee 1965) were used for some of the observational studies in the laboratory. The cages were 15.5 cm in diam and 4.0 cm in ht with mesh covers and a 1.5 cm hole in the side wall. Through this hole, a broad bean shoot was inserted and held in place with plasticine. All shoots were maintained in bottles full of water during the studies. Large plexi-glass cages (27 cm wide x 42 cm high x 36 cm long) with mesh screens on the top, back, and both side walls were used for rearing colonies or large-scale foraging studies. All observational studies were conducted in the laboratory at 21-24°C, 25-40% RH, and under continuous light. All studies in growth chambers were conducted at 20 $\pm$ 1°C, 40-60% RH and under continuous light.

Unless otherwise stated, I used the SYSTAT statistical package (Wilkinson *et al.* 1989) for both analysis and evaluation of experimental data.

CHAPTER 2

Effects of Immature Aphidiids on Aphid Behaviour

INTRODUCTION

Immature endoparasitoids, especially koinobionts, must live with their hosts over an extended interval. During this interval, they avoid killing their hosts by selectively feeding on only certain tissues or organs (Godfray 1994). In addition to these passive adaptations, some parasitoids may actively manipulate the physiology, biochemistry, or behaviour of the host to their own advantage (Vinson and Iwantsch 1980). Identification of active host manipulation from the metabolic and physiological consequences of parasitism can be difficult (Schmid-Hempel and Schmid-Hempel 1990, 1991). The normal behaviour of hosts may be changed by parasitism but, if the costs and underlying mechanisms are uncertain, it may be difficult to interpret changes in host behaviour from an evolutionary perspective. Three explanations for modified behaviour of hosts are commonly cited in the literature. First, the parasitoid or parasite may improve its chances of survival by manipulating the behaviour of the host (Fritiz 1982, Schmid-Hempel and Müller 1991, Müller and Schmid-Hempel 1992, Müller 1994). Second, the host may alter its behaviour to improve its inclusive fitness (Shapiro 1976, Smith Trail 1980) or minimize the effect of parasitism on its reproductive process (Minchella 1985). Third, modified behaviour of hosts may be selectively neutral and result from either altered physiology or the trauma of parasitism (Thompson 1983, Beckage 1985).

Several studies have examined the behaviour of aphids after successful parasitism by aphidiid wasps; however, the benefactor of the change appears to vary with the aphid-aphidiid association. Changes in aphid behaviour after parasitism may benefit the aphid under certain conditions. McAllister and Roitberg (1987) examined the effect of parasitism by *A. ervi* on the behaviour of pea aphids from wet and dry regions of British Columbia. They found that pea aphids parasitized by *A. ervi* were more likely to drop from plants than non-parasitized pea aphids, if the aphids came from dry areas where the risk of subsequent death was high. This behaviour was interpreted as 'adaptive suicide' and it was suggested that parasitized aphids benefit from a higher

dropping tendency if their reproductive value were zero and the behaviour reduced the probability of kin being parasitized in the future. Tomlinson (1987) proposed that these observations could be explained by changes in host behaviour caused by different strains of *A. ervi*, rather than by host suicide. McAllister *et al.* (1990) subsequently showed that the behaviour of parasitized aphids did not differ between those containing immature *A. ervi* from wet or dry regions. However Godfray (1994) suggested it was possible that in the absence of selection for suicide, the pea aphid biotype from the dry region may react to parasitism in a way that makes falling off the plant a more likely accident of attempted escape from predation. The degree of pathology experienced by a host may vary with the type of host or parasitoid and their respective stages of development. Trauma due to parasitism may be higher in certain host-parasitoid associations and result in different behavioural responses.

Changes in aphid behaviour after parasitism may also benefit the parasitoid under different conditions. Brodeur and McNeil (1989, 1990, 1992) found that potato aphids parasitized by *Aphidius nigripes* left their colonies prior to mummification. Potato aphids containing diapausing individuals mummified off their host plant and those containing non-diapausing individuals mummified within the top stratum of their host plant. Potato aphids containing diapausing individuals apparently showed negative phototaxis and sought sheltered sites prior to mummifying. It was argued that immature parasitoids benefited if their host aphids mummified in sites where the micro-climate was more favorable or where the incidence of hyperparasitism was lower. Other studies have also shown that aphids parasitized by other aphidiid species tend to mummify away from their colonies and host plants (Behrendt 1968, Lykouressis and van Emden 1983, Höller 1991).. A common assumption of all these studies is that parasitized aphids move (i.e. walk) away from their colonies and host plants to find sheltered or concealed sites. However, an alternative explanation is that parasitized aphids disperse randomly from feeding sites within the plant canopy and frequently fall from host plants because of the pathology resulting from parasitism.

In this chapter, I separately examined the effects of the immature stages of five aphidiid species (*Aphidius ervi*, *A. pisivorus*, *Ephedrus californicus*, *Monoctonus paulensis*, and *Praon pequodorum*), the density of aphids, and the absence of light on the behaviour of parasitized pea aphids. I used these primary parasitoids because they are attacked by *Dendrocerus* sp., have different developmental physiology in the pea aphid, and their immature stages appear to affect host behaviour differently. At 20°C, second-instar pea aphids die and mummify approximately ten days after being parasitized by *E. californicus* or *M. paulensis*, in comparison, pea aphids die and mummify approximately eight days after being parasitized by one of the other three aphidiid species. I found from preliminary observations that mummies of aphids parasitized by *E. californicus* are frequently found off plants, but mummies of aphids parasitized by one of the other four aphidiid species are usually found on plants. I show that the behaviour of pea aphids containing immature *E. californicus* differed from the behaviour of pea aphids containing immature of other aphidiid species and apparently resulted from the pathology induced by *E. californicus*. The effects of aphid density and absence of light on dispersal by parasitized aphids supported the hypothesis of dispersal being a consequence of the trauma of parasitism. I discuss possible explanations for the different behaviour of aphids parasitized by these aphidiid species.

METHODS

Influence of Aphidiid Species

I tested the hypothesis that dispersal of parasitized pea aphids off their host plants is influenced by the species of primary parasitoid. Females of *A. ervi*, *A. pisivorus*, *E. californicus*, *M. paulensis*, and *P. pequodorum* were used to parasitize aphids. Six female parasitoids of the same species were taken from stock colonies and placed in a plastic cup with 50 second nymphal instars (72 $\pm$4 h old) of pea aphid. The cup was covered with a clear plastic lid and the wasps were left with the aphids for 2 h and then removed. Aphids from two cups were mixed and the 100 aphids were placed on a pot (13.0 cm, 'standard', Kord Products, Toronto, Ontario) containing a single broad-bean plant. A transparent plastic cylinder was placed over the pot to prevent the aphids from wandering off. The pot was placed on a dish and transferred to a large plexi-glass cage. I removed the cylinder 24 h later and watered the plant with 50 ml of water every 48 h over a period of 120 h. Daily observations were made on the behaviour of these aphids until most of the parasitized individuals had mummified. The numbers of mummies recovered on or off the plant and the number of aphids that did not mummify were recorded at the end of the trial. Ten trials (= one set) were completed for each aphidiid species for a total of 50 trials. I completed one set for a single aphidiid species before proceeding to complete another set for a different aphidiid species. However, the order in which I completed the sets for the five aphidiid species was not predetermined and subject to the availability of the insects.

I also conducted trials with colonies consisting of two types of parasitized aphids. The same design as in the first set of trials was used except that 50 of the aphids were exposed to female *E. californicus* and the other 50 were exposed to females of a different aphidiid species. I used, as the other species, *A. ervi* in 20 trials and *M. paulensis* in 10 trials. These two aphidiid species were choosen because they have different developmental times but their hosts tend

to mummify on plants. With this second experimental design, I attempted to address two issues: (1) reduction of behavioural variation among parasitized aphids that may have resulted from differences in quality among host plants, (2) determination of the influence, if any, of aphids containing immature of one aphidiid species on the behaviour of aphids containing immature of a different aphidiid species. As in the sets with only one aphidiid species, I completed one set of trials for a single combination of aphidiid species before proceeding to complete another set for a different combination. Again, the order in which I completed the sets for each combination was not predetermined and subject to the availability of the insects.

Female *E. californicus* were 5-8 d old and other female parasitoids were 4-6 d old when used for experiments. The broad bean plants had two pairs of mature leaves, one pair of apical leaves and were 11-14 cm tall when parasitized aphids were transferred onto them. All studies were conducted in growth chambers at 20 $\pm$ 1°C, 40-60% RH and under continuous light.

Influence of Aphid Density

I was motivated by the results of the first study to test the hypothesis that the density of an aphid colony will influence the dispersal of aphids parasitized by *E. californicus*, but have no effect on the behaviour of aphids parasitized by *A. ervi*. I conducted trials with parasitized aphids at intermediate and low densities. To set up colonies at an intermediate density, I used a similar protocol as in the first study to parasitize aphids. However, after parasitization, I transferred only forty aphids to each broad bean plant. The locations of the aphids were recorded both approximately 96 h after they were introduced onto plants and also after mummification. I measured the height of each plant and divided it into apical, top, middle, and bottom strata according to the location of leaves on the main stalk. For example, the apical stratum consisted of the immature leaves at the growing tip while the top stratum consisted of the two mature leaves closest to the

growing tip and the portion of the stalk above the next pair of mature leaves. The bottom stratum consisted of the two mature leaves closest to the ground and the portion of the stalk below these leaves. The middle stratum consisted of the portion of the plant between the mature leaves of the bottom and top strata. I recorded the location of each aphid or mummy according to stratum. Plants from experiments with aphids parasitzed by *A. ervi* were destructively sampled to determine the vertical distribution of leaf area. I completed three sets of trials, one for aphids exposed to *E. californicus*, one for aphids exposed to *A. ervi*, and one for aphids that were not exposed to any parasitoids. A total of 12 trials was completed for each set. I completed an entire set of trials for a single aphidiid species before proceeding to complete another set for a different aphidiid species or a set with aphids that had not been exposed to parasitoids. The order in which I completed the sets for the different treatments was not predetermined and subject to the availability of the insects.

I used a different protocol to set up colonies at a low density. An individual female *E. californicus* was placed in a plastic petri-dish (5.5 cm in diam by 1.5 cm in ht) with 10-15 second nymphal instars of pea aphid on a circular (4.2 cm diam) piece of filter paper. The wasp was allowed to freely encounter and parasitize aphids. I classified parasitized aphids as individuals that a female struck and left her ovipositor in for at least seven seconds. All parasitized aphid were immediately removed and replaced with unparasitized individuals. Parasitized aphids were held in a plastic cup, for no longer than 1 h, before they were transferred to broad bean plants that were 5 $\pm$ 1 cm tall. A replicate (= trial) consisted of three parasitized aphids on a single plant. The plant was contained in a square pot (8.5 cm, Premo, Victoria, BC) placed within a petri-dish lid (9.0 cm in diam by 1.5 cm in ht). I used 16 female wasps to complete 86 replicates and each replicate was kept in a growth chamber at 20 $\pm$ 1^{o}C, 65-80 % RH, and continuous light. The plants were watered every second day for 10 days and behaviour of the parasitized aphids were observed on a daily basis. On the eleventh day after the aphids had been parasitized, I recorded the number of mummies found on each plant, pot, petri-dish

lid, or any surface of the chamber. I used a different procedure for dividing the plants into strata because the plants in the low density treatment grew more rapidly than those in the medium density treatment and usually had more than three levels of mature leaves at the end of the trials. The height of each plant was measured and divided it into top, middle, and bottom thirds based on the length of the main stem. Leaves were assigned to a stratum (top, middle, or bottom) based upon the location of the leaf stem on the main stalk. The location of each mummy was classified according to strata. If the mummy was on a leaf, I recorded if it was found on the top or bottom of the leaf.

Influence of Light

It has been suggested, particularly by Brodeur and McNeil (1990), that the movement of parasitized aphids are the result of a photo-negative response by the host-parasitoid complex. In this experiment, I tested the hypothesis that the behaviour of parasitized pea aphids is not dependent on the presence of light. It is possible that dispersal by parasitized aphids, containing immature *E. californicus*, is stimulated or triggered by light. I would expect the absence of this behaviour if the stimulus was removed. However, if dispersal is a consequence of the trauma of parasitism, we would expect that the absence of light might inhibit but not entirely eliminate this behaviour. I followed the same design as in the first study except that half of the replicates were kept under continuous light for 120 h and then in the absence of light for an additional 120 h. The other half of the replicates were kept under continuous light for 240 h. In previous studies, I did not find parasitized pea aphids, containing immature *E. californicus*, off their host plants prior to 144 h after parasitism. Pea aphids parasitized by *E. californicus* remain alive until 216-240 h after parasitism, but appear to stop feeding approximately 168 h after parasitism (personal observations). The choice of light-dark treatment for half of the replicates was made to minimize effects to plant quality prior to and during the 'critical window for dispersal' (144-216 h after

parasitism) by aphids containing immature *E. californicus*. If plant quality was severly compromised by this light-dark treatment, we would expect that aphids parasitized by *A. ervi* would leave the plants prior to mummification. Pea aphids containing immature *A. ervi* remain alive for 168-192 h after parasitism and appear to feed until near death (personal obserations). I conducted two sets of trials, using aphids parasitized by *E. califonicus* in one set and aphids parasitized by *A. ervi* in the other. A total of twenty replications were completed for each set. I completed a entire set of trials for one aphidiid species before proceeding with a set for the other aphidiid species or a set. The order in which I completed the sets for the different aphid species was not predetermined and subject to the availability of the insects.

Statistical Analysis

For ANOVA, I transformed all proportions to the their arcsine values by equation 14.5 in Zar (1984, p. 186). To evaluate the influence of the species of the immature parasitoid on aphid behaviour, I used a fully randomized one-way ANOVA to compare proportions of recovered mummies found off plants. I also used ANOVA to compare proportions of released aphids that were recovered as mummies in each set. To determine if aphid mortality varied among sets, ANOVA was used to compare the proportions of released aphids that were not recovered as mummies or live aphids.

To evaluate the influence of density on the behaviour of aphids parasitized by *E. californicus*, I used linear regression to analyze data from the sets of 3, 40, and 100 aphids that were reared for 240 h under continuous light. I obtained the data for sets of 100 aphids from the experiment that evaluated the influence of aphidiid species and the experiment that evaluated the influence of light. I transformed the numbers of mummies recovered to the natural log scale to straighten the initial curve of the relationship. The proportions of mummies that formed off plants were then regressed against the transformed values for the numbers of mummies recovered. To determine if the

distribution of aphids or mummies differ between strata within sets of plants with 3 or 40 aphids, I used one-way ANOVA to compare the proportions of aphids and mummies in each stratum. ANOVA was also used to compare the leaf area of the four strata in the set of plants with 40 aphids that were exposed to *A. ervi*.

RESULTS

Influence of Aphidiid Species

I observed that the aphids tended to aggregate and feed within the top half of the plants. Aphids containing immature *E. californicus* began to disperse from their feeding sites on the seventh day of the experiment. Some aphids were on the floor of the cage and others appeared to move randomly within the plant canopy. Most of the aphids parasitized by *E. californicus* were found on the pots, dishes and cage floors on the eighth day and had mummified by the tenth day. In comparison, very few aphids containing immature of one of the other aphidiid species dispersed from their feeding sites prior to mummification. Most of these aphids appeared to die and mummify at their feeding sites on the seventh or eighth day of the experiment.

Parasitism of aphids was high among all sets exposed to one aphidiid species, but I found significant differences among sets in the proportion of released aphids that mummified ($F = 5.637$, $df = 4, 45$, $P < 0.001$) (Table 1). Slightly fewer mummies were recovered from the set of aphids exposed to *E. californicus* than from the other sets. There were significant differences in the location of mummies belonging to different aphidiid species ($F = 369.730$, $df = 4,45$, $P < 0.001$). Most of the *E. californicus* mummies were found off plants, but mummies belonging to other aphidiid species were almost always found on plants (Table 1). Aphids parasitized by *E. californicus* showed an equal tendency to mummify off plants when mixed with aphids parasitized by either *A. ervi* or *M. paulensis* (Table 2). However when mixed with aphids parasitized by *E. californicus*, aphids parasitized by *M. paulensis* mummified more frequently off plants than those parasitized by *A. ervi* (Table 2). Aphid mortality did not vary significantly between sets of 100 aphids in which I exposed all the aphids to one aphidiid species and sets in which I exposed half the aphids to *E. californicus* and the other half to a different aphidiid species ($F = 0.2115$; $df = 6, 73$; $P = 0.9721$) and was 0.097 ± 0.008 (mean $\pm$ SE).

Table 1. Location of mummified *Acyrthosiphon pisum* containing immature of one of five species of primary parasitoids. One hundred aphids were confined with six female parasitoids of the same species for 2 h. The aphids were released onto a potted broad-bean plant and kept within a plexi-glass cage at 20 ± 1°C, 50 ± 10 % RH, with continuous light until all parasitized aphids had mummified. All aphids were reared at 20°C and 72 ± 4 h old when exposed to parasitoids.

Parasitoid species[1]	Percentage mummified[2, 3] (±SE)	Mummies off plant[4] (±SE)	Percentage of mummies off plant[3, 5] (±SE)
Aphidius ervi	79.20 ± 2.55[ab]	1.40 ± 0.45	1.77 ± 0.58[a]
A. pisivorus	88.30 ± 2.28[a]	5.20 ± 0.99	6.00 ± 1.18[b]
Ephedrus californicus	71.80 ± 5.27[b]	64.40 ± 5.05	89.61 ± 2.34[c]
Monoctonus paulensis	89.90 ± 1.87[a]	1.10 ± 0.38	1.21 ± 0.41[a]
Praon pequodorum	89.90 ± 2.56[a]	4.90 ± 0.96	5.34 ± 0.95[b]

[1]Ten trials, consisting of 100 aphids each, were completed for each species of primary parasitoids.

[2]Mean percentage of 100 aphids released onto plants and recovered as mummies.

[3]Means, within columns, showing the same letter are not significantly different ($P \geq 0.05$) by Tukey's test.

[4]Mean number of mummies found off plants.

[5]Mean percentage of mummies found off plants.

Table 2. Location of mummified *Acyrthosiphon pisum* containing immature of one of three species of primary parasitoids. Fifty aphids were confined for 2 h with six female *Ephedrus californicus* and another fifty were confined with six female *Aphidius ervi* or *Monoctonus paulensis*. The two groups of aphids were combined and released onto a potted broad-bean plant and confined in a plex-glass cage at 20 ± 1°C, 50 ± 10 % RH, with continuous light until all parasitized aphids had mummified. All aphids were reared at 20°C and 72 ± 4 h old when exposed to parasitoids.

Parasitoid species[1]	Percentage mummified[2, 3] (± SE)	Mummies off plant[4] (± SE)	Percentage of mummies off plant[3, 5] (± SE)
1. *Aphidius ervi*	88.30 ± 2.91[a]	0.20 ± 0.12	0.42 ± 0.25[a]
Ephedrus californicus	73.30 ± 3.12[b]	29.10 ± 1.64	79.43 + 2.75[b]
2. *Monoctonus paulensis*	84.90 ± 2.85[a]	3.10 ± 0.88	7.29 ± 2.02[c]
Ephedrus californicus	86.60 ± 3.00[a]	37.50 ± 2.18	86.13 ± 2.87[b]

[1]Twenty trials, consisting of 100 aphids each, were completed for the set with *A. ervi* and *E. californicus*. Ten trials were completed for the set with *M. paulensis* and *E. californicus*.

[2]Mean percentage of 50 aphids released onto plants and recovered as mummies.

[3]Means, within columns, showing the same letter are not significantly different ($P \geq 0.05$) by Tukey's test.

[4]Mean number of mummies found off plant.

[5]Mean percentage of mummies found off plants.

Influence of Aphid Density

The proportion of *E. californicus* mummies that formed off plants increased with the total number of mummies recovered (Fig. 1). A regression of the proportion of mummies that formed off plants against the natural log transformation of the total number of mummies was highly significant ($F = 2419.139$; $df = 1, 34$; $P < 0.001$). The equation of the regression was "$Y = 0.2005 \ln X$" and correlation was high ($r^2 = 0.986$). Mean percentages of *E. californicus* mummies that formed off plants in sets with 3, 40, and 100 aphids were 18.60 ± 2.86, 65.10 ± 3.21, and 89.61 ± 2.34, respectively.

Aphids exposed to parasitoids and aphids from the control treatment tended to be found in the apical stratum of plants (Table 3). At a density of 40 aphids, all of the *A. ervi* mummies were found on plants and most of the mummies were in the apical stratum (Table 4). *E. californicus* mummies that formed on plants at this density were rarely found in the apical stratum, but there frequency among the other three strata were not significantly different (Table 4). There were also significant differences among strata in the proportion of *E. californicus* mummies that formed on plants at a density of three aphids ($F = 9.4113$; $df = 2, 252$; $P < 0.001$). Half of the mummies, 54.1 ± 5.4 % (mean $\pm$ SE), were in the top stratum and the other half were evenly distributed among the middle stratum, 30.5 ± 5.0 %, and bottom stratum, 24.70 ± 4.1 %. The mean height of plants in the medium and low density studies were 18.46 ± 0.25 and 27.75 ± 0.50 cm ($\pm$SE), respectively.

In the medium density study, there was significant variation among strata of broad bean plants in leaf area ($F = 37.2193$; $df = 3, 44$; $P < 0.001$). Leaf area was least in the apical stratum and greatest in the top and middle strata (Table 4).

Figure 1. Dispersal from the feeding site of *Acyrthosiphon pisum* containing immature *Ephedrus californicus* in relation to the number of parasitized aphids in a colony. Different numbers of aphids were exposed to attack by female *E. californicus* and then reared on broad bean at the following densities: o, 100 aphids per plant ($N = 20$); Δ, 40 aphids per plant ($N = 12$); ❑, 3 aphids per plant ($N = 86$). Numbers of mummies that formed off or on plants were recorded after parasitized aphids had mummified. Mean ($\pm$ SE) values were plotted for the proportion of mummies that formed off plants at a density of 3 aphids. Hosts were second nymphal instars, 72 $\pm$ 4 h old, reared at 20°C. The regression line was hand-fitted.

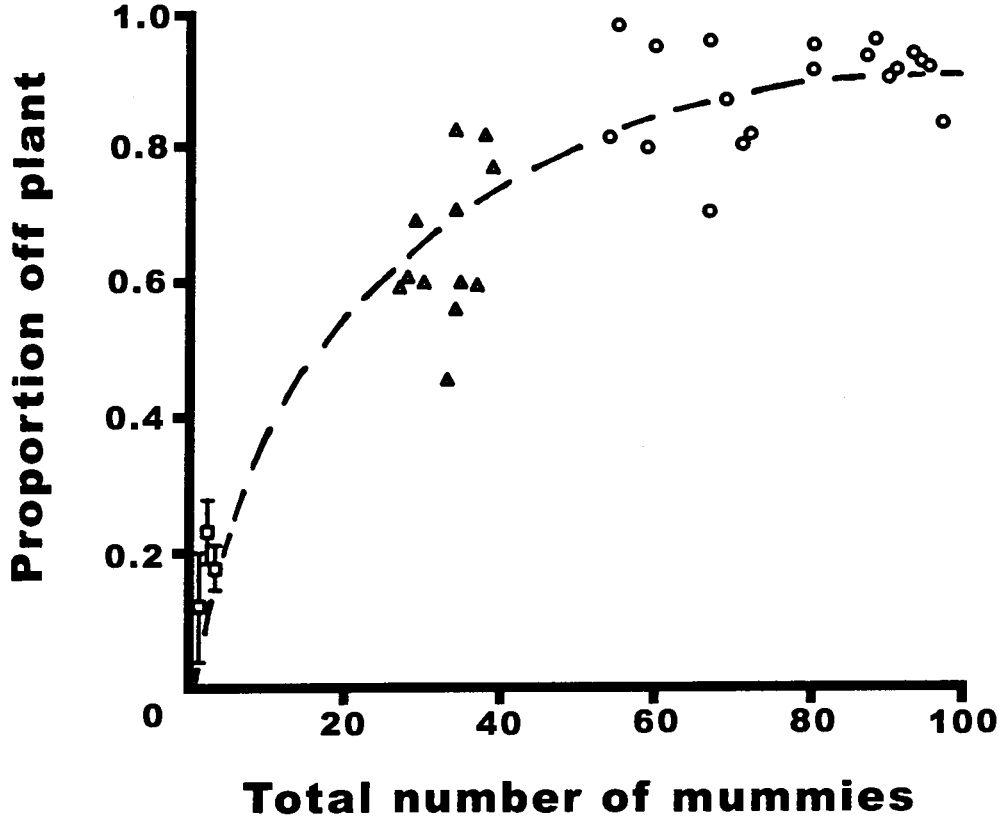

Proportion off plant
1.0
0.8
0.6
0.4
0.2
0
20
40
60
80
100
Total number of mummies

Table 3. Distribution of parasitized and non-parasitized *Acyrthosiphon pisum* within the canopy of broad bean plants. Forty aphids (72 ± 4 h old) were confined in a cup with six female parasitoids of the same species or without parasitoids for 2 h. The aphids were released onto a potted broad-bean plant and kept within a plexi-glass cage at 20 ± 1°C, 50 ± 10 % RH, with continuous light and their locations were recorded 96 h later. The aphidiid species used to parasitize aphids were *Aphidius ervi* and *Ephedrus californicus*.

Plant strata	Control aphids[1, 2, 3] (±SE)	Exposed to *A. ervi*[1, 2, 3] (±SE)	Exposed to *E. californicus*[1, 2, 3] (±SE)
Apical	63.69 ± 7.79[a]	72.82 ± 4.36[a]	66.28 ± 7.84[a]
Top	26.86 ± 6.00[b]	12.17 ± 2.66[b]	22.95 ± 7.78[b]
Middle	1.10 ± 0.51[c]	6.99 ± 2.99[b]	5.62 ± 1.48[b]
Bottom	0.23 ± 0.23[c]	8.04 ± 1.81[b]	5.15 ± 1.54[b]

[1]Twelve trials, consisting of 40 aphids each, were completed for each treatment.

[2]Mean percentage of total aphids found on plants.

[3]Means, within columns, showing the same letter are not significantly different ($P \geq 0.05$) by Tukey's test.

Table 4. Distribution of mummified *Acyrthosiphon pisum* containing *Aphidius ervi* or *Ephedrus californicus*. Forty aphids (72 ± 4 h old) were confined in a cup with six female parasitoids of the same species for 2 h. The aphids were released onto a potted broad-bean plant and kept within a plexi-glass cage at 20 ± 1°C, 50 ± 10 % RH, with continuous light until all parasitized aphids had mummified.

Location	Leaf area[1, 2, 4] (cm^2)	Mummies *A. ervi*[1, 3, 4] (%)	Mummies *E. californicus*[1, 3, 4] (%)
Off plant	——	0	65.10 ± 3.21[a]
Apical stratum	4.65 ± 0.52[a]	73.88 ± 4.36[a]	0.90 ± 0.90[b]
Top stratum	16.80 ± 0.85[b]	14.00 ± 2.80[b]	8.88 ± 2.03[c]
Middle stratum	16.58 ± 1.45[b]	6.77 ± 1.93[b]	16.14 ± 2.12[c]
Bottom stratum	11.15 ± 0.64[c]	5.36 ± 1.63[b]	8.98 ± 1.20[c]

[1]Twelve trials were completed for each treatment.

[2]Mean (± SE) leaf area of strata from plants that *A. ervi* mummies formed on.

[3]Mean percentage (± SE) of recovered mummies.

[4]Means, within columns, showing the same letter are not significantly different ($P \geq 0.05$) by Tukey's test.

Behaviour of Parasitized Aphids

At a density of three aphids per plant, I was able to observe the behaviour of parasitized individuals more easily than that at higher densities. Aphids, containing immature *E. californicus*, seem to undergo three distinct stages of parasitism before death. I could distinguish parasitized aphids aproximately five to six days after parasitism because a white patch, the immature *E. californicus* larva, appeared within their abdomens. During this early stage of parasitism, both parasitized and non-parasitized aphids behaved similarly but the former were more prone to falling off plants when disturbed by watering or other sources of vibrations. Aphids that fell off during this early stage of parasitism were usually able to climb back onto their plants.

Seven to eight days after parasitism, I observed distinct changes in the behaviour and appearance of parasitized aphids. The aphids stopped feeding and seemed to spend most of their time walking rapidly within the plant canopy. Their patterns of movement appeared random and they often fell from leaves while trying to cross over to adjacent leaves or different sides of the same leaf. Some individuals that fell and landed off their plants during this intermediate stage of parasitism were unable to locate their plants during the period of observation. None of the parasitized aphids walked off and away from a plant during my periods of observation. During this intermediate stage of parasitism, the white patch within the abdomen of parasitized aphids was generally larger than in the previous stage.

Approximately nine to ten days after parasitism, the aphids tended to be stationary but had not resumed feeding and were still prone to dropping when disturbed. Some individuals were observed to drop without any apparent disturbance. During this advanced stage of parasitism, individuals that fell from plants appeared very sluggish and usually did not move far from where they fell. The abdomen of parasitized aphids during this stage was completely white and visibly filled with an *E. californicus* larva.

Influence of Light

I found no significant differences among sets of aphids exposed to different parasitoid species or light treatments in the proportion of released aphids that were recovered as mummies ($F = 2.5519$; $df = 3, 36$; $P = 0.0708$). Parasitism was high and the mean number of mummies recovered from the four sets was 85.63 ± 9.38 ($\pm$SE). There were significant differences among sets in the proportion of mummies found off plants ($F = 192.1070$; $df = 3, 36$; $P < 0.001$). The absence of light greatly reduced the proportion of *E. californicus* mummies that formed off plants, but did not affect the distribution of *A. ervi* mummies (Table 5). The proportions of *A. ervi* mummies that formed off plants in the absence or presence of light were significantly less than the proportion of *E. californicus* mummies that formed off plants in the absence of light.

Table 5. Influence of light on the location of mummified *Acyrthosiphon pisum* containing immature of *Ephedrus californicus* or *Aphidius ervi*. One hundred aphids were confined with six female parasitoids of the same species for 2 h. The aphids were released onto a potted broad-bean plant and kept at 20 ± 1°C, 50 ± 10 % RH, with continuous light for 240 h or continuous light for 120 h followed by120 h without light. All aphids were reared at 20°C and 72 ± 4 h old when exposed to parasitoids.

Parasitoid species[1]	Light treatment[2]	Mummies off plant[3] (±SE)	Percentage off plant[4, 5] (±SE)
Aphidius ervi	240-h *L*	1.20 ±0.29	1.30 ±0.31[a]
	120-h *L* + 120-h *D*	1.30 ±0.26	1.48 ±0.30[a]
Ephedrus californicus	240-h *L*	72.20 ±4.39	88.53 ±2.37[b]
	120-h *L* + 120-h *D*	13.10 ±3.78	16.41 ±4.98[c]

[1]Ten trials were completed for each species of primary parasitoids.

[2]*L*, continuous presence of light; *D*, continuous absence of light.

[3]Mean number of mummies found off plants.

[4]Mean percentage of mummies found off plants.

[5]Means, within columns, showing the same letter are not significantly different ($P \geq 0.05$) by Tukey's test.

DISCUSSION

If aphid behaviour is altered after parasitism, there are several alternative explanations of the underlying mechanisms: (1) control by the aphid, (2) control by the parasitoid, and (3) trauma or pathology. If both organisms exert control, but the degree varies at different stages of the association, we may see transitions in host behaviour. For the aphid-aphidiid interactions that I studied, I favor the pathology hypothesis over the alternatives for two reasons. First, if the aphid is in control, why does aphid behaviour change only when it is parasitized by *E. californicus* and not the other four aphidiid species? All five species of wasps are related and, in fact, belong to the same family. Second, if the parasitoid is in control, why is movement of parasitized aphids off the host plant apparently the result of chance or 'random' events?

In my studies, the behaviour of the pea aphid varied with the type of aphidiid wasp that it was infected with. Pea aphids containing *E. californicus* larvae, 6-d old or older, stopped feeding, seemed to randomly disperse or 'wander' from their feeding sites, and had a high tendency to drop from plants. These behaviours were largely responsible for the location of *E. californicus* mummies away from colonies and plants. 'Wandering' was not observed among pea aphids parasitized by any one of the other four aphidiid species. Dropping is a common response to disturbance in both healthy and parasitized pea aphids; however, individuals containing third- or fourth-instar *E. californicus* exhibited not only the highest tendency to drop when disturbed but also a high tendency to drop while wandering.

Other studies have also found that the behaviour of parasitized aphids will vary with the parasitoid. Höller (1991) found that the mummification sites of parasitized cereal aphids varied with the aphidiid species they were infected with. Similarly, mummification sites often differ for aphids containing diapausing and non-diapausing parasitoids of the same species (Behrendt 1968; Brodeur and McNeil 1989, 1990). McAllister *et al.* (1990) did not find significant variation between the behaviour of pea aphids parasitized by *A. ervi* from the

coast or interior of BC and argued that it was unlikely for modified behaviour of infected interior aphids to be merely a proximate effect of interior parasitoids. However, the interior aphids could have been more susceptible than the coastal aphids to the pathological effects of feeding by immature *A. ervi*. Dropping behaviour may be accentuated by trauma resulting from parasitism, and this could be an alternative explanation for the differences in dropping behaviour observed for the two biotypes of pea aphid.

Movement of aphids parasitized by *E. californicus* appeared to be random within the plant canopy and the result of pathology induced by immature *E. californicus* prior to the death of the aphid. Aphids containing immature *E. californicus* rarely walked off a host plant, but they frequently fell while wandering and mummified away from the plant. Physical interactions between parasitized aphids wandering on a crowded plant may result in a 'cascade effect' and increase the proportion of parasitized aphids that fall from the plant. Interestingly, a small number of the *E. californicus* parasitized aphids from my studies always mummified on their host plants. I suggest that developmental variation may result in some parasitized aphids being in a less advanced stage of parasitism when the majority of the colony disperses. Parasitized aphids that remained, after the colony dispersed, would have a high probability of mummifying on their plants. An important assumption of most studies on the selection of micro-habitats by parasitized aphids is that parasitized individuals move (i.e. walk) away from their host plants to find sheltered or concealed sites. My studies show that falling, as a result of the pathology or trauma associated with parasitism, is also a viable explanation for the location of mummies away from the host plant.

It is interesting that aphids containing *A. ervi* or *M. paulensis* did not change their tendency to mummify on plants when mixed with aphids containing *E. californicus*. Wandering by *E. californicus* parasitized aphids seemed to have little effect on where *A. ervi* or *M. paulensis* parasitized aphids mummified. I propose that only parasitized aphids in advanced stages of parasitism become too

'disoriented' to stay on or return to plants. Delayed feeding on the organs and tissues of the aphid may enable *A. ervi* or *M. paulensis* larvae to minimize pathological effects until they are ready to kill the host. Polaszek (1986) found that parasitism of the pea aphid by *E. plagiator*, as opposed to *A. ervi*, resulted in more rapid embryonic degeneration. Immature *Ephedrus* may affect the physiology and/or biochemistry of aphids differently and disrupt the normal behaviour of their host aphids well in advance of host death.

Höller (1991) proposed that parasitized aphids, containing immature aphidiids of species with long post-larval development times, tend to mummify away from their original feeding sites. He examined the behaviour of cereal aphids parasitized by a several aphidiid species, including *E. plagiator*, but his results were not convincing. This pattern of host behaviour is also not evident among other aphid-aphidiid associations studied in the literature. In my studies, *M. paulensis* and *E. californicus* had similar developmental times but aphids parasitized by the former tended to mummify on plants. Developmental time of the parasitoid may be inconsequential if the proximate mechanism of aphid dispersal is trauma induced by the feeding of the immature parasitoid. In fact, the rate or extent of feeding may be less important than which aphid tissues are affected by parasitoid feeding.

In my studies the absence of light greatly reduced, but did not eliminate, the tendency of *E. californicus* parasitized aphids to mummify away from their host plants. Parasitized aphids that mummified away from their plants, were probably individuals that fell because they were moving or disturbed. These results do not support the hypothesis that modified behaviour of parasitized pea aphids is an induced phototactic response. Brodeur and McNeil (1990) proposed that potato aphids, containing diapausing larvae of *A. nigripes*, exhibited negative photo-taxis and chose dark- rather than light-colored substrate when seeking a site to mummify. However, they did not substantiate this claim by examining the behaviour of parasitized aphids in the absence of light. Aphid activity is probably reduced in the absence of light. The flight activity of alate aphids is inhibited by

darkness (Lewis and Taylor 1964), and it is likely that movement of apterous aphids is similarly affected. My findings suggest that aphids, containing immature *E. californicus*, still 'wander' in the absence of light but to a much lesser degree than in the presence of light.

From my studies, I conclude that pea aphid parasitized by some species of aphid parasitoids clearly show altered behaviour. But whether this altered behaviour benefits the parasitoid is another matter. Future investigations of aphid-aphidiid interactions should include studies on the biochemical and physiological changes induced by immature aphidiids in their aphid hosts. A better understanding of this topic is generally hampered by difficulties in separating active intervention by the parasitoid from parasitic trauma. Although the resulting behaviour of this aphid-aphidiid association may not be under the clear control of either the aphidiid wasp or aphid, it can result in a distribution of mummies that is both variable and uncertain for hyperparasitoids. Dispersal of aphids containing immature of *E. californicus* or other aphidiid species may have adaptive value if it reduces the incidence of hyperparasitism or predation under certain conditions. The following chapters of this thesis will present studies that identify some of these conditions.

CHAPTER 3

Host Handling and Acceptance by *Dendrocerus carpenteri*

INTRODUCTION

Foraging behaviour is an important aspect of the reproductive strategey of parasitoids. The foraging strategy of a primary parasitoid or hyperparasitoid is facilitated by a set of species-specific decision rules that determine which of several alternative behaviour patterns is optimal under certain conditions. Examination of the behaviour of female hyperparasitoids at a 'patch' level may yield a better understanding of both the foraging and reproductive strategies of hyperparasitoid species and their aphidiid hosts. A patch may consist of many levels or components with properties that affect, to varying degrees, the foraging behaviour of parasitoid wasps. The most fundamental component of a patch is the host. At this level, properties such as host quality and handling effort will influence the behaviour of female parasitoids.

Aphidiid wasps are common primary parasitoids of aphids over a broad range of plants (Mackauer and Stary 1967). Many field studies have reported differential hyperparasitism of aphidiid species sharing the same plant-aphid complex (Guitierrez and van den Bosch 1970, Sullivan and van den Bosch 1971, Höller *et al.*, 1993). Female hyperparasitoids may attack some aphidiid species more than others because of differences in availability, in suitability for larval development, or in the amount of time and energy needed for handling (examination and oviposition). Knowledge of the proximate causes behind differential hyperparasitism in the field may provide additional insight on the composition and dynamics of host-parasitoid communities.

All known species of aphidiid wasps are solitary endoparasitoids; supernumerary larvae are eliminated by physical combat or physiological suppression so that normally only one larva completes development (Mackauer and Chow 1986, Stary 1988). There are four larval instars; the last one will kill and mummify the host aphid. Many aphidiid species are susceptible to attack by species of *Dendrocerus* and other ectoparasitic hyperparasitoids during their last larval instar and

pupal stages of development inside the mummy (Bennett and Sullivan 1978, Walker and Cameron 1981, Takada 1973; Stary 1977). However, several factors may influence the vulnerability of primary parasitoids within mummies. Mummy morphology will vary with both the species of the primary parasitoid and the host aphid. Some mummies may be more difficult for hyperparasitoids to handle than others. Moreover, the age and mobility of the immature primary parasitoid inside the mummy can influence the oviposition success of a female hyperparasitoid (Bennett and Sullivan 1978). Finally, the suitability of primary parasitoids as hosts for hyperparasitoids may vary with the plant-host complex that the former exploits (Godfray 1994).

In this study, I tested two hypotheses: (1) host acceptance by *D. carpenteri* is influenced by the handling time, developmental stage and species of the immature primary parasitoid, (2) *D. carpenteri* attacks immature primary parasitoids only within mummified aphids. If aphid mummies of different aphidiid species vary in shape and texture, female *D. carpenteri* should select those that are least costly in terms of handling time. However if immature aphidiids differ in quality or vulnerability, the females should select those that are most suitable for progeny development. To evaluate host handling and preference of *D. carpenteri* in the laboratory, I used three aphidiid species: *A. ervi*, *E. californicus*, and *P. pequodorum*. I selected these three because they form distinctive mummies. *Praon* pupates in a separate cocoon below the dead aphid, whereas *Aphidius* and *Ephedrus* pupate inside the mummified host. *Ephedrus* mummies are bluish-black, whereas those of *Praon* and *Aphidius* range in color from white to brown.

D. carpenteri is a common hyperparasitoid of aphidiid wasps on the pea aphid (Stary 1977, Mertins 1985a, Mackauer and Lardner 1995), but little is known about its host preferences under field conditions. Hyperparasitism by *D. carpenteri* is random with regard to either mummy size or the sex of *A. ervi* collected from populations of pea aphids on alfalfa (Mackauer and Lardner 1995). However, *Dendrocerus* species appear to 'prefer' *Ephedrus* mummies over those of other aphidiid species that attack cereal aphids (Höller *et al.* 1993)

and the potato aphid (Sullivan and van den Bosch 1971). I show that the handling and acceptance of host by female *D. carpenteri* are influenced by both the species and the developmental stage of the primary parasitoid. In dichotomous choice tests, females 'preferred' immatures of *E. californicus* over those of *A. ervi* that were of the same developmental age and had not sclerotized. However, female *D. carpenteri* equally accepted these two species after the immature wasps had partially sclerotized. As female hyperparasitoids gained experience, they modified their handling behaviour for 'novel' hosts. I suggest that host preference by *D. carpenteri* is influenced more by the developmental stage than the species of aphidiid wasp.

An interesting question is whether *D. carpenteri* can exploit immature aphidiids within dead, non-mummified aphids. I am not aware of any studies that have properly addressed this topic. Walker and Cameron (1981) reported that *Aphidius smithi* is vulnerable to attack by *D. carpenteri* from the time of aphid death until the formation of the adult primary parasitoid. However, they described a dead aphid as one that had turned brown and was attached to a substrate by the immature aphidiid. Hence, Cameron and Walker probably used partially or fully mummified aphids. Pea aphids killed by immature aphidiids may remain non-mummified for several hours (Chow, personal observation). There also appears to be little information on the frequency at which immature aphidiids within dead, non-mummified aphids are encountered and attacked by natural enemies. Yet, in order to better understand differential parasitism of *Ephedrus* and other aphidiid species in the field, I felt that it was important to identify all the developmental stages of the host that were vulnerable to *D. carpenteri*. I examined the susceptibility of immatures of *A. ervi* and *E. californicus* to attack by *D. carpenteri* when the aphid host was alive and also when it was dead but non-mummified. I show that *D. carpenteri* is unable to hyperparasitize immature aphidiids prior to mummification of the host aphid.

OVIPOSITION AND HOST HANDLING BEHAVIOUR OF *DENDROCERUS CARPENTERI*

D. carpenteri is a solitary hyperparasitoid of the prepupal and pupal stages of various aphidiid species inside mummies (Takada 1973, Stary 1977). The oviposition behaviour of *D. carpenteri* has been described by Bennett and Sullivan (1978) and Sullivan (1988). After the female has examined a suitable mummy with her antennae, she turns around, with the abdomen directed towards the mummy. Anchoring herself with the prothoracic, and occasionally the mesothoracic, legs to the substrate, she extends her ovipositor, drills a hole with a jackhammer-like motion through the mummy shell, and deposits an egg externally on the primary parasitoid. During oviposition, a venom is applied by the female that does not immediately kill the host but prevents further development after 24 - 48 h (Bocchino and Sullivan 1981). Females produce eggs throughout their life and have a total fecundity of about 75 progeny (Walker and Cameron 1981). When deprived of hosts, they survive an average of 48 days at 20°C in the laboratory and store between 10-15 mature eggs in the ovaries (Le Ralec 1991); this number approximates the average daily fecundity (Walker and Cameron 1981). Mated females lay both unfertilized eggs, which produce sons, and fertilized eggs, which produce daughters, whereas unmated females lay only unfertilized eggs. The actual mechanism by which sex is determined is not known. To avoid superparasitism, females mark already parasitized hosts (Höller *et al.* 1991).

METHODS

Host Handling

In my first study, I evaluated the influence of host development and species on the handling time and oviposition behaviour of *D. carpenteri*. I set up four experiments, with 10 replicates (= trials) in each, as follows: 9-day old *A. ervi* vs 11-day old *A. ervi*, 11-day old *E. californicus* vs 15-day old *E. californicus*, 9-day old *A. ervi* vs 11-day old *E. californicus*, and 9-day old *A. ervi* vs 9-day old *P. pequodorum*. *Ephedrus californicus* develops more slowly than *A. ervi* and *P. pequodorum*, I thus standardized the immature primary parasitoids by developmental stage, as opposed to chronological age. In the experiments involving one species of primary parasitoid, *A. ervi* or *E. californicus*, the first group of mummies contained immature wasps that were in the last larval stage prior to pupation. The second group contained immature wasps that were in the pupal stage just prior to sclerotization. In the experiments involving two species of primary parasitoids, both groups of mummies contained immature wasps that were in the last larval stage prior to pupation. Experimental females provided with both *A. ervi* and *E. californicus* mummies were reared on *E. californicus*, whereas those provided with *A. ervi* and *P. pequodorum*. mummies were reared on *A. ervi* mummies.

To evaluate the responses of *D. carpenteri* to different kinds of mummies, I introduced a single female hyperparasitoid into a plastic petri-dish (9.0 cm in diam by 1.5 cm in ht). Each dish contained six mummies, three each of two kinds, which I glued in alternating pairs on the upper surface of a bean leaf. I observed the wasp continuously, distinguishing between the following behaviours: *examination* (the female examined a mummy externally with her antennae), and *attack* (the female drilled holes into the mummy, inserted her ovipositor and probed for the immature primary parasitoid; in the case of a *P. pequodorum* mummy, I distinguished between attack on the mummified aphid body and attack on the *P. pequodorum* cocoon below the mummy). A female hyperparasitoid could examine and probe a

particular mummy several times in sequence and either accept or reject it. I defined *handling time* as the total time from first encounter to the wasp's leaving a mummy, summed over all examinations and attacks. Oviposition could not be observed directly, but I found that attack duration and wasp behaviour could be used as an indicator (A. Chow, personal observation). I counted as oviposition any ovipositor insertion that lasted 3 min or longer and was followed by the wasp's sudden departure. A female *D. carpenteri* could re-encounter a previously accepted mummy; however, I prevented further attacks, and potentially superparasitism, by placing one half of a gelatin capsule (size 00) over such mummies. I manipulated and recorded the order in which mummies of each kind were encountered and handled. Each female encountered a sequence of one kind of mummy followed by the other kind. In replicates with different developmental stages of the same aphidiid species, the first mummy encountered contained the younger stage. In replicates with *A. ervi* and another aphidiid species, the former was the first encountered. Mummies that I did not want the female to attack were covered with halves of gelatin capsules. To prevent the accumulation of mummies that, for unknown reasons, might not be suitable, I immediately replaced any mummy that was examined but not accepted by another one of the same kind. A trial was concluded when the female hyperparasitoid had accepted three mummies of each kind. The mummies were stored individually in coded gelatin capsules at room temperature until adult wasps eclosed, which I identified to species and counted. Mummies from which no wasps eclosed were dissected four to five weeks after the experiment and their contents identified; these data were added to the numbers of eclosed parasitoids.

Host Preference

I defined preference as the ranked order of different kinds of hosts that are suitable. In my second study, I set up experiments to test if *D. carpenteri* females accept mummies according to the developmental stage and/or species of the primary parasitoid within a

mummy. In all experiments the null hypothesis is that immature primary parasitoids within each kind of mummy have the same probability of being hyperparasitized. Implicit in this hypothesis is the assumption that oviposition success is the same on both kinds of mummies. I tested eight different combinations of immature *A. ervi* and *E. californicus* (Table 6).

Table 6. Combinations of immature *A. ervi* and *E. californicus* used in preference tests for females of *Dendrocerus carpenteri.*

Species (Host 1)	Age (days)	Species (Host 2)	Age (days)	Replicates
1. *A. ervi*	8	*A. ervi*	11	10
2. *A. ervi*	9	*A. ervi*	11	10
3. *A. ervi*	9	*A. ervi*	12	10
4. *E. californicus*	10	*E. californicus*	15	10
5. *E. californicus*	11	*E. californicus*	15	10
6. *E. californicus*	11	*E. californicus*	16	10
7. *A. ervi*	9	*E. californicus*	11	20
8. *A. ervi*	12	*E. californicus*	16	20

As in the first study, I standardized the immature primary parasitoids by developmental stage. In the experiments involving one species of primary parasitoid, I compared larvae of different ages with pupae just prior to and after the start of sclerotization. In the experiments involving two species of primary parasitoids, I compared larvae just prior to pupation or pupae just after the start of sclerotization. I conducted 10 replicates for each experiment involving one species of primary parasitoid. In the experiments involving two species of primary parasitoids, I completed 10 replicates with *D. carpenteri* females reared on, respectively, *A. ervi* and *E. californicus*

for a total of 20 replicates in each experiment. Female hyperparasitoids were reared on two different primary parasitoids so that I could also test the influence of the rearing host on mummy choice in *D. carpenteri.*

In each replicate, I introduced a single female into a plastic petri dish (14.0 cm in diam by 1.5 cm in ht). Each dish contained 60 mummies, 30 of each kind, glued onto a 6 x 10 matrix laid out on a circle of bond paper (13.0 cm diam) with the two kinds of mummies alternating in sequence within each row. All hosts (mummies or dead, unmummified aphids) were glued 16 to 24 h prior to the start of an experiment and each dish was stored at 20°C until needed. When a female was introduced into a dish, she was released onto the mummy that occupied a site on the matrix that was selected by a random number generator. I allowed the female to forage freely and concluded the replicate 5 h after the time of release. All mummies were stored individually in coded gelatin capsules at room temperature until adult wasps eclosed. Mummies from which no wasps eclosed were dissected four to five weeks after the experiment and their contents identified; these data were added to the numbers of eclosed parasitoids.

Immature Aphidiids within Non-mummified Aphids

I conducted two experiments to determine if *D. carpenteri* could successfully hyperparastize immature *A. ervi* or *E. californicus* within live aphids or dead non-mummified aphids. In the first experiment, I tested female *D. carpenteri* under two conditions: (1) live pea aphids containing either 8-d old *E. californicus* or (2) live pea aphids containing 6-d old *A. ervi.* I placed six aphids, parasitized by same aphidiid species, on a circular piece (4.2 cm diam) of filter paper within a plastic petri dish (5.5 cm in diam by 1.5 cm in ht). All parasitized aphids were selected for uniformity in size and removed from their host plants approximately 30 min prior to the start of each trial. Each parasitized aphid was mobile and contained a single aphidiid larva that could be clearly seen within the aphid's abdomen. A female *D*

carpenteri was released into the dish and allowed to forage freely. I observed and recorded the behaviour of the wasp for 20 min. Both a new group of parasitized aphids and a naive female was used for each replicate. Twelve replicates were completed for each test condition for a total of 24 replicates.

In the second experiment, I also tested female *D. carpenteri* under two conditions: (1) dead, non-mummified aphids containing either 9-d old *E. californicus* or (2) dead non-mummified aphids containing 7-d old *A. ervi.* I used the same design as the first experiment, except the six aphids were arranged in a 3 x 2 matrix on the piece of filter paper. Aphids that began to mummify near the end of a replicate, turning brown or black, were covered with half of a gelatin capsule (size 00). Each female was allowed to forage for approximately 60 min before being removed from the arena. Each aphid was placed in a coded gelatin capsules and stored at 20 $\pm$ 1°C until offspring eclosed, which I both identified and counted. Mummies from which no insects emerged were dissected and their contents identified. I completed 80 replicates for each test condition for a total of 160 replicates.

Influence of Mating Status

'Host quality' models predict that females should differentially allocate fertilized and nonfertilized eggs to low- and high-quality hosts respectively (Charnov *et al.* 1981, Charnov 1982). Mated females may have longer handling times if they evaluate hosts more carefully than do non-mated females. I was motivated by the results of the host handling study to determine if mating status would influence handling behaviour by female *D. carpenteri* for 9-day old *A. ervi.* A single female was placed in a plastic petri dish (3.5 cm in diam by 1.0 cm in ht) containing a single mummy glued onto the middle of a circular piece of filter paper (2.0 cm diam). After the female encountered the mummy, I recorded the number and duration of all examination and attack bouts until she left the mummy. Each mummy was placed in a

gelatin capsule and stored in a growth chamber until offspring eclosed. After a period of 3 weeks, all mummies from which no wasps eclosed were dissected and their contents identified. I counted only those trials in which adult or immature *D. carpenteri* were recovered. Thirty mated and non-mated females were tested for a total of 60 replicates. All females had no previous experience with hosts prior to the experiment.

Statistical Analysis

To evaluate the influence of encounter sequence and host type on handling behaviour, I used two-way ANOVA to compare the following: total examination time, total attack time, total handling time, total number of holes drilled into the mummy, oviposition success (proportion of attacked mummies on which adult or immature hyperparasitoids developed on). The factors examined were the kind of mummy and the sequence of encounter for each kind of mummy [i.e. 1st 9-day old *A. ervi*, 1st 11-day old *E. californicus*]. One-way ANOVAs were used to compare the effect of encounter sequence on both the number and duration of attack bouts on the body and cocoon of *P. pequodorum* mummies. I also used a one-way ANOVA to compare the oviposition success of each kind of mummy among the four experiments. When significant effects were found, I used Tukey's test to compare the dependent variables. All proportions were transformed into their arcsine values by equation 14.5 in Zar (1984, p. 186). Differences between the frequency of examination and attack bouts on each kind of mummy were assessed by paired-difference t-test (two-tailed). Differences in the total handling time and both the number and duration of examination or attack bouts by mated and non-mated females were compared with one-way ANOVAs.

In each specificity experiment, I compared observed and expected frequencies of each kind of mummy that were hyperparasitized and not hyperparasitized using the log-likelihood ratio test with Williams' correction for continuity (Sokal & Rohlf 1981,

p. 737). In experiments involving one species of primary parasitoid, I also used the paired-difference t-test (two-tailed) to compare the numbers of each kind of mummy on which a hyperparasitoid developed (hyperparasitism). In experiments involving two species of primary parasitoids, I transformed the same dependent variables into the arcsine values of their proportions and used two-way ANOVA to analyze the data. The factors examined were the kind of mummy and the rearing host for *D. carpenteri* females. When significant main effects or interactions were found, I used Tukey's test to compare the dependent variables. When the rearing host of the female was found to have no significant effect on or interaction with hyperparasitism, I pooled the data from all replicates and analyzed the numbers of each kind of mummy that were hyperparasitized with the paired-difference t-test (two-tailed) and the log-likelihood ratio test. The total number of both kinds of hosts hyperparasitized (total hyperparasitism) in each of the six experiments with only one primary parasitoid species were transformed into the arcsine values of their proportions and compared with one-way ANOVA. Similarly, total hyperparasitism in both experiments with two primary parasitoid species were also compared with one-way ANOVA. To compare the mortality of the two kinds of primary parasitoid in each experiment, I used paired-difference t-tests (two-tailed).

RESULTS

Host Handling

The handling behaviour of *D. carpenteri* varied with the species of primary parasitoid, but differences were more evident between *P. pequodorum* and *A. ervi* than between *E. californicus* and *A. ervi* (Table 7). Examination ($F = 36.900$; $df = 1, 54$; $P < 0.001$) and attack ($F = 29.604$; $df = 1, 54$; $P < 0.001$) times of females were two to three times longer on *P. pequodorum* than on *A. ervi* mummies. In comparison, examination times of females were slightly longer on *A. ervi* than *E. californicus* mummies ($F = 4.908$; $df = 1, 54$; $P = 0.031$) but their attack ($F = 1.084$; $df = 1, 54$; $P = 0.302$) and handling ($F = 1.384$; $df = 1, 54$; $P = 0.245$) times on these mummies were not significantly different. Females drilled, on average, 3.5 times as many holes into *P. pequodorum* than *A. ervi* mummies ($F = 40.114$; $df = 1, 54$; $P < 0.001$) and 1.5 times as many holes into *A. ervi* than *E. californicus* mummies ($F = 12.033$; $df = 1, 54$; $P = 0.001$).

The handling efficiency of *D. carpenteri* for different species of hosts seemed to increase with experience. In trials with both *E. californicus* and *A. ervi* mummies, females had considerably longer attack ($F = 6.114$; $df = 1, 54$; $P = 0.004$) and handling times ($F = 6.324$; $df = 1, 54$; $P = 0.003$) on first than second or third mummies (Fig. 2), but the number of holes drilled did not vary with encounter sequence ($F = 1.218$; $df = 1, 54$; $P = 0.304$). In trials with both *P. pequodorum* and *A. ervi* mummies, handling behaviour of females on *A. ervi* mummies was not significantly affected by encounter sequence; however, females had fewer examination bouts and shorter examination times as they gained experience on *P. pequodorum* mummies (Fig. 3, 4). *Dendrocerus carpenteri* apparently learned to invest less time in handling the part of the *Praon* mummy that did not contain the larva. Both the number of attacks ($F = 6.512$; $df = 2, 27$; $P = 0.005$) and attack time ($F = 5.157$; $df = 2, 27$; $P = 0.013$) by females on the mummified aphid body were significantly greater for first than second or third *P. pequodorum* mummies, but the number of attacks

Table 7. Handling behaviour by females of *Dendrocerus carpenteri* for mummies of three aphidiid species. A female wasp sequentially handled six mummies, three of each kind of mummy, in each of 10 replicates for each combination of hosts.[1]

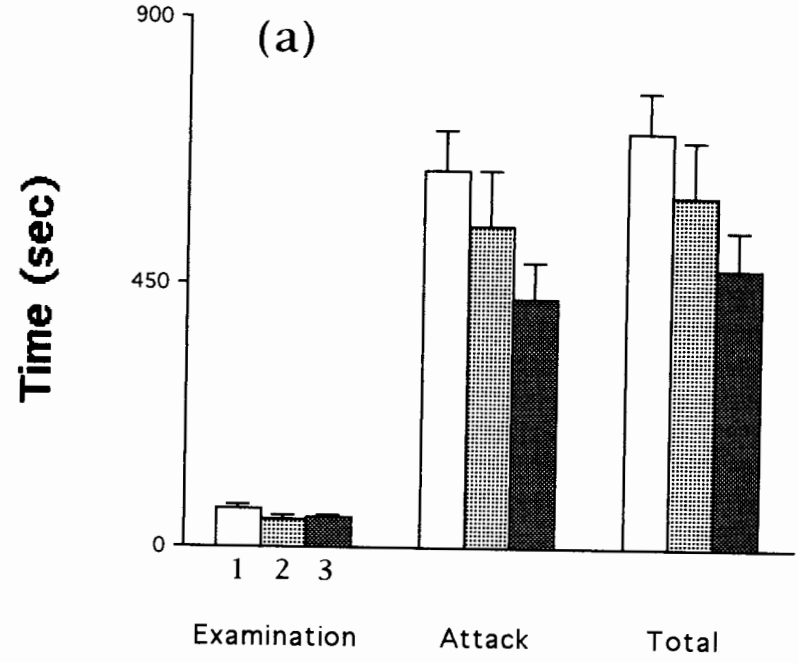
Host species	Age (days)	Total Examination (sec)[2]	Total Attack (sec)[2]	Total Handling (sec)[2]	Total Holes Drilled[2,3]
1. *A. ervi*	9	38.83 ± 3.76[a]	448.13 ± 37.13[a]	486.97 ± 40.45[a]	1.60 ± 0.25[a]
	11	40.90 ± 2.70[a]	455.23 ± 25.13[a]	496.13 ± 26.79[a]	1.53 ± 0.17[a]
2. *E. californicus*	11	41.07 ± 3.29[a]	367.73 ± 22.17[a]	408.80 ± 23.38[a]	1.43 ± 0.14[a]
	15	42.93 ± 4.27[a]	484.07 ± 32.78[b]	527.00 ± 34.86[b]	1.33 ± 0.10[a]
3. *A. ervi*	9	55.27 ± 3.28[a]	535.97 ± 45.74[a]	591.23 ± 46.96[a]	2.03 ± 0.18[a]
E. californicus	11	44.63 ± 3.87[b]	472.33 ± 48.14[a]	516.97 ± 50.30[a]	1.30 ± 0.11[b]
4. *A. ervi*	9	36.43 ± 2.50[a]	298.50 ± 17.39[a]	334.93 ± 17.89[a]	1.30 ± 0.10[a]
Praon pequodorum	9	92.77 ± 10.38[b]	760.30 ± 82.13[b]	853.07 ± 86.09[b]	4.60 ± 0.60[b]

[1]A total of thirty mummies of each kind were handled in each combination.

[2]Means (± SE), within columns, for the same host combination showing the same letter are not significantly different ($P > 0.05$) by Tukey's test.

[3]Mean (± SE) number of holes that female wasps drilled, with their ovipositors, into each kind of mummy.

Figure 2. Mean handling times by females of *Dendrocerus carpenteri* for sequentially encountered mummies of two kinds: (a) mummified pea aphids containing 9-d old *Aphidius ervi*; (b) mummified pea aphids containing 11-d old *Ephedrus californicus*. Females (N= 10) handled three mummies of each kind in one foraging bout. Open columns = first mummy; lightly shaded columns = second mummy; heavily shaded columns = third mummy. Bars indicate + 1 SE.


(a)
900
450
0
Time (sec)
1 2 3
Examination
Attack
Total

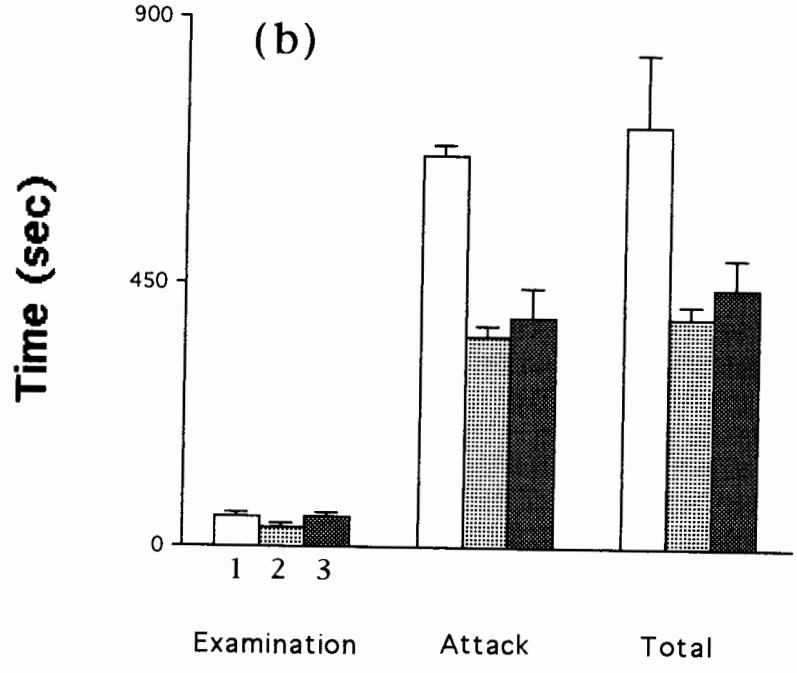

(b)
900
450
0
Time (sec)
1 2 3
Examination
Attack
Total

Figure 3. Mean number of handling events by females of *Dendrocerus carpenteri* for sequentially encountered mummies containing 9-d old *Praon pequodorum*. Females (N = 10) handled three *Aphidius ervi* and three *P. pequodorum* mummies in one foraging bout. Open columns = first *Praon* mummy; lightly shaded columns = second *Praon* mummy; heavily shaded columns = third *Praon* mummy. Bars indicate + 1 SE.

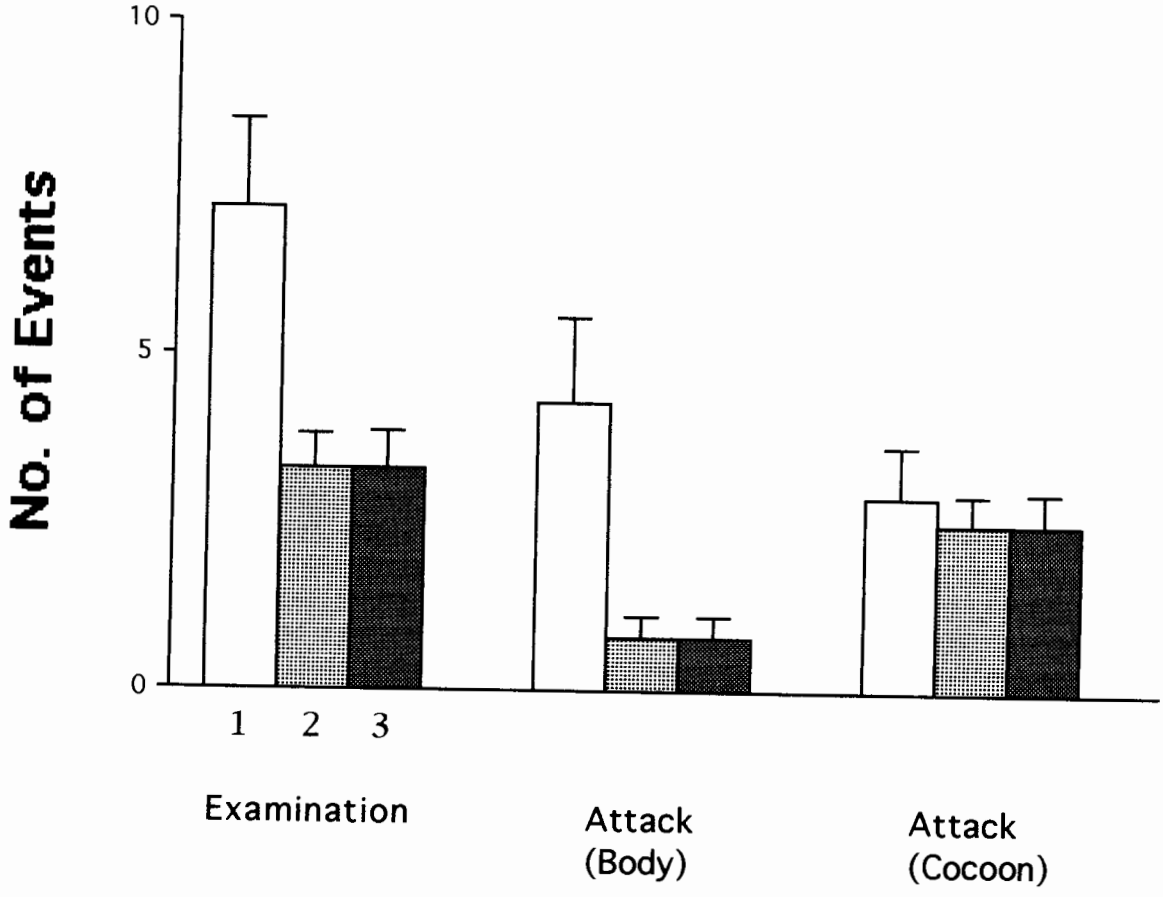
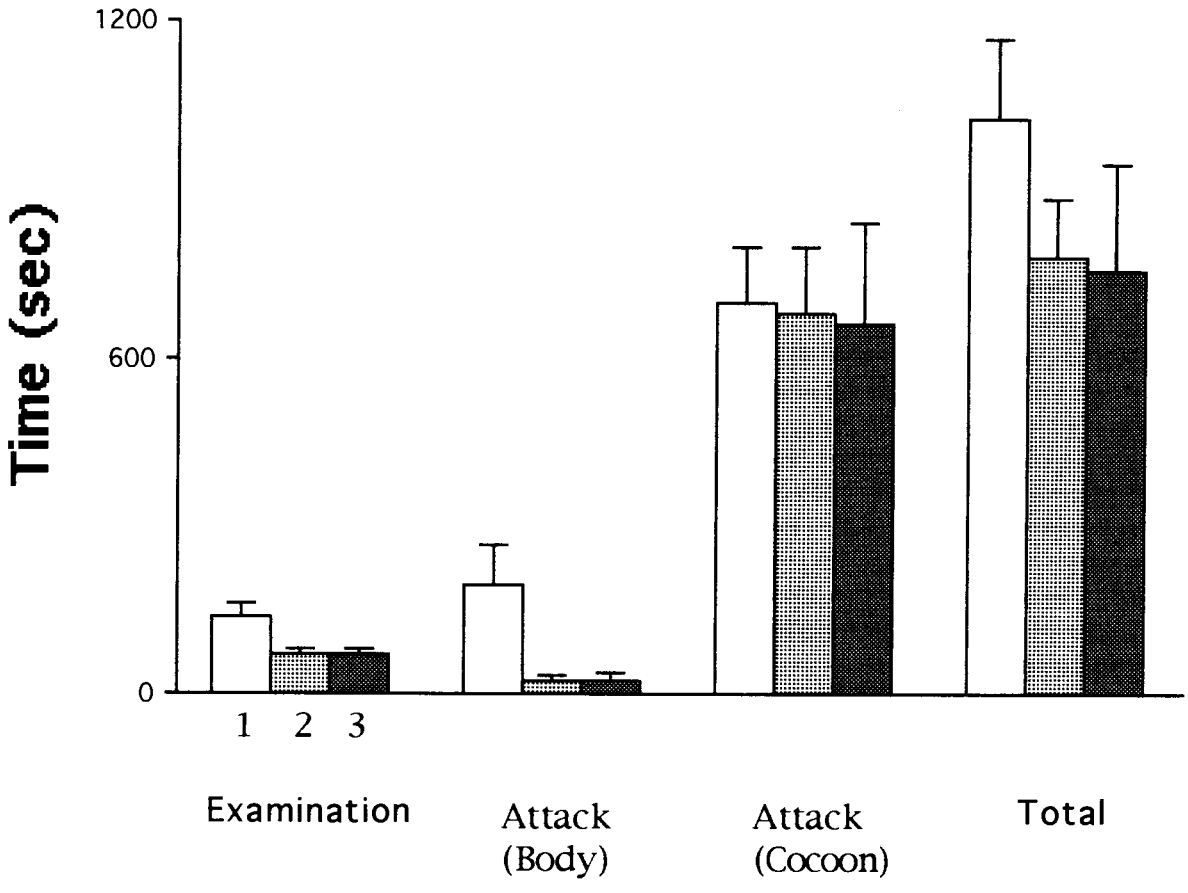

No. of Events
10
5
0
1
2
3
Examination
Attack
(Body)
Attack
(Cocoon)

Figure 4. Mean handling time by females of *Dendrocerus carpenteri* for sequentially encountered mummies containing 9-d old *Praon pequodorum*. Females (N= 10) handled three *Aphdius ervi* and three *P. pequodorum* mummies in one foraging bout. Open columns = first *Praon* mummy; lightly shaded columns = second *Praon* mummy; heavily shaded columns = third *Praon* mummy. Bars indicate + 1 SE.


1200
600
0
Time (sec)
1 2 3
Examination
Attack (Body)
Attack (Cocoon)
Total

(F = 0.160; df = 2, 27; P = 0.853) and attack time (F = 0.019; df = 2, 27; P = 0.981) by females on the cocoon were not significantly affected by encounter sequence. Total handling time by females for *P. pequodorum* mummies did not vary with encounter sequence (F = 1.291; df = 2, 54; P = 0.283) (Fig. 4).

The handling behaviour of *D. carpenteri* for mummies containing different developmental stages of *A. ervi* or *E. californicus* did not vary with experience (Table 7). Females also did not handle mummies containing *A. ervi* pupae or larvae differently. However, attack (F = 8.967; df = 1, 54; P = 0.004) and handling (F = 8.096; df = 1, 54; P = 0.006) times by females were longer on mummies containing *E. californicus* pupae than on mummies containing *E. californicus* larvae.

Oviposition success of *D. carpenteri* was high among all the types of immature aphidiids that were tested. There was little variation among the kinds of hosts tested and the mean percentage of attacked mummies that I recovered hyperparasitoids from was 88.75 $\pm$2.63 ($\pm$ SE, N= 240). The proportion of hyperparasitoids that died within their mummies did not vary with the type of host and was on average less than 3 %. There were also no differences between the numbers of examination and attack bouts by females on each kind of mummy. I observed that a female regularly followed an examination bout with one attack bout and drilled one hole in each attack bout. Females rarely rejected mummies and usually attacked each kind of host encountered.

Host Preference

Among the six choice tests with different developmental stages of the same aphidiid species, host acceptance by *D. carpenteri* was influenced by the physiological state of the primary parasitoid, but the effects differed with the species of primary parasitoid (Table 8). Females strongly preferred mummies containing non-sclerotized pupae over mummies containing partially sclerotized pupae, regardless of

Table 8. Specificity by females of *Dendrocerus carpenteri* for larval and pupal stages of *Aphidius ervi* and *Ephedrus californicus*. A female wasp was singly confined for 5 h with thirty mummies of each kind of host in each combination of hosts.[1]

Host species	Age (days)	No. Mummies Hyperparasitized[2] (30)	Choice Index[3] (a/b)	G_W[4]
1. *A. ervi*	8 days	4.10 ± 0.64**		
	11 days	9.60 ± 0.64	0.427	29.224***
2. *A. ervi*	9 days	9.90 ± 1.20*		
	11 days	5.10 ± 0.59	1.941	20.734***
3. *A. ervi*	9 days	13.50 ± 0.73**		
	12 days	2.00 ± 0.30	6.750	-79.196***
4. *E. californicus*	10 days	3.70 ± 0.45**		
	15 days	10.40 ± 1.15	0.356	42.914***
5. *E. californicus*	11 days	6.40 ± 0.83		
	15 days	7.00 ± 0.80	0.914	0.346
6. *E. californicus*	11 days	10.80 ± 0.95**		
	16 days	3.30 ± 0.76	3.273	54.262***
7. *A. ervi*	9 days	6.90 ± 0.94**		
E. californicus	11 days	13.65. ± 0.97	0.504	69.288***
8. *A. ervi*	12 days	3.35 ± 0.57		
E. californicus	16 days	4.30 ± 0.40	0.791	2.417

[1]The numbers of replicates completed for combinations that involved one and two species of primary parasitoid were respectively ten and twenty.

[2]Statistical significance of difference between means (± SE), within columns, of each choice condition (by paired-difference t-test, two-tailed): *, $P < 0.05$; **, $P < 0.01$.

[3]Ratio of total numbers of first ('a') and second ('b') kinds of hyperparasitized hosts among all replicates of each combination.

[4]Fit of observed frequencies of hyperparasitized hosts of each kind with their expectations from a 1:1 ratio of 'no preference' (by loglikelihood ratio test with Williams' correction; ***$P < 0.001$).

host species. In trials with only *E. californicus* or only *A. ervi* mummies, *D. carpenteri* females preferred mummies containing aphidiids that were in the larval stages just prior to pupation over mummies containing aphidiids at younger larval stages, but they showed no preference between mummies containing either the oldest larval stage or pupal stages that had not sclerotized.

Although *D. carpenteri* females foraged selectively when given a choice of non-sclerotized hosts, the proportion of all available hosts (60 per trial) that was hyperparasitized did not vary significantly among the six combinations tested ($F = 0.561$; df 5, 54; $P = 0.729$) and was 0.238 ± 0.007 (mean $\pm$ SE). Females apparently allocated most of their foraging time to handling preferred hosts and were able to efficiently recognize and reject less preferred hosts.

Mortality of immature primary parasitoids differed among developmental stages. In trials that compared two different larval stages, the younger larval stage had considerably higher mortality. The number of dead 8-day old *A. ervi* and 11-day old *A. ervi* larvae were respectively 19.600 ± 0.846 and 0.800 ± 0.327 (mean $\pm$ SE) ($t = 22.512$, df = 9, $P < 0.001$). Likewise, the number of dead 10-day old *E. californicus* and 15-day old *E. californicus* larvae were respectively 14.300 ± 0.790 and 2.200 ± 0.629 (mean $\pm$ SE) ($t = 10.307$, df = 9, $P < 0.001$). Mortality between the two kinds of hosts in other experiments was not significantly different and was on average less than 10% for each kind.

I did not find any significant differences in host selection among female *D. carpenteri* reared on *A. ervi* or *E. californicus* in choice tests with larval ($F = 1.999$; df = 1, 36; $P = 0.166$) or late pupal stages ($F = 0.417$; df = 1, 36; $P = 0.522$) of the two aphidiid species. However, host selection by *D. carpenteri* appeared to be influenced by both the species and the developmental stage of the host in these tests. Females showed differential acceptance of *A. ervi* and *E. californicus* mummies containing larvae; they hyperparasitized more *E. californicus* and fewer *A. ervi* than predicted by the null model of "no preference" (G_W =

69.288, df = 1, P < 0.001), but equally accepted *E. californicus* and *A. ervi* mummies containing pupae that had partially sclerotized (Table 8). *Dendrocerus carpenteri* seemed less efficient at handling hosts that were partially sclerotized because females hyperparasitized three times as many larvae than late pupae. The proportions of available hosts hyperparasitized in experiments with only larval and only late pupal stages were respectively 0.343 ±0.020 and 0.128 ±0.012 (mean ±SE) (F = 87.859; df = 1, 38; P < 0.001). Host mortality was low in both tests, but it was slightly higher among immature *E. californicus* than *A. ervi*. The mean numbers of dead *E. californicus* and *A. ervi* larvae were respectively 4.400 ±0.499 and 1.450 ±0.359 (±SE) (t = 4.844, df = 19, P < 0.001). Similarly, the number of dead *E. californicus* and *A. ervi* pupae were respectively 5.35 ±0.504 and 1.500 ±0.295 (±SE) (t = 6.568, df = 19, P < 0.001).

Immature Aphidiids within Non-mummified Aphids

None of the *D. carpenteri* females attempted to oviposit into live aphids containing either immature *A. ervi* or *E. californicus*. Females frequently encountered live aphids, but they quickly moved away from the aphids after contact. Following repeated encounters with live aphids, most females attempted to leave their dish. In comparison, I observed that females usually examined dead, non-mummified aphids containing aphidiid larvae. Most females rejected dead aphids after a few seconds of examination. Many females attempted to probe at least one dead aphid, but the attempts usually lasted less than 30 seconds. I did not recover any adult or immature *D. carpenteri* from any of the trials with dead aphids that contained immature *A. ervi* or *E. californicus*. Pre-emergence mortality of *A. ervi* and *E. californicus* were respectively 14.0 ±15.8 % and 9.4 ±14.5 %.

Influence of Mating Status

There were no significant differences between the total handling time or the number and duration of examination or attack bouts by mated and non-mated females for mummies containing 9-day old *A. ervi* (Table 9).

Table 9. Handling behaviour of mated and non-mated *Dendrocerus carpenteri* on *Aphidius ervi* mummies. Each female was given a mummy containing a 9-day old *A. ervi*. Female *A. ervi* were given second-instar pea aphids (72 ± 4 h) to parasitize and all parasitized aphids were reared on broad bean at 20 ± 1°C, 40-60% RH, with continuous lighting. Female *D. carpenteri* were 6 to 8 days old when used for experiments.

Mating Status of Female[1]	Examination Bouts[2]	Examination Time (sec)[2]	Attack Bouts[2]	Attack Time (sec)[2]	Total Handling Time (sec)[2]
Mated Female	1.17 ± 0.08[a]	31.70 ± 3.68[a]	1.17 ± 0.08[a]	278.47 ± 14.12[a]	310.17 ± 15.54[a]
Non-mated Female	1.10 ± 0.06[a]	26.17 ± 2.67[a]	1.10 ± 0.06[a]	277.50 ± 17.42[a]	303.67 ± 18.31[a]

[1]Thirty trials were completed for each type of female.

[2]Means (± SE), within columns showing the same letter are not significantly different by paired-difference t-test (two-tailed).

DISCUSSION

There are several important steps necessary for successful parasitism: host habitat location, host location, host acceptance, host suitability, and host regulation (Vinson and Iwantsch 1980). Host recognition and host evaluation are two important behavioural events that precede the acceptance of a potential host. For ectoparasitoids, host recognition often involves association with some physical or chemical cue(s) derived from the host. Females can obtain information by either external or internal examination of the host; the former usually by antennal or tarsal contact and the latter by ovipositor probing (Godfray 1994). Ectoparasitoids that exploit concealed hosts often assess, to some degree, the suitability of a host through external examination and will reject some without probing. Both the duration and sequence of events during examination or probing may provide some indication of the suitability of the host.

Rivers (1996) showed that the duration of antennal drumming by *Nasonia vitripennis* and *Muscidifurax zaraptor* varied among the puparia of different species of flies. The duration of drumming was shortest on flies that were most suitable for oviposition and increased as host suitability decreased. However, the amount of time invested in probing and oviposition were not predictive measures of host preference. I found the same behavioural pattern in *D. carpenteri*; females preferred to attack mummies containing *E. californicus* larvae over those containing *A. ervi* larvae and had shorter examination times on the former. No differences were found in the examination time for hosts that *D. carpenteri* accepted equally in choice tests. Oviposition (attack) times and total handling times of *D. carpenteri* did not differ among preferred and less preferred hosts, but females did drill more holes into the latter. Drilling through the mummy and locating the immature primary parasitoid may represent a considerable investment in both time and energy for *D. carpenteri*. The longer examination times on less preferred hosts may indicate that females required more information or time before they committed to ovipositor probing.

Endophagous aphid hyperparasitoids are somewhat host specific while ectophagous aphid hyperparasitids are generally not host specific (Hafez 1961, Sullivan 1987). Gauld and Bolton (1988) suggested that a likely explanation for this trend was that ectophagous species do not have to contend with the physiological stresses of living within a live host. *Dendrocerus carpenteri* has very wide host range and it would be adaptive for this species to recognize different kinds of hosts and modify handling behaviour to increase handling efficiency. Rivers (1996) showed that some polyphagous ectoparasitoids of fly pupa have slightly different handling behaviour among different species of hosts. However, the puparia of the tested fly species were similar in morphology. *Praon* species are unique among Aphidiidae because the immature wasp completes development within a cocoon situated under the mummified body of the dead aphid. All other aphidiid species complete development within the mummified body of their aphid host. *Dendrocerus carpenteri* females initially invested considerable time in examining and attacking the empty bodies of *P. pequodorum* mummies, but they quickly learned to concentrate their handling efforts on the cocoon. Interestingly, this did not significantly decrease the handling times of females for *P. pequodorum* mummies because attack time for the cocoon accounted for the greatest proportion of total handling time. Nevertheless, the behaviour of *D. carpenteri* towards the mummified body did change significantly with experience and indicates an ability to both recognize and adjust handling behaviour for a novel host.

The unique structure of the *Praon* mummy did not reduce the susceptibility of immature *Praon* to attack by *D. carpenteri.* Female hyperparasitoids had considerably longer handling times on mummies containing *P. pequodorum* than *A. ervi* larvae, but they readily accepted both kinds of mummies. Handling time appears to have little influence on host acceptance by *D. carpenteri.* When hosts are rare and dispersed, as is often with aphid mummies (Mackauer and Vöelkl 1993), it may be adaptive for *D. carpenteri* to reject only low quality or unsuitable hosts. Unfortunately, due to difficulties in transferring *Praon* mummies, I was unable to test this hypothesis by conducting choice tests with adequate sample sizes. It is possible that the unique

configuration of the *Praon* mummy reduces the susceptibility of this aphidiid species to either predators or other species of ecto-hyperparasitoids, but this topic requires further study.

Female *D. carpenteri* apparently ranked mummies containing larval stages of *E. californicus* highest for oviposition. This behaviour is consistent with the patterns of hyperparasitism found for *Ephedrus* species in the field. Field studies of hyperparasitism among primary parasitoids have shown that *Ephedrus* species are disproportionately attacked by *Dendrocerus* species. Höller et al. (1993) examined hyperparasitism of *Ephedrus*, *Aphidius*, and *Praon* mummies by *Dendrocerus* species on wheat and found that it was twice as high among *Ephedrus* as among the other two aphidiid species. Similarly, Sullivan and van den Bosch (1971) studied hyperparasitism of the primary parasitoid complex of the potato aphid on *Iris germanica* L. by *Dendrocerus* species and found that it was approximately six times as high among *E. californicus* as among *A. nigripes* and an unidentified species of *Monoctonous*. It is possible that these results were biased by the sampling procedure. Only mummies on plants were collected and *Ephedrus* mummies are often found away from the plant that the host aphid was feeding on (Behrendt 1968, Höller 1991). However, parasitized aphids that mummify off plants may escape attack by hyperparasitoids (Brodeur and McNeil 1989) and *Ephedrus* species may only be hyperparasitized if they mummify on plants.

It is difficult to explain why *D. carpenteri* should prefer *E. californicus* larvae over *A. ervi* larvae. Handling times and oviposition success of *D. carpenteri* among mummies containing *E. californicus* and *A. ervi* larvae were not significantly different. Host conditioning on *A. ervi* did not reverse preference; female wasps reared on *A. ervi* still preferred *E. californicus* larvae. I have an explanation for the behaviour of *D. carpenteri*, but it requires an important assumption. *Ephedrus californicus* develops at a slower rate than *A. ervi* and requires approxmately 24 h longer to develop from a larva to a pupa. The venom of *D. carpenteri* paralyzes immature aphidiids and prevents further development, but it requires time to take effect (Bocchino and

Sullivan 1981). *Dendrocerus carpenteri* may prefer to attack *E. californicus* larvae because this host has a greater probability of being a larvae or a non-sclerotized pupae when the eggs of the hyperparasitoid hatch.

Parasitoids that are idiobionts usually face the problem of reduced host quality among the more advanced stages of their host. Bocchino and Sullivan (1981) transferred eggs of *D. carpenteri* to non-paralyzed larvae of 8-day old *A. ervi* and found that the former had fully sclerotized by the time the hyperparasitoid eggs hatched. All of the *D. carpenteri* larvae died because they were unable to pierce the hardened exoskeleton and feed, but the primary parasitoids developed normally and eclosed. It may be adaptive for *D. carpenteri* to prefer slower developing hosts for oviposition. Additional studies, beyond the scope of this thesis, are required to test predictions from this hypothesis.

The developmental stage of the host appears to be more important than the species when *D. carpenteri* selects hosts for oviposition. Female wasps were able to hyperparasitize partially sclerotized pupae, but they show a preference for non-sclerotized hosts when given a choice. Partially sclerotized hosts could be less preferred because they are more difficult to handle or less suitable hosts. Dissection of the mummies containing partially sclerotized hosts revealed that only the non-sclerotized parts of the hosts were consumed. Head and thorax tissue were usually inaccessible because of sclerotization. Otto (1996) showed that *D. carpenteri* that developed on partially sclerotized hosts had longer development times and were significantly smaller than those that developed on non-sclerotized hosts. *Dendrocerus carpenteri* will attack very low quality hosts, such as 13-day old (adult) *A. ervi*, in 'no-choice' situations but they have extremely long handling times and low oviposition success on them (Otto 1996).

Michaud and Mackauer (1995) found that mating status could influence the foraging or reproductive behaviour of female parasitoids

within a patch of hosts. They showed that young, non-mated females of *Monoctonus paulensis* abandoned host patches earlier and superparasitized fewer pea aphids than did mated females. Differences in host handling behaviour among mated and non-mated females have been more difficult to quantify. 'Host quality' models predict that females should differentially allocate fertilized and nonfertilized eggs to low- and high-quality hosts respectively (Charnov *et al.* 1981, Charnov 1982). We may expect mated females to have longer handling times if they evaluate hosts more carefully than non-mated females. Mated and non-mated *D. carpenteri* females did not differ significantly in their handling times or behaviour for hosts in my study. However, the behaviour of mated and non-mated females may become evident while foraging within patches of hosts. This hypothesis warranted further study and it was evaluated in experiments that will be covered later in this thesis.

Aphid hyperparasitoids belonging to the genus *Aphidencyrtrus* can attack the larvae of primary parasitoids either while the aphid is alive or after the mummy is formed (Sullivan 1988). However, aphid hyperparasitoids with dual oviposition behaviour have not been recorded in any other genus. I found that *D. carpenteri* ignored live, parasitized aphids and was unable to successfully attack immature aphidiids within dead parasitized aphids that had not mummified. The ovipositor of *D. carpenteri* appears to be adapted for perforating thick and hard integuments and not thin, flexible integuments (Le Ralec *et al.* 1996). Gauld and Bolton (1988) proposed that some obligate hyperparasitoids evolved from parasitoids of predators of their primary hosts. Megaspilids closely related to *Dendrocerus* sp. are known to be primary parasitoids of various *Syrphidae*, *Hermerobiidae*, *Chrysopidae* and *Coccinellidae* associated with aphids.

Mortality of the youngest larvae (8-day old *A. ervi* and 10-d old *E. californicus*) was the highest among all the kinds of hosts in my studies and I believe that it resulted from (1) my inability to consistently select parasitized aphids that contained healthy, immature primary parasitoids and (2) injuries to immature primary parasitoids

due to handing of parasitized aphids prior to mummification. I ruled out higher mortality due to handling by *D. carpenteri* because the numbers of mummies hyperparasitized in these studies were approximately the same as those in studies where host mortality was much lower. It is unlikely that the higher mortality for these hosts was due to unsuccessful attempts at hyperparasitism because we would expect much lower levels of hyperparasitism if this was the case. *Dendrocerus carpenteri* females probably showed preference for mummies containing older larvae because of the large number of unhealthy or dead hosts among mummies containing young larvae.

In summary, the host handling behaviour of *D. carpenteri* enables it to exploit a wide range of hosts. Generalist hyperparasitoids are often 'opportunists' that do reasonably well in a variety of hosts and habitats. Because *D. carpenteri* exploit hosts that are often rare and dispersed, it would be adaptive to reject only very low quality or unsuitable hosts. I found that host preference of *D. carpenteri* was influenced by both the developmental stage and the species of the primary parasitoid. The most important criteria for host acceptance was the developmental stage of the host. Sclerotized hosts were less acceptable for oviposition than non-sclerotized hosts. The species of the host only influenced preference when hosts were non-sclerotized and available in large numbers. Handling time varied with the species of the primary parasitoid but not among non-sclerotized stages of the same species. Examination time for mummies appeared to be an indicator of host quality. Experienced *D. carpenteri* modified their handling behaviour for different hosts and increased their handling efficiency.

Host preference of *D. carpenteri* for *E. californicus* was consistent with results from the field and warrants additional testing of hypotheses regarding the effect of mummy location and distribution on the foraging efficiency of this hyperparasitoid. The choice of hosts will have a significant influence on the results of studies on foraging or reproductive behaviour. *Dendrocerus carpenteri* was able to exploit immature primary parasitoids after but not prior to mummification of

the host aphid. Early larval and late pupal stages of *A. ervi* or *E. californicus* would be unsuitable hosts for foraging and reproductive studies of *D. carpenteri* because of the high risk of mortality in the former and the risk of partial sclerotization in the latter if development varied among individuals. The use of 9-day old *A. ervi* or 11-day old *E. californicus* would minimize variablity in host quality and thus behaviour of *D. carpenteri*. Studies in the next chapter will investigate the effect of host distribution and density on the foraging and reproductive behaviour of *D. carpenteri*.

CHAPTER 4

Foraging Behaviour of *Dendrocerus carpenteri*

INTRODUCTION

Patterns of progeny allocation and host exploitation in the parasitic Hymenoptera have been the subjects of extensive research (Godfray 1994). Parasitoid hosts are rarely random in distribution and often exist in discrete groups or patches in the environment. The fitness of a female parasitoid is largely determined by her ability to locate host patches, assess risk within and between patches, and allocate progeny accordingly. Mackauer and Völkl (1993) observed that most aphidiid parasitoids parasitize only a small proportion of the suitable hosts in an aphid colony. Female parasitoids may reduce offspring mortality by "spreading the risk" of hyperparasitism over several patches. Support for this hypothesis is found in both field data (Horn 1989, Mackauer and Völkl 1993) and theoretical models (Ayal and Green 1993, Weisser *et al.* 1994). Implicit assumptions of these models are (1) a direct correlation exists between the size of host aggregations and the probability of discovery and attack by hyperparasitoids and (2) hyperparasitoids forage for hosts in a density-dependent manner. One of the goals of my studies was to test these assumptions by evaluating offspring allocation and foraging behaviour of *D. carpenteri.*

The foraging and reproductive strategies of a female wasp will determine allocation of time and offspring among individual hosts or a patch of hosts. Whether a patch is considered to be an infested leaf, plant, or clump of plants, the offspring allocated by a female to a patch can be considered a brood. Brood size is an important life-history trait and female parasitoids may have evolved various strategies for distributing broods among host patches (Stearns 1976). The time that a female stays in a patch, the proportion of available hosts parasitized, and the proportion of eggs fertilized may be influenced by host density, host quality, and the physiological state or experience of the wasp (Van Alphen and Vet 1985). Even mating status may influence a female wasp's reproductive strategy (Michaud and Mackauer 1995). Where and how efficiently a female wasp searches for hosts will be influenced by the distribution of hosts and the structural complexity of the

environment. Plant architecture can influence both the distribution of hosts and the search efficiency of hyperparasitoids (Horn 1984). Immature primary parasitoids may benefit from reduced hyperparasitism if parasitized hosts leave their feeding sites or usual habitat (Stamp 1981; Brodeur and McNeil 1992).

In this chapter, I present a series of experiments using broad bean plants and two species of host, *E. californicus* and *A. ervi*, to evaluate how the structure of the habitat and distribution of hosts affects the allocation of progeny and patch time by female *D. carpenteri*. I was especially interested in (1) how females allocate offspring among clumped and uniform distributions of hosts, (2) how habitat structure influences searching behaviour and efficiency, (3) how females respond to different host densities and whether their responses will vary with the species of host or the mating status of females, and (4) what factors may influence their decision to leave a patch. I tested the prediction that females will adjust patch time according to both host density and previous foraging experience. I also tested the prediction that brood size is dependent on host density, but brood sex ratio is relatively independent of the density or species of hosts. Finally, I tested the prediction that non-mated females will hyperparasitize proportionately fewer hosts and leave patches sooner than mated females. I discuss how the foraging behaviour of *D. carpenteri* may be adaptive when the availability of hosts are patchy and uncertain.

METHODS

Influence of Host Density

In this first experiment, I examined the behaviour of female *D. carpenteri* on broad bean leaves with different numbers of mummies and tested three hypotheses: (1) the time and number of progeny that a female will invest in a patch is dependent on the density of hosts, (2) a female will leave a patch only if all hosts have been parasitized, and (3) the brood sex ratio will not vary significantly with brood size. I define 'sex ratio' as the proportion of daughters among all offspring. A female was first conditioned so that she would gain experience with hosts and the structure of the test arena. Each female was placed singly into a screened plastic cage (15.5 cm in diam by 4.0 cm in ht) which contained the apical portion of a bean shoot with four mature and two apical leaves. Each mature leaf had a single mummy on its ventral surface. I allowed the female to forage freely for 1 h and then transferred her to a new cage. The new cage contained a new shoot and mummies, evenly spaced (approx. 0.5 cm apart) on a single mature leaf (leaf area = 17 ± 1 cm^2) and arranged in one of the following square matrixes: 2x3 (6 mummies), 3x4 (12 mummies), 4x5 (20 mummies), 5x6 (30 mummies). I released the female onto the shoot and directly observed her behaviour. 'Patch time' was defined as the period from a female's discovery of a mummy to when she left the leaf with mummies and proceeded to search a different leaf on the shoot. I concluded a trial if a female left the treatment leaf and did not return after 3 min. All mummies were placed into gelatin capsules and stored at $22 \pm 1^{o}C$ and 50-70% RH until offspring eclosed, which I identified and counted. Mummies from which no offspring eclosed were dissected and their contents identified; these data were added to the numbers of eclosed primary parasitods and hyperparasitoids. Separate trials were conducted with mummified pea aphids that contained either 9-day old *A. ervi* or 11-day old *E. californicus*. Twenty trials (= one set) were completed for each host species and density for a total of 160 trials. I completed one set for a single combination of host species and density before proceeding to complete another set for a different

combination. The order in which I completed the sets for each combination was not predetermined and subject to the availability of the insects.

I also measured the residence time of female *D. carpenteri* on a leaf with only one mummy. The same experimental procedure was used except that I did not count the number of mummies that were attacked in the conditioning cages or record the sex of the hyperparasitoid's offspring from the single mummy. Twenty trials (= one set) were completed for each host species for a total of 40 trials. I completed one set for a single host species before proceeding to complete another set for the other host species. The order in which I completed the sets for each host species was not predetermined and subject to the availability of the insects. To evaluate search behaviour in the absence of hosts, I released experienced *D. carpenteri* into cages containing a shoot with four mature leaves but no mummies. A single wasp was released onto a leaf and I recorded the time that each wasp stayed on the second and third leaves that they came across. The time for the first leaf was not recorded because the wasps required a period to settle down after being released. A plant was never used to test more than one female. I observed 20 females conditioned on *A. ervi* mummies and 20 wasps conditioned on *E. californicus* mummies. All studies were conducted in the laboratory under fluorescent light at 22-24°C and 25-40% RH.

Influence of Mating Status

I tested the hypothesis that the patch time and oviposition behaviour of female *D. carpenteri* are influenced by their mating status. I used the same design as in first experiment and conditioned each unmated female *D. carpenteri* (N= 20) before introducing her into a cage with a bean shoot that had 12 *A. ervi* mummies on one of its four mature leaves. The unmated females were from the same cohort as the mated females that were tested on 20 *A. ervi* mummies in the first

experiment. I completed 20 trials (= one set) for mated females before proceeding to complete another set for unmated females.

Allocation of Time within a Patch

I examined if female *D. carpenteri* respond to host density and foraging experience by adjusting the time they allocate to different activities on leaves with mummies. The variables that I manipulated were the number of mummies on a leaf and the sequence that females encountered leaves with small and large batches of mummies. Females were conditioned as in the first experiment and then placed into a new cage containing a bean shoot that had leaves with either one or six *E. californicus* mummies. I observed the wasp continuously, distinguishing between the following behaviours: *search* (the movement of a female between encounters with mummies), *examination* (the examination of a mummy externally by a female with her antennae), *attack* (the drilling and insertion of her ovipositor into a mummy), and *rest* (grooming or absence of activity). I defined *handling time* as the sum of a female's examination and attack times on a mummy. *Search* was further distinguished into three categories: (1) before the female encountered its first host on the leaf, (2) between the first and last host attacked, and (3) after all hosts were attacked. The *terminal search interval* was the total period of time that the female continued searching after attacking the last unmarked mummy and before abandoning the leaf. Because oviposition could not be observed directly, I used attack duration as an indicator of oviposition and acceptance; I counted as oviposition any ovipositor insertion that lasted $\geq$3 min, usually followed by the female's sudden departure. Accepted mummies were not removed and a female could re-encounter and attack a previously accepted mummy. I recorded the frequency and duration of each type of behaviour as a female searched a leaf and also the number of times that she re-encountered each mummy during the terminal search interval. A trial was terminated after a wasp had searched two different leaves. All mummies from the two leaves were stored in coded gelatin capsules at room temperature until adult wasps

eclosed. Mummies from which no wasps eclosed were dissected and their contents identified; these data were added to the numbers of eclosed parasitoids. I observed females under two trial conditions: (1) encounter with a single host on a leaf followed by six hosts on a different leaf, and (2) encounter with six hosts on a leaf followed by a single host on a different leaf. Fifteen trials (= one set) were completed for each condition at 20-22°C and 40-60% RH. I completed one set for the first condition before proceeding to complete another set for the second condition.

Influence of Host Distribution and Habitat Structure

I varied host distribution and habitat structure in large foraging arenas in order to determine if the distribution and location of mummies in the habitat will affect (1) their likelihood of being attacked by *D. carpenteri* and (2) the allocation of sons and daughters by *D. carpenteri*. The foraging success of female wasps was tested under three trial conditions (= sets): hosts among separate plants, hosts among and off separate plants, and hosts among and off a continuous canopy of plants. In each trial, I introduced a single female into a screened plexi-glass cage (27 cm wide x 42 cm high x 36 cm long) which contained 24 *E. californcius* mummies distributed throughout a canopy of six broad-bean plants. The plants were14-19 cm in height and each plant had four mature and 2 apical leaves. For each trial, I selected six plants of approximately the same height and total leaf area. Each plant was planted in soil within a plastic container (9.0 cm wide x 6.0 cm high x 13.5 cm long, 'Pak' plant starter, Kord Products, Toronto, Ontario) and the six containers were arranged to form a continuous floor (27 x 27 cm) with the six plants evenly spaced apart in two rows of three. I identified all leaves with mummies by strata, upper or lower, to determine the location of attacked mummies after the trial. The female was released into the cage and allowed to forage freely for 5 h. I observed and recorded the movement of females within and between plants. At the end of the trial, the female was recovered from the cage and all mummies were placed into gelatin capsules and stored

until offspring eclosed, which I identified and counted. Mummies from which no wasps eclosed were dissected and their contents identified; these data were added to the numbers of eclosed parasitods. All trials were conducted under continuous light at 20 ± 1°C and 50-60% RH.

For this study, I completed three different sets of trials. In the first set (42 trials), each of the six plants were evenly spaced without any overlapping or contact of their leaves. Each plant was assigned four *E. californicus* mummies, but half of the plants had a single mummy assigned to each of its mature leaves (4 L) and the other half had all four mummies assigned to a single mature leaf (1 L). The assignment of mummy distributions to the front and back row of plants were respectively '4 L, 1 L, 4 L' and '1 L, 4 L, 1 L' (Fig. 5). In half of the trials, I randomly assigned mummies of the 1-L plants to one of two lower leaves; in the other half I randomly assigned them to one of two upper leaves. The floor of each arena was a continuous layer of soil. In each trial, I released a female onto the center of the arena floor.

The second set was similar to the first except I laid a thin layer of dry alfalfa, *Medicago sativa* L., over the soil floor of the arena, glued 24 *E. californicus* mummies onto alfalfa leaves and uniformly distributed the mummies (approx. 2-3 cm apart) throughout the cover (Fig. 6). The female was released onto the center of the soil floor (20 trials) or one of the apical leaves of a randomly chosen 4-L plant (10 trials) or 1-L plant (10 trials). In the third set (20 trials), I placed the six plants so that their leaves overlapped and were in contact to form a continuous canopy. A single *E. californicus* mummy was assigned to each of the 24 mature leaves that made up the canopy. As in the second set, alfalfa was laid over the soil floor of the arena and 24 *E. californicus* mummies were glued onto alfalfa leaves and uniformly distributed throughout the cover. In each trial, a wasp was released onto one of the apical leaves of a randomly chosen plant.

Figure 5. Arrangement of three broad-bean plants with one *Ephedrus californicus* mummy on each of their four mature leaves '1 L' and three plants with four *E. californicus* mummies on a single mature leaf '4 L'. All six plants were planted in Kord Pak containers and arranged to form a continuous floor of soil (27 x 27 cm) within a screened plexi-glass cage (27 cm wide x 42 cm high x 36 cm long).

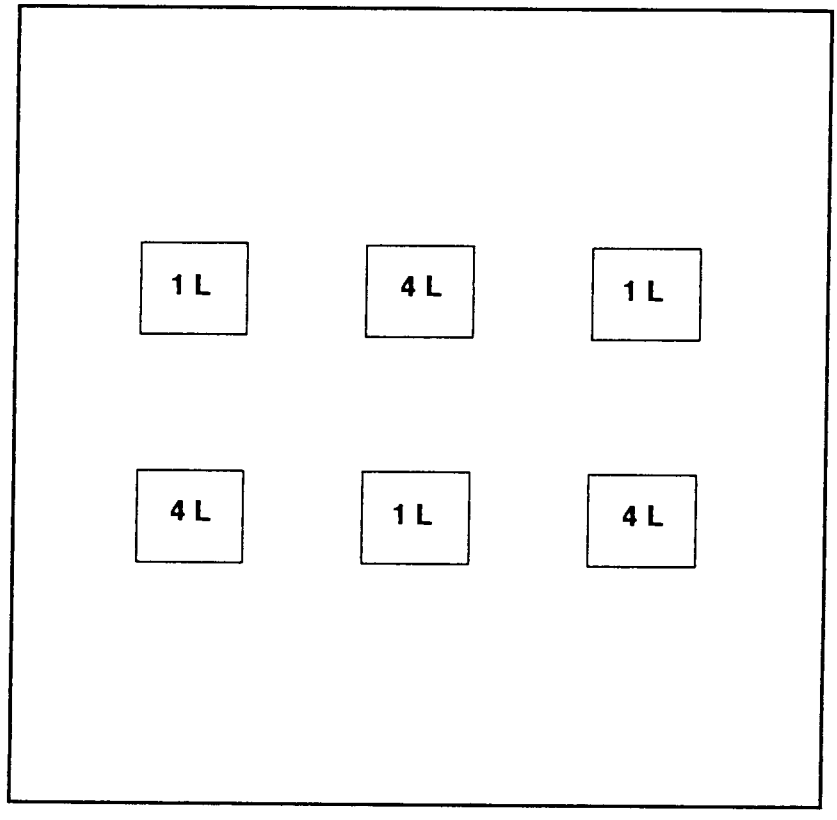
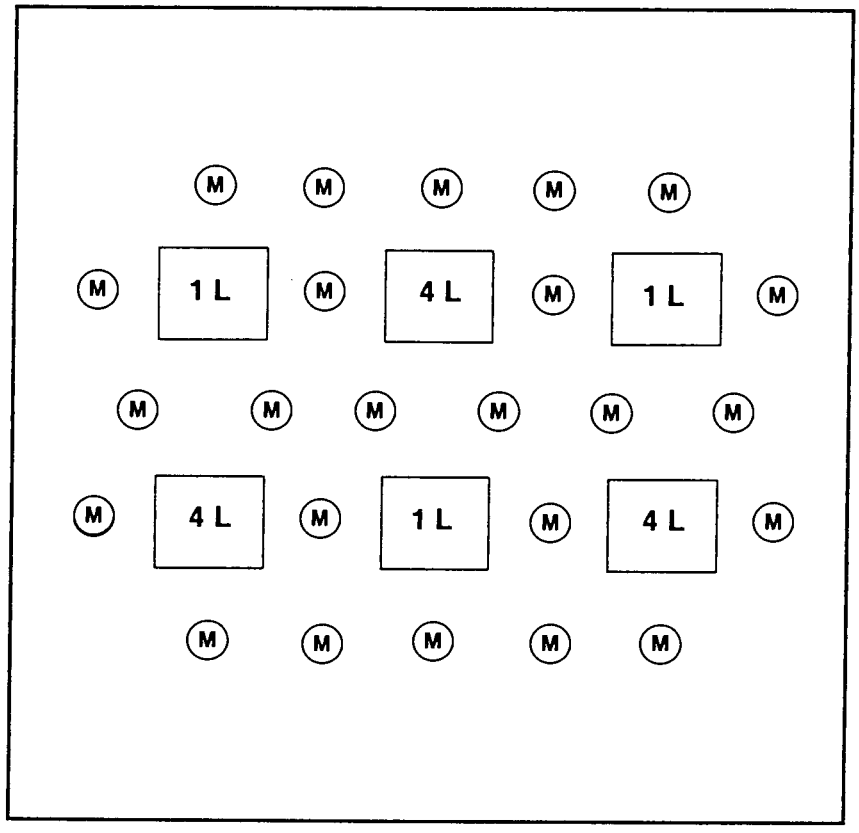

1 L
4 L
1 L
4 L
1 L
4 L

Figure 6. Arrangement of three broad-bean plants with one *Ephedrus californicus* mummy on each of their four mature leaves '1 L' and three plants with four *E. californicus* mummies on a single mature leaf '4 L'. All six plants were planted in Kord Pak containers and arranged to form a continuous floor of soil (27 x 27 cm) within a screened plexi-glass cage (27 cm wide x 42 cm high x 36 cm long). A total of 24 *E. californicus* mummies 'M' were evenly distributed among a layer of dry alfalfa on top of the soil floor.


M
M
M
M
M
M
1 L
M
4 L
M
1 L
M
M
M
M
M
M
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4 L
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1 L
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4 L
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M
M
M

Statistical Analysis

Two-way ANOVA was used to compare the proportion of hosts hyperparasitized by mated female *D. carpenteri* in the conditioning cages. The factors examined were the species of host and the density of hosts that mated females were later tested on. I also used one-way ANOVAs to compare the data from trials with mated and unmated females tested on 20 *A. ervi* mummies. Separate ANOVAs were run for the following dependent variables: the proportions of hosts hyperparasitized in the conditioning cages, the proportions of hosts hyperparasitized in the trial, and patch time. All proportions were transformed into their arcsine values by equation 14.5 in Zar (1984, p. 186).

I compared the number of hosts hyperparasitized and the sex ratio of *D. carpenteri* with a test of equality of means based on the method of Games and Howell (Sokal and Rolf 1981, p. 408); the factors that I examined were the type and density of hosts. Patch times for female *D. carpenteri*, tested on different densities of *A. ervi* or *E. californicus* mummies, were compared by analysis of covariance (ANCOVA) after host density and time were transformed to the logarithmic scale. Two-way ANOVA was used to compare the time that female *D. carpenteri* stayed on leaves without hosts. The factors that I compared were the type of host that females were conditioned on and the sequence in which a leaf was encountered.

Fully randomized ANOVAs were used to compare the components of *D. carpenteri*'s behaviour within a patch. The two factors that I examined were the previous foraging experience of females (whether the largest batch of mummies that she recently encountered was one or six) and the number of mummies on a leaf. Dependent variables were the behavioural components defined in real time or as proportions of the patch time, the terminal search interval, or the total time that females used to handle mummies. To test the hypothesis that a female *D. carpenteri* re-encounters each marked mummy the same number of times before she abandons a leaf, I used two-way ANOVA to analyze

the data for females when they foraged on leaves with six mummies. The factors were the previous experience of females and the sequence in which a mummy was attacked and presumably marked. The dependent variable was the proportion of total re-encounters that was allocated to each mummy. I used an approximate t-test (Sokal and Rolf 1981, p. 411) to compare the number of times that marked mummies were re-encountered on leaves with one and six mummies. All proportions were transformed into their arcsine values by equation 14.5 in Zar (1984, p. 186).

To evaluate the data on foraging by *D. carpenteri* among different habitats and distributions of hosts, I tested four null hypotheses. The first null hypothesis was that the distribution of mummies does not influence their probability of being hyperparasitized or not. This first hypothesis predicts the emergence of equal numbers of hyperparasitoid progeny from mummies that were uniform or clumped in their distribution among plants. The second null hypothesis was that the location of a mummy within the habitat does not influence its probability of being hyperparasitized or not. This hypothesis predicts equal numbers of hyperparasitized mummies among different plants, among different strata, and among mummies off or on plants.

My third null hypothesis was that females do not control offspring sex allocation and allocate sons and daughters randomly to hosts. From the results of the first experiment, I found that female *D. carpenteri* consistently produce offspring sex ratios of approximately 0.70 among uniform hosts that belong to the same brood. If the probability of an egg being fertilized is 0.70, the null hypothesis predicts (1) an offspring sex ratio of 2.33 daughters for every son, that will not vary significantly with the distribution of hosts; (2) a binomial distribution of sexes between broods. An implicit assumption of the third hypothesis is that immature mortality is random for both sexes (Cloutier *et al.* 1991). I compared observed and expected frequencies, using the log-likelihood ratio test with William's correction for continuity (Sokal and Rolf 1981, p. 737). I used one-way ANOVA to determine if the distribution of mummies influenced the proportion of

available mummies hyperparasitized on plants that female *D. carpenteri* were released on. All proportions were transformed into their arcsine values by equation 14.5 in Zar (1984, p. 186).

RESULTS

Influence of Host Density

Female *D. carpenteri* were able to locate and attack most of the four available hosts in the conditioning cages. There were no significant differences among the species of host ($F = 2.027$; $df = 1, 3$; $P = 0.157$) or sets of females tested on different densities of hosts ($F = 1.461$; $df = 1, 3$; $P = 0.227$) in the proportion of hosts hyperparasitized by mated females in the conditioning cages. The mean number of hyperparasitized hosts recovered from the conditioning cages was 3.24 ± 0.07 ($\pm$SE, $N = 160$).

The density and type of host on a leaf had greater influence on the patch time than on the number of hosts attacked by female *D. carpenteri*. There were no significant differences between the number of immature *A. ervi* or *E. californicus* that were hyperparasitized in trials with the same density of mummies (Table 10). However, the numbers of hosts hyperparasitized by *D. carpenteri* increased with density until the treatment of 30 hosts per leaf. The numbers of hyperparasitized hosts were not significantly different among trials with 20 and 30 hosts. Sex ratio did not vary significantly among the type or density of hosts and was 0.711 ± 0.008 (mean $\pm$SE, $N = 160$). Patch time of *D. carpenteri* increased linearly with an increase in the number of hosts on a leaf (Fig. 7). Although there were no significant interactions between the density or the type of hosts ($F = 1.4104$; df 1, 196; $P = 0.2364$), ANCOVA showed that females stayed longer in patches with *A. ervi* than *E. californicus* mummies ($F = 10.9913$; $df = 1$, 197; $P = 0.001$). Differences in the patch time of *D. carpenteri* among the two types of mummies increased with density (Fig. 7).

Female *D. carpenteri* spent progressively less time on empty leaves. Patch time on empty leaves did not vary significantly among females conditioned on different types of hosts ($F = 0.1667$; $df = 1, 76$; $P = 0.6842$), but it was shorter on the third than the second leaf ($F =$

Table 10. Hyperparasitism of immature aphidiid wasps by female *Dendrocerus carpenteri* among different numbers of mummified pea aphids on a broad bean leaf. Hosts were 9-day old *Aphidius ervi* or 11-day old *Ephedrus californicus* reared on pea aphid at 20°C. Only one aphidiid species was used as a host in each trial. Female *D. carpenteri* were 6 to 8 days old.

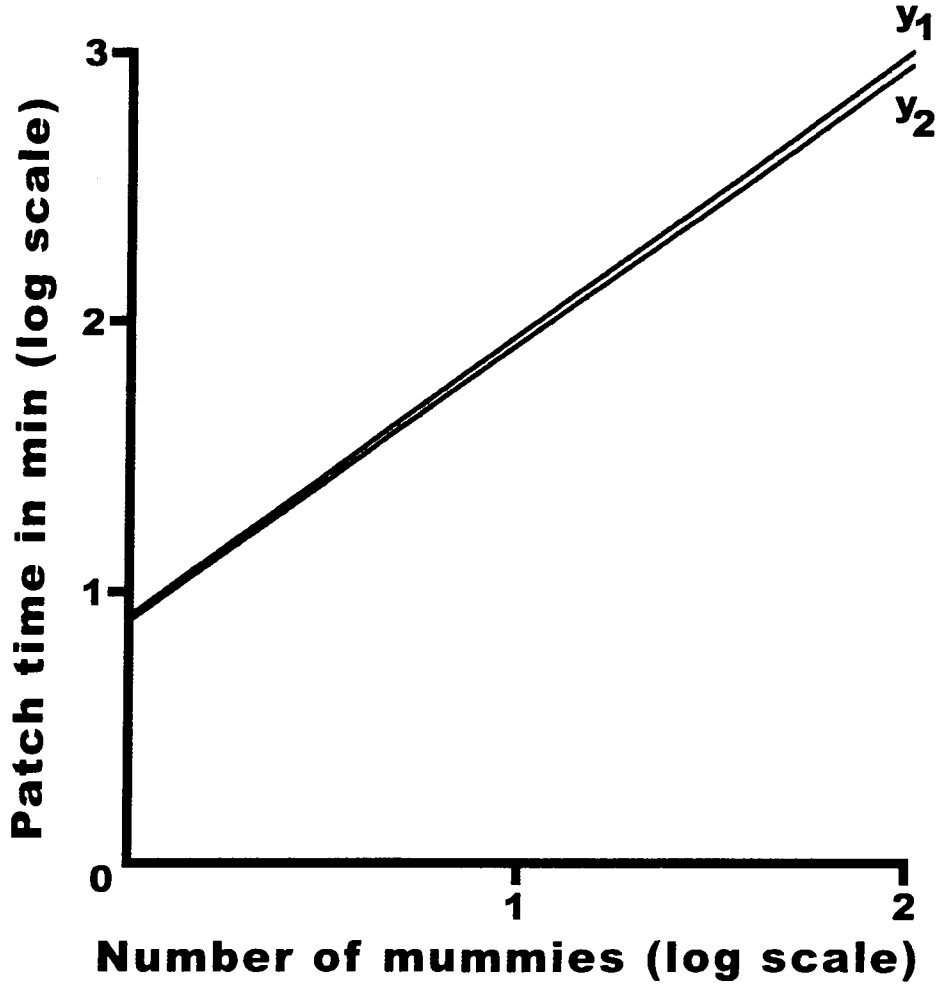
Parasitoid species	Mummy density[1]	Hyperparasitized hosts[2] (± SE)	Patch time (min)[3] (± SE)
Aphidius ervi	6	5.75 ± 0.10[a]	45.65 ± 1.90
	12	10.35 ± 0.32[b]	133.00 ± 8.70
	20	15.05 ± 0.63[c]	206.65 ± 11.36
	30	17.95 ± 1.20[c]	349.50 ± 26.91
Ephedrus californicus	6	5.70 ± 0.11[a]	57.00 ± 4.19
	12	9.40 ± 0.43[b]	110.80 ± 4.74
	20	13.95 ± 0.56[c]	160.40 ± 8.75
	30	14.85 ± 0.83[c]	259.30 ± 18.62

[1]Twenty trials were completed for each aphidiid species and density.

[2]Mean number of mummies that contained *D. carpenteri*. Means showing the same letter are not significantly different ($P > 0.05$) by unplanned comparisons among pairs of means using the Games and Howell method.

[3]Mean time that female wasps stayed on the leaves with mummies.

Figure 7. Influence of the host density (x) on the patch time of female *Dendrocerus carpenteri* on leaves with mummies of *Aphidius ervi* (y_1) or *Ephedrus californicus* (y_2). Exact densities were 1, 6, 12, 20, and 30 mummies on a single broad bean leaf. A single female hyperparasitoid was released onto each leaf. The regression equations were: $y_1 = 0.913 + 1.071\ (\pm 0.026)x$; significance of regression, $F = 1685.7$; $df = 1, 98$; $P < 0.001$; and $y_2 = 0.891 + 1.029\ (\pm 0.015)x$; significance of regression, $F = 1892.6$; $df = 1, 98$; $P < 0.001$.


y1
y2
3
2
1
0
1
2
Patch time in min (log scale)
Number of mummies (log scale)

10.1067; df = 1, 76; P = 0.0021). The mean patch time of females on their second and third leaves was 17.48 ± 1.49 and 12.03 ± 0.81 sec (± SE, *N* = 40), respectively.

Allocation of Time within a Patch

The foraging experience of a female influenced the time that she required to locate a single mummy on a leaf (F = 32.2009; df = 1, 28; P < 0.001). Female *D. carpenteri* that recently foraged on leaves with six mummies required more than twice as much time to locate a solitary mummy, 17.60 ± 0.80 sec (mean ± SE, *N* = 15), than females that had foraged only on leaves with one mummy, 7.07 ± 0.80 sec (mean ± SE, *N* = 15). However, foraging experience did not influence the patch time (F = 0.002; df = 1, 28; P = 0.9898), the terminal search interval (F = 0.0447; df = 1, 28; P = 0.8340), or the total time that females allocated to host handling (F = 0.1874; df = 1, 28; P = 0.6684) on leaves with a single mummy.

In real time, females had longer patch times (F = 216.2602; df = 1, 58; P < 0.001) and terminal search intervals (F = 32.2009; df = 1, 58; P < 0.001) on leaves with six mummies than on leaves with one mummy (Table 11). *Dendrocerus carpenteri* also allocated proportionately more time resting and handling hosts among leaves with six mummies than among leaves with one mummy (F = 9.7749; df = 1, 58; P = 0.0028) (Table 11). However, females had proportionately longer terminal search intervals (F = 14.2066; df = 1, 58; P = 0.0004) and spent proportionately more time searching on leaves with one mummy than on those with six mummies (F = 5.7910; df = 1, 58; P = 0.0193) (Table 11). Most of a female's time on leaves with mummies was allocated to handling mummies, but the number of mummies on a leaf did not significantly affect the proportion of time that she allocated to examining (F = 0.3635; df = 1, 58; P = 0.5489) or attacking mummies (F = 3.8600; df = 1, 58; P = 0.0542). *Dendrocerus carpenteri* allocated 16.90 ± 0.39 % and 89.33 ± 1.26 % (mean ± SE, *N* = 60) of their total handling time to examination and attack of mummies, respectively.

Table 11. Allocation of time by females of *Dendrocerus carpenteri* on broad bean leaves with one or six hosts. Hosts were mummified pea aphids containing 11-day old, immature *Ephedrus californicus*. Female *E. californicus* were given second-instar pea aphids (72 ± 4 h) to parasitize and all parasitized aphids were reared on broad bean at 20 ± 1°C. Female *D. carpenteri* were 6 to 8 days old and mated.

Behaviour on leaf[1,2]	Residence time (sec)[3]		Percentage of residence time[3]	
	Six mummies / leaf	One mummy / leaf	Six mummies / leaf	One mummy / leaf
Host handling	3414.80±192.51***	420.63±39.56	85.16±1.30**	80.51±1.93
Search	461.63± 45.63***	96.47±14.99	13.48±1.21*	18.86±1.92
Rest	49.27± 11.37***	2.73± 1.55	1.36±0.29**	0.62±0.35
Terminal search interval	308.33± 36.28***	86.87±14.38	9.29±1.09***	16.81±1.87
Patch time	3464.07±195.49***	519.83±43.22	———	———

[1]Each female *D. carpenteri* foraged on both a leaf with six mummies and a leaf with one mummy in each of 30 trials.

[2]'Host handling' was examination and attack of a mummy by female *D. carpenteri*. 'Search' was movement of females between mummies. 'Rest' was grooming or absence of visible activity. 'Terminal search interval' was the period that females stayed on a leaf after attacking the last unmarked host. 'Patch time' was the total time that females stayed on a leaf.

[3]Means (± SE) of the accumulative durations of each behaviour in seconds and percentages of patch time were compared separately for statistical significance by ANOVA. Statistical significance of differences between means within rows: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Females re-encountered each marked mummy the same number of times during the terminal search interval before abandoning leaves with 6 mummies ($F = 0.226$; $df = 5, 168$; $P = 0.951$). The experience of females did not significantly affect the number of times that marked mummies were re-encountered ($F = 0.636$; $df = 1, 168$; $P = 0.426$). Each marked mummy was re-encountered 3.92 ± 0.2187 times (mean $\pm$SE, $N = 180$) by a female before she left the leaf. However, the number of times that females re-encountered marked mummies during the terminal search interval was significantly different among leaves with six mummies or a single mummy ($t'_S = 2.386$, $df = 29$, $P > 0.05$). Marked mummies were re-encountered 2.80 ± 0.42 times (mean $\pm$SE, $N = 30$) before females abandoned leaves with only one mummy. Females usually required only a few seconds to examine and reject marked hosts. The tendency of female *D. carpenteri* to confirm the status of hosts before they abandon a patch appears to increase with the density of hosts.

Influence of Mating Status

Mating status did not apparently influence the foraging or oviposition behaviour of female *D. carpenteri*. Patch time did not vary significantly among mated and unmated females ($F = 0.639$; $df = 1, 38$; $P = 0.429$). The mean time that females stayed on leaves with 12 *A. ervi* mummies was 128.40 ± 5.73 min. ($\pm$SE, $N = 40$). Mated and unmated females hyperparasitized the same percentage of hosts in the conditioning cages ($F = 0.006$; $df = 1, 38$; $P = 0.941$). The mean number of hyperparasitized hosts recovered from the conditioning cages was 2.80 ± 0.17 ($\pm$SE). Similarly, the mean percentage of the 12 hosts hyperparasitized by unmated females, 69.17 ± 2.91 %, was only slightly less than the mean percentage hyperparasitized by mated females, 86.25 ± 2.66 % ($F = 9.576$; $df = 1, 38$; $P = 0.004$).

Influence of Host Distribution and Habitat Structure

D. carpenteri rarely flew and primarily moved within or between plants by walking. Females seemed to follow a systematic pattern of search for hosts. When the plants formed a continuous canopy, females showed a preference for searching within the canopy and rarely attacked mummies on the floor (Table 12). However when the plants were separate, I frequently observed *D. carpenteri* searching on both the floor and plants. In trials with mummies located on both separate plants and the floor of the arena, I always recovered hyperparasitized mummies from both parts of the habitat. However, females hyperparasitized more hosts in the part of the habitat that they were released in (Table 12). Among females released on plants with single or clumped mummies, the proportion of mummies that they attacked on their first plant did not vary significantly with the distribution of mummies on the plant ($F = 0.076$; $df = 1, 18$; $P = 0.079$). The mean proportion of mummies hyperparasitized by these females on their first plant was 0.875 ± 0.039 ($\pm$SE, $N = 20$). Similarly, the total numbers of plants with single or clumped mummies that had hyperparasitized mummies were not significantly different (Table 13). Hyperparasitism of single or clumped mummies did not vary significantly among plant strata (Table 14). Among trials with or without mummies on the floor, the ratio of single and clumped mummies that females hyperparasitzed on plants was not significantly different from a 1:1 ratio. (Table 15).

Offspring sex allocation by female *D. carpenteri* was neither random or apparently influenced by the distribution of mummies. Among trials with mummies found only on separate plants, the overall sex ratio among broods of four hyperparasitoids from plants with clumped mummies ($N = 26$) or single mummies ($N = 29$) did not differ significantly from the expected ratio of 0.70 (Table 16); however, the differences between observed and expected binomial frequencies of broods were highly significant (Table 17). In these trials, females often hyperparasitized only a portion of the available mummies on a plant. I found that 20%, 12%, and 8% of broods of hyperparasitoids from

Table 12. Influence of host location, plant architecture, and release site of hyperparasitoids on the the foraging success of female *Dendrocerus carpenteri*. Hosts were mummfied pea aphids containing 11-day old *Ephedrus californicus*. A *D. carpenteri* female was singly confined for 5 h with 24 mummies distributed among six broad-bean plants and 24 mummies distributed among the floor of the arena in each study.

Study[1]	Hyperparasitoid release site[2]	Mummy location[3]	No. mummies hyperparasitized[4]	Choice index[5] (a/b)	G_W[6]
1. Separate plants	Plant	Plant	5.60 ± 0.62		
		Floor	3.40 ± 0.88	1.603	11.722**
	Floor	Plant	2.70 ± 0.81		
		Floor	6.25 ± 1.02	0.419	36.926***
2. Continuous canopy	Plant	Plant	14.15 ± 0.88		
		Floor	0.35 ± 0.21	41.429	469.781***

[1]Twenty trials were completed for both studies involving separate plants and studies involving continuous canopies. For studies involving separate plants, I released females on plants in ten trials and on the arena floor in ten other trials.

[2]Female *D. carpenteri* were released on the apical leaf of a plant or the center of the arena floor.

[3]Half of the separate plants had four mummies on one of four leaves and the other half had one mummy on each leaf. Plants in a continuous canopy had one mummy on each leaf. Floor mummies were uniformly distributed.

[4]Mean (± SE) number of mummies that contained *D. carpenteri*.

[5]Ratio of total numbers of first ('a') and second ('b') kinds of hyperparasitized mummies among all trials of each study.

[6]Fit of observed frequencies of hyperparasitized mummies of each kind with their expectations from a 1:1 ratio of 'no preference' (by loglikelihood ratio test with Williams' correction): **, $P < 0.01$; ***, $P < 0.001$.

Table 13. Foraging success of female *Dendrocerus carpenteri* among broad bean plants with clumped and uniform distributions of hosts. Hosts were mummfied pea aphids containing 11-day old *Ephedrus californicus*. A *D. carpenteri* female was singly confined for 5 h in an arena with six separate plants and mummies.

Study[1]	Hyperparasitoid release site[2]	Mummy distribution[3]	No. plants with hyperparasitized mummies[4]	Choice index[5] (a/b)	G_W[6]
1. Floor mummies present	Floor	Clumped	0.40 ± 0.13		
		Uniform	0.50 ± 0.14	0.800	0.260 (NS)
	Plant	Clumped	0.70 ± 0.15		
		Uniform	0.95 ± 0.17	0.737	1.041 (NS)
2. Floor mummies absent	Floor	Clumped	1.02 ± 0.11		
		Uniform	1.26 ± 0.13	0.811	1.680 (NS)

[1]In 42 trials, females were released on the floors of arenas with mummies distributed among only plants. Twenty trials were completed for studies involving mummies distributed among both the plants and floor of the arena. For studies involving floor mummies, I released females on plants in ten trials and on the arena floor in ten other trials.

[2]Female *D. carpenteri* were released on the apical leaf of a plant or the center of the arena floor.

[3]Half of the plants had four mummies on one of four leaves (clumped) and the other half had one mummy on each of four leaves (uniform). In studies with floor mummies, 24 mummies were uniformly distributed on the arena floor.

[4]Mean (± SE) number of plants with mummies that contained *D. carpenteri*.

[5]Ratio of total numbers of first ('a') and second ('b') kinds of hyperparasitized mummies among all trials of each study.

[6]Fit of observed frequencies of hyperparasitized mummies of each kind with their expectations from a 1:1 ratio of 'no preference' (by loglikelihood ratio test with Williams' correction); NS, $P > 0.05$.

Table 14. Foraging success of female *Dendrocerus carpenteri* for hosts among different stratum of broad bean. Hosts were mummfied pea aphids containing 11-day old *Ephedrus californicus*. A *D. carpenteri* female was singly confined for 5 h with 24 mummies distributed among six broad-bean plants.

Study[1]	Mummy distribution[2]	Stratum[3]	No. Mummies hyperparasitized[4]	Choice index[5] (a/b)	G_W[6]
1. Separate plants	Uniform	Upper	2.00 ±0.24		
		Lower	2.17 ±0.25	0.903	0.704 (NS)
	Clumped	Upper	1.88 ±0.37		
		Lower	1.69 ±0.43	1.263	0.878 (NS)
2. Continuous canopy	Uniform	Upper	7.05 ±0.51		
		Lower	7.10 ±0.51	0.993	0.009 (NS)

[1]The number of trials completed for studies that involved separate plants or continuous canopies were respectively 20 and 42. Studies with continuous canopies also had 24 mummies that were uniformly distributed on the floor.

[2]In the studies involving separate plants, half of the plants had four mummies on one of four leaves (clumped) and the other half had one mummy on each of four leaves (uniform). Plants among continuous canopies had a single mummy on each mature leaf (uniform).

[3]The upper stratum consisted of the pair of mature leaves found in the top half of the plant. The lower stratum consisted of the pair of mature leaves found in the lower half of the plant.

[4]Mean (± SE) number of mummies that contained *D. carpenteri*.

[5]Ratio of total numbers of first ('a') and second ('b') kinds of mummies among all trials of each study.

[6]Fit of observed frequencies of hyperparasitized mummies of each kind with their expectations from a 1:1 ratio of 'no preference' (by loglikelihood ratio test with Williams' correction); NS, $P > 0.05$.

Table 15. Foraging success of female *Dendrocerus carpenteri* among clumped and uniform distributions of hosts on broad bean plants. Hosts were mummfied pea aphids containing 11-day old *Ephedrus californicus*. A *D. carpenteri* female was singly confined for 5 h in an arena with six separate plants and mummies.

Study[1]	Hyperparasitoid release site[2]	Mummy distribution[3]	No. Mummies hyperparasitized[4]	Choice index[5] (a/b)	G_W[6]
1. Floor mummies present	Floor	Clumped	2.75 ± 0.57		
		Uniform	2.85 ± 0.42	0.946	0.107 (NS)
	Plant	Clumped	1.60 ± 0.54		
		Uniform	1.10 ± 0.33	1.167	0.345 (NS)
2. Floor mummies absent	Floor	Clumped	3.69 ± 0.40		
		Uniform	4.21 ± 0.45	0.876	2.173 (NS)

[1]In 42 trials, females were released on the floors of arenas with mummies distributed among only plants. Twenty trials were completed for studies involving mummies distributed among both the plants and floor of the arena. For studies involving floor mummies, I released females on plants in ten trials and on the arena floor in ten other trials.

[2]Female *D. carpenteri* were released on the apical leaf of a plant or the center of the arena floor.

[3]Half of the plants had four mummies on one of four leaves (clumped) and the other half had one mummy on each of four leaves (uniform). In studies with floor mummies, 24 mummies were uniformly distributed on the arena floor.

[4]Mean (± SE) number of mummies that *D. carpenteri* emerged or was dissected from among 24 plant or 24 floor mummies.

[5]Ratio of total numbers of first ('a') and second ('b') kinds of hyperparasitized mummies among all trials of each study.

[6]Fit of observed frequencies of hyperparasitized mummies of each kind with their expectations from a 1:1 ratio of 'no preference' (by loglikelihood ratio test with Williams' correction); NS, $P > 0.05$.

Table 16. Mean sex ratio and distribution of sons and daughters among broods produced by mated females of *Dendrocerus carpenteri*. Host were 11-day old *Ephedrus californicus* in mummified pea aphids. Broods of four offspring were from individual broad bean plants with clumped ($N = 26$) or uniform ($N = 29$) distributions of mummies. Large broods with 14.15 ± 0.88 (mean $\pm$ SE, $N = 20$) offspring were from continuous canopies with uniform distributions of mummies.[1]

Study	Mummy distribution[2]	Female Sex Ratio (Mean $\pm$ SE)	Frequencies of offspring			Predicted Sex Ratio	G_W
			Males (Obser.)	Females (Obser.)	n		
Separate plants	Clumped	0.654 ± 0.034	36	68	104	0.7	1.0220 (NS)
	Uniform	0.647 ± 0.034	41	75	116	0.7	1.5302 (NS)
Continuous canopy	Uniform	0.689 ± 0.019	87	197	284	0.7	0.0334 (NS)

[1]Abbreviations: n, total number of offspring that emerged from all broods; G_W, fit of observed frequencies with their predicted values (by log-likelihood ratio test with Williams' correction); NS, $P > 0.05$.

[2]Plants had four mummies on one of four leaves (clumped) or a single mummy on each of four leaves (uniform).

Table 17. Mean sex ratio and distribution of daughters among broods of four offspring produced by mated females of *Dendrocerus carpenteri*. Hosts were 11-day old *Ephedrus caliifornicus* in mummified pea aphids. Broods were from broad bean plants with clumped and uniform distributions of mummies[1].

Mummy distribution[2]	Frequencies of broods						
	0	1	2	3	4	n	G_W
Clumped	0	2	7	16	1	26	17.585***
Uniform	0	2	10	15	2	29	18.848***

[1]Abbreviations: n, total number of broods with 0, 1, 2, 3, and 4 daughters among four offspring; G_W, fit of observed frequencies with their binomial expectations (by log-likelihood ratio test with Williams' correction); ***, $P < 0.001$.

[2]Plants had four mummies on one of their four leaves (clumped) or a single mummy on each of their four leaves (uniform).

plants with single mummies (N= 49) had three and two offspring, respectively. In comparison, 24% and 5% of broods of hyperparasitoids from plants with clumped mummies (N= 37) had three and two offspring, respectively. *Dendrocerus carpenteri* always hyperparasitized more than one host when they encountered a batch of four mummies on a single leaf. The ratio of daughters to sons was usually 2:1 when the brood size was three and always 1:1 when the brood size was two. When the brood consisted of only one offspring, it had an equal probability of being a male or female. In trials with plants forming continuous canopies, the observed frequencies of offspring of *D. carpenteri* were also not significantly different from the expected ratio of 0.70 (Table 16).

DISCUSSION

The results of my studies show that *D. carpenteri* located single or clumped mummies on plants with equal success, but the structure of the plant canopy influenced searching behaviour and efficiency. When the density of mummies on a leaf was increased, females responded by increasing patch time and brood size in a density-dependent manner. Giving-up time increased with host density and the decision to leave a patch was apparently influenced by re-encounters with self-marked hosts, but not by prior foraging experience. Brood sex ratio did not vary with the density or species of hosts. Mating status did not apparently influence patch exploitation by females. I will now discuss the behaviour of *D. carpenteri* within the context of parasitoid foraging and reproductive theory.

A successful approach to understanding the evolution and dynamics of parasitoid-host systems has been to examine it from the perspective of parasitoid foraging behaviour and host distribution (Rosenheim *et al.* 1989, Mackauer and Völkl 1993, Ayal and Green 1993). Some of the more important questions are: how do individual parasitoids allocate their foraging effort over different parts of the habitat, and what are the consequences for hosts with different kinds of distributions? Ecologists usually study the foraging behaviour of animals under the assumption that natural selection will favour those individuals that exploit their resources most efficiently (Krebs and McCleery 1984). Parasitoids are often expected to allocate their foraging effort in a manner that maximizes encounter rate with hosts within the habitat. The marginal value theorem predicts that each patch within a habitat should be exploited until the encounter rate within the patch has decreased to a marginal value which is the same for all patches (Charnov 1976). However, because parasitoids are unlikely to know the exact abundance and distribution of hosts in the habitat, they may use simple rules to decide how long they stay and to what extent should they exploit a patch.

In the case of parasitoids that forage in patches where all hosts are easy to discover and handle, the optimal strategy for individual females is to parasitize all suitable hosts in a patch and then search for a new patch (Godfray 1994). The females of several species of mymarid wasps which attack leaf hopper egg masses do not leave an egg mass until all the eggs have been parasitized and the mass has been repeatedly checked for unparasitized eggs (Sahad 1982, 1984). However, females of some mymarid species apparently employ a 'spread the risk' strategy because these females regularly leave host egg masses although they still have mature eggs left and many host eggs are unparasitized (Waloff and Jervis 1987, Cronin and Strong 1990). In low density patches, *D. carpenteri* females attacked and marked all suitable hosts and did not leave until they had re-encountered each marked hosts several times. In higher density patches, *D. carpenteri* females probably left their patches before all the suitable hosts were attacked because they had laid all of their eggs, or the non-marked mummies were 'hidden' among marked mummies and missed. *Dendrocerus carpenteri* females will apparently invest all of their eggs into a single brood if enough suitable hosts are available. If hosts are patchy in their distribution and the cost or risks of traveling between patches are high or the likelihood of finding another suitable patch is low, it may be adaptive for female parasitoids to invest most of their eggs in a single patch if mortality risks for progeny are low (Weisser *et al.* 1994). *Dendrocerus carpenteri* avoid ovipositing into self-marked hosts or hosts marked by conspecifics (Höller *et al.* 1991) and their progeny may also be rarely attacked by other hyperparasitoid species (Scholz and Höller 1992). Further studies should investigate the response of females to patches that have been partially exploited by conspecifics or different species of hyperparasitoids.

The total time that a female parasitoid spends on a patch has been shown to increase with density among a diverse range of species (Waage 1979, Galis and van Alphen 1981, van Alphen and Galis 1983, Nelson and Roitberg 1995). Patch times for *D. carpenteri* also do not fit the predictions of models using simple rules of thumb such as 'fixed

time' (Krebs 1973), 'fixed number' (Gibb 1962), or 'fixed giving up time' (Murdoch and Oaten 1975, Cook and Hubbard 1977). The giving up time, as estimated by the terminal search interval, increased with host density and does fit into the density-dependent 'giving up time' model proposed by McNair (1982). According to McNair's model, in patches that a parasitoid perceives as relatively 'good', the parasitoid should be more persistant and have longer giving up times. The increase in the patch time of *D. carpenteri* with host density could have resulted from (1) females investing more time in handling hosts, (2) females re-encountering a larger number of marked hosts, (3) females spending more time on non-foraging behaviours such as rest or grooming. In Chapter 3, I showed that *D. carpenteri* females had shorter examination times on *E. californicus* than on *A. ervi* mummies. The difference in examination time for individual mummies of the two aphidiid species was small, but it could help to explain why the difference between the patch times of *D. carpenteri* on leaves with similar numbers of *E. californicus* or *A. ervi* mummies increased with density.

Michaud and Mackauer (1995) showed that the mating status of female *Monoctonus paulensis* affected both the patch time and the number of eggs laid into host aphids. Young non-mated females abandoned host patches earlier and superparasitized fewer aphids than mated females of the same age. It was suggested that young, non-mated females may benefit from distributing their progeny among many patches and increasing the chances of their sons mating with daughters of other females. The foraging behaviour of non-mated *D. carpenteri* did not fit this hypothesis. As discussed earlier, it may not be adaptive for *D. carpenteri*, regardless of mating status, to under-exploit patches because their hosts are rare and widely distributed.

When the broods or clutches of hosts are large, the number of offspring present can far exceed the number that can be attacked by a single parasitoid. Godfray (1986, 1987) suggested that parasitoid attack can lead to selection in favor of the production of large egg clutches by herbivorous insects. The advantage of large clutches depend largely on both the number of hosts that a single parasitoid can

attack and the relative rate of discovery of different-sized clutches by parasitoids. Most aphid hyperparasitoids have much lower daily and lifetime fecundities than their aphidiid hosts (Mackauer and Völkl 1993). It is possible that under certain conditions, selection may favor aphidiid wasps that produce large broods within individual aphid colonies. However, studies have shown that it is more likely that primary parasitoids reduce offspring mortality by under exploiting aphid colonies and 'spreading the risk' of hyperparasitism over several patches (Horn 1989, Mackauer and Völkl 1993, Ayal and Green 1993, Weisser *et al.* 1994).

Kfir *et al.* (1976) found that two hyperparasitoid species, *Cheiloneurus paralia* and *Marietta exitiosa*, had greater search efficiency than its host, *Microterys flavus*, a primary parasitoid of the brown soft scale. The greater search efficiency of these hyperpasitoids enabled them to maintain their population when the population of the primary host was low. If aphid hyperparasitoids are more efficient foragers than aphidiid wasps, hyperparasitism of immature aphidiids may actually increase with the number of mummies within an aphid colony. The rate of discovery could be disproportionately higher for large than for small aggregations of aphid mummies. Under the conditions of my studies, the distribution of mummies among separate plants did not affect their probability of discovery by *D. carpenteri*. I observed females searching the foliage of each plant systematically and repetitively. The behaviour of female *D. carpenteri* increased their chances of finding all available hosts on a plant that was searched, but it may have also prevented them from searching many of the plants in the habitat during the experiments. Primary parasitoids that spread their progeny among many patches may minimize the chances of their progeny being attacked by *D. carpenteri* and other species of hyperparasitoids with similar searching behaviour.

There is very little data on the foraging behaviour of aphid hyperparasitoids under field conditions. Shon *et al.* (1996) examined the pattern of hyperparasitism of *A. ervi* by *Asaphes lucens* at different spatial scales. In laboratory cages and small plots from a

single alfalfa field, they found no evidence of density-dependent hyperparasitoid aggregation. However when entire alfalfa fields were sampled, they found evidence of both density-dependent and density-independent hyperparasitoid aggregation. These results suggest that hyperparasitoids may aggregate in habitats where mummy densities are high. Unfortunately, Shon *et al.* did not compare aphid densities among the different fields that were sampled; therefore, its equally possible that *A. lucens* and other hyperparasitoid species aggregate in fields where aphid densities are highest. Extrapolation of the results from my cage studies suggests that patterns of *D. carpenteri* aggregation in the field would be similar to that found for *A. lucens.*

Some aphidiid species appear to modify the behaviour of their aphid hosts so that the latter will mummify either off the host plant or in the upper strata of the canopy (Brodeur and McNeil 1989, Höller 1991, Brodeur and McNeil 1992). It was suggested that the primary parasitoid may benefit from reduced hyperparasitism in these parts of the habitat. Under the conditions examined in my studies, the foraging behaviour of *D. carpenteri* do not support this hypothesis. *Dendrocerus carpenteri* showed a preference for searching within a plant canopy and rarely attacked mummies located away from plants when the plants formed a continuous canopy. However, females attacked mummies on both the ground and plants when they foraged in habitats with separate plants. *Dendrocerus carpenteri* was observed to primarily move within and between plants by walking and flying was rare. It appears that both the distance between host patches and the structure of the habitat influenced the foraging behaviour of females. *Dendrocerus carpenteri* females that were released on the ground also tended to attack mummies in this part of the habitat, if mummies were distributed among both the plants and the ground cover. Females of this species seem to retain a great deal of behavioural flexibility in their foraging patterns, they apparently learned to concentrate their efforts within 'productive' habitats in a simple laboratory enviroment. Other parasitoid species have also demonstrated the ability to adapt to foraging for hosts in novel habitats (Arthur 1966, Taylor 1974).

Hamilton (1967) showed that the optimal sex ratio in offspring produced by female parasitoids depends on the number of females (termed foundresses) colonizing a patch. When there are few foundresses, the optimal sex ratio is highly female biased, thereby reducing competition among males for mates. Sex ratio can also be influenced by the degree of inbreeding among emergent offspring (Herre 1985). Hardy (1994) defined 'partial local mating' as an intermediate mating structure, between panmixis and fully local mating, that arises due to a mixture of local and non-local mating. In my experiments, female *D. carpenteri* produced sex ratios that were highly female-biased and apparently varied little with brood size. Information on the mating structure of this hyperparasitoid species is generally lacking. However, some characteristics of *D. carpenteri* lead us to believe that partial local mating may be common for this species. Both male and females are long lived and disperse by flight, asynchronous development of progeny within broods of single foundress has been regularly observed in the laboratory, and overlap of generations in the field may be common. The lack of variation in progeny allocation by *D. carpenteri* with brood size may also be due to the inability of females to estimate the number of hosts present in a patch or even the inability to adjust the sequences of male and female eggs laid during a single oviposition bout. Fitness in parasitoid species with local mating may also be affected by host quality. Ikawa *et al.* (1993) found that increasing the probability of non-local mating leads to progressively male bias of sex ratio in patches if hosts are poor, and a progressively female bias in patches if hosts are good. My experiments were conducted with only high quality hosts and this may have contributed to the highly female-biased sex ratios that I obtained. However, an issue crucial to our understanding of sex allocation in *D. carpenteri* is whether females can produce 'precise sex ratios'.

In summary, the foraging behaviour of *D. carpenteri* enables females to search for potential hosts over a wide range of habitats and thoroughly exploit aggregations of hosts when they are found. It may be suggested that the foraging behaviour of *D. carpenteri* is not particularly efficient because it does not maximize the number of host

patches exploited. However, the foraging strategy of this species may be adaptive when host patches are rare and dispersed. Offspring sex allocation by *D. carpenteri* was neither random or apparently influenced by the brood size, species, density or distribution of hosts in the habitat. The sex ratios that I obtained in my laboratory studies were also similar to sex ratios of *D. carpenteri* that emerged from *A. ervi* mummies of pea aphids collected from field surveys (Mackaeur and Lardner 1995). Information on the proximate mechanisms that determine sex ratio allocation in this species is lacking. Further investigations into the sex allocation patterns of *D. carpenteri* are needed to gain a more complete understanding of the reproductive strategy of *D. carpenteri.*

Chapter 5

Progeny and Sex Allocation by *Dendrocerus carpenteri*

INTRODUCTION

One of the more important and interesting aspects of the reproductive strategy of a parasitoid species is the sex ratio of offspring. Sex ratios are female-biased and have less than binomial variance in many species of parasitoid wasps (Hardy 1994). Sex determination by haplo-diploidy provides females of parasitoid wasps with a mechanism for facultative control over the sex of offspring. Diploid (i.e. fertilized) eggs normally develop as females and haploid (i.e. unfertilized) eggs as males (Luck *et al.* 1993); thus, mated females can determine offspring sex by controlling fertilization. Female manipulation of offspring sex is predicted by theory (Godfray 1994; Hardy 1994) and supported by many empirical studies (reviewed in King 1987, 1993a). If siblings compete for mates within local mating groups, 'local mate competition' (LMC) theory predicts that a female should adjust her progeny and sex allocation pattern in accordance with the reproductive behaviour of other females exploiting the same patch and the probability of developmental mortality, among other factors. Allocation of offspring may also be influenced by variations in host quality if, for example, one sex gains more in fitness from increased size than the other.

In this chapter, I am concerned with how *D. carpenteri* allocate offspring to hosts that they sequentially encounter while foraging. Assuming that a female wasp can control offspring sex, one of the decisions that she needs to make when exploiting a patch of hosts is what sex ratio to produce amongst the eggs laid. The proximate mechanisms that result in precise sex ratios are only poorly understood. A foraging wasp encounters hosts one at a time and presumably has only incomplete knowledge of her environment. At each encounter, her sex ratio response is constrained to one of two alternative decisons. If she decides to release sperm from the spermatheca, the next egg laid will be fertilized and develop as a daughter; if no sperm is released however, the egg will be unfertilized and develop as a son. This sequence of fertilized and unfertilized eggs ultimately determines the offspring sex ratio, regardless of whether sex

ratio is considered at the level of an individual host, a patch, or over a female's lifetime. Patterns of offspring sex allocation can be quite simple, suggesting that females 'count' eggs. Males may be produced either early or late in an oviposition bout. The sex ratio can also be constrained mechanically, however. For example, King (1961) proposed that the random orientation of eggs during their passage through the egg canal results in a ratio of three daughters to one son in the parasitoid wasp, *Nasonia vitripennis.*

LMC theory predicts that mated females produce only as many sons as required to fertilize all daughters, including the daughters of other females. An implicit assumption of many adaptive sex-ratio models is that 'precise' females control offspring allocation and produce sons and daughters in a fixed sequence that is independent of host quality. In contrast, 'host quality' models predict that females differentially allocate sons and daughters to low- and high-quality hosts if host quality varies. This prediction assumes a flexible, rather than fixed, production schedule of sons and daughters. Parasitoids may also sample their environments and modify their reproductive behaviour in response to recent information, such as chemical markers left on hosts (Visser *et al.* 1992).

My experimental objectives were to evaluate the separate and combined influences of encounter sequence and variation in host quality on the pattern of offspring sex allocation in *D. carpenteri.* I designed six experiments to evaluate the influence of encounter sequence on offspring sex allocation if host quality is uniform or variable. I show that *D. carpenteri* females typically produced one son and one daughter among the first two offspring per oviposition bout, regardless of host quality. With additional ovipositions, the sex ratio asymptotically approached an equilibrium value that varied with host quality. Females repeat non-random patterns of offspring sex allocation with each new oviposition bout, suggesting control by an 'oviposition clock'. I used sex allocation patterns of female wasps to distinguish different oviposition bouts among clumped and solitary hosts and suggest that offspring sex allocation may be useful for

understanding this species' perception of a patch. Female *D. carpenteri* may use self-marked hosts to determine if they are still in the same patch of hosts.

METHODS

Experimental Design

I first designed three experiments to test whether *D. carpenteri* females control offspring sex and allocate sons and daughters according to the sequence of encountered mummies, host quality, or both. I define host quality as the total amount of resources available to the immature parasitoid (Mackauer and Sequeira 1993). This amount generally depends on host size, with large hosts assumed to be of higher quality than small hosts. An exception is hosts that continue to feed, grow and metamorphose during the initial stages of parasitism, such as aphids parasitized by aphidiid wasps. In such associations, quality is influenced by the host's future growth potential, rather than by its size at the time of parasitization (Sequeira and Mackauer 1992a, 1993). Because growth and resource exploitation are individual and species-specific, same-sized mummies containing different primary parasitoids may vary in quality (Mackauer and Lardner 1995).

The concept of a 'patch' is vital to optimal foraging theory (Stephens and Krebs 1986) and it is often described as a limited area in which a female parasitoid will search for hosts (van Alphen and Vet 1986). Ideally, the boundaries of a patch should be defined by parasitoid behaviours relevant to exploitation of hosts and production of offspring. I use the term "brood" to refer to all offspring produced by a female *D. carpenteri* during a single oviposition bout. A brood represents the elementary unit of reproductive behaviour and, in this regard, is analogous to a patch. When a female starts a new oviposition bout, it is analogous to starting a new brood or exploiting a new patch of hosts. I designed three additional experiments to evaluate conditions under which females reset the production schedule of offspring. In all three experiments, the prediction is that a female will produce broods with the same sex ratio when she starts a new oviposition bout and both the number and quality of hosts attacked are the same.

For this study I used *A. ervi* and *E. californicus* as the host species. Because *E. californicus* has a longer time-to-adult than *A. ervi* (Sequeira and Mackauer 1992b, 1993), I standardized the immature parasitoids so that they had the same physiological age when used, i.e. the last larval instar just prior to pupation. At that stage, immature *A. ervi* and *E. californicus* were respectively 9- and 11-d old.

In all experiments, the null hypothesis was that each egg has the same probability of being fertilized (p) or not ($q = 1-p$), i.e. females do not control offspring sex and allocate sons and daughters randomly to hosts. For $p = q = 0.5$, the null hypothesis predicts an offspring sex ratio of 1:1 sons:daughters, with a binomial distribution of the sexes between broods. Implicit in this hypothesis is the assumption that immature mortality is random for both sexes, i.e. that the sex ratio at eclosion provides an unbiased estimate of the primary ratio at oviposition (Cloutier *et al.* 1991).

Experiment 1: Influence of Encounter Sequence

For my first experiment, I tested the hypothesis that offspring sex allocation is influenced by the sequence of encountered hosts. Each *D. carpenteri* female ($N = 126$) was placed singly in a plastic petri dish (5.5 cm in diam by 1.5 cm in ht) which contained eight mummies with immature *A. ervi*; the mummies were arranged in a 4 x 2 square matrix on a circular piece (4.2 cm diam) of filter paper. I observed the wasp's foraging behaviour and recorded encounter sequences. Host acceptance (i.e. oviposition) was defined as ovipositor probing that lasted 3 min or longer and was followed by the female leaving the host (A. Chow, personal observation). I did not remove an encountered mummy that was accepted, allowing the female to re-encounter already parasitized hosts. To prevent superparasitism, however, I placed one half of a gelatin capsule (size 00) over a re-encountered mummy if the wasp attempted to attack it a second time. I immediately replaced a rejected mummy with a new one to avoid the accumulation in the arena of mummies that, for unknown reasons,

might not be perceived as suitable by a foraging wasp. I terminated a trial when the wasp had accepted, and presumably parasitized, eight mummies. The mummies were placed individually in numbered gelatin capsules and stored at 22 ± 1°C and 50-70% RH until offspring eclosed, which I then counted and sexed.

Experiment 2: Influence of Host Size

I tested the hypothesis that host quality, as indexed by mummy size, influenced offspring sex allocation. To obtain mummies that differed in size, I used first-instar (24 ± 4 h old) and third-instar (120 ± 4 h old) pea aphids. I used two sets of trials, using only small mummies in one set and large mummies in the other. In both sets, the mummies contained 9-day-old *A. ervi* immatures when they were exposed to hyperparasitoids.

I placed each *D. carpenteri* female (small mummies, $N = 45$; large mummies, $N = 34$) singly in a petri dish (5.5 cm in diam by 1.5 cm in ht) containing four mummies arranged in a 2 x 2 square matrix, on a circular piece (4.2 cm diam) of filter paper, with approximately 1 cm between mummies. I observed wasps and noted the sequence of accepted mummies, as above. Once each hyperparasitoid had attacked four mummies, I terminated the trial and stored the mummies individually in numbered gelatin capsules. After 16 days, I examined the capsules and counted, by sex, the hyperparasitoids that had eclosed. All *D. carpenteri* females used in trials were preserved, oven-dried for 24 h, and weighed on a Cahn 21 electronic microbalance (sensitivity to 0.0001 mg) to determine their dry mass.

Experiment 3: Influence of Host Species and Immature Experience

In this experiment, I tested (1) whether offspring sex allocation by *D. carpenteri* is influenced by the species of primary parasitoids, assuming that the latter differ in quality, and (2) whether host

acceptance and offspring sex allocation are dependent on female conditioning by immature experience. The protocol was the same as that for Experiment 1, except that each arena contained eight mummies, four each containing *A. ervi* and *E. californicus*, arranged in alternating pairs; the mummies were selected for uniformity in size and contained immature wasps of the same physiological age. Although the total number of mummies was fixed, females were able to accept one kind of mummy in preference to the other or allocate offspring sexes differentially to these hosts. The experiment included 40 trials, 20 trials each with *D. carpenteri* females reared on, respectively, *A. ervi* and *E. californicus* as the conditioning hosts.

Experiment 4: Resetting the Oviposition Clock

I was motivated by the results of the earlier experiments to determine if a female's sex ratio response is controlled by an 'oviposition clock' that is reset after each oviposition bout. I use 'oviposition clock' to refer to a possible mechanism in a female wasp which resets the sequence of son and daughter production after an interval without hosts. For this experiment, I used the same design as in Experiment 2. I provided each female (N= 32) with four mummies containing immature *A. ervi* in each of two trials, the second trial was conducted after a 24-h ovipositional rest. During that time, I kept females in numbered petri-dishes (2.75 cm in diam by 1.50 cm in ht) and fed them with a honey-water solution.

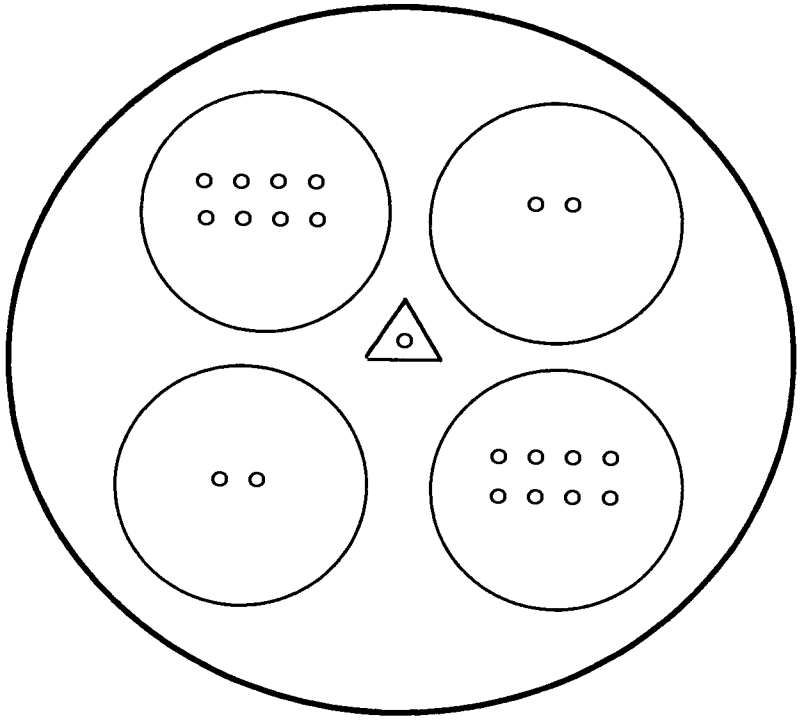
Experiment 5: Sequences of Small and Large Broods

I was motivated by the results of Experiment 4 to test the hypothesis that female *D. carpenteri* reset their pattern of offspring sex allocation at the beginning of each new brood. I compared the brood sex ratios of female wasps that consecutively encountered a series of small and large batches of mummies containing immature *A. ervi*. A *D. carpenteri* female (N= 30) was placed near a single mummy on an

equilateral triangle of filter paper (1 cm per side) in the center of a large plastic petri-dish (14.0 cm in diam by 2.5 cm in ht) which contained four small plastic petri-dishes (5.5 cm in diam by 1.5 cm in ht) equally spaced apart. Two of the small dishes contained two mummies and the other two small dishes contained eight mummies; the mummies were arranged as either a pair or a 4 x 2 square matrix on a circular piece (4.2 cm diam) of filter paper, with a space of approximately 1 cm between mummies. The small dishes were initially arranged so that the two dishes closest to any given dish always contained a different number of mummies from the latter (Fig. 8). At the beginning of the experiment, all of the mummies in the small dishes were covered with one half of a gelatin capsule. The single mummy on the triangle was used to give the female wasp experience in host handling. After the wasp had examined and probed the single mummy on the triangle of filter paper, she was allowed to forage freely throughout the arena. I directly observed the wasp's foraging behaviour and both manipulated the content of each dish and uncovered mummies so that she would sequentially encounter small dishes with two, eight, two, and finally eight mummies.

Host acceptance (= oviposition) was defined as ovipositor probing which lasted 3 min or longer and was followed by the wasp leaving the host. I did not remove an encountered mummy that was accepted, allowing the female to re-encounter already parasitized hosts. However, to prevent superparasitism, I placed one half of a gelatin capsule over a re-encountered mummy if the wasp attempted to attack it a second time. Rejected mummies were replaced immediately by a new ones to avoid the accumulation of unsuitable mummies in the arena. Each of the mummies in a small dish was covered with half of a gelatin capsule after the female wasp had finished attacking hosts and left the dish. I adjusted both the content of each dish and the removal of capsules so that each female foraged for 15-25 min. before encountering a group of new mummies. Females were not given an opportunity to re-encounter patches of mummies that were previously exploited. A trial was terminated when the wasp had examined and left the dish containing the last group of eight mummies. The

Figure 8. Arrangement of four small petri-dishes (5.5 cm in diam by 0.9 cm in ht) around a single *Aphidius ervi* mummy on a triangle of filter paper. Two of the small dishes contained two *A. ervi* mummies and the other two small dishes contained eight *A. ervi* mummies. All the small dishes were arranged within a large petri-dish (14.0 cm in diam by 2.5 cm in ht).

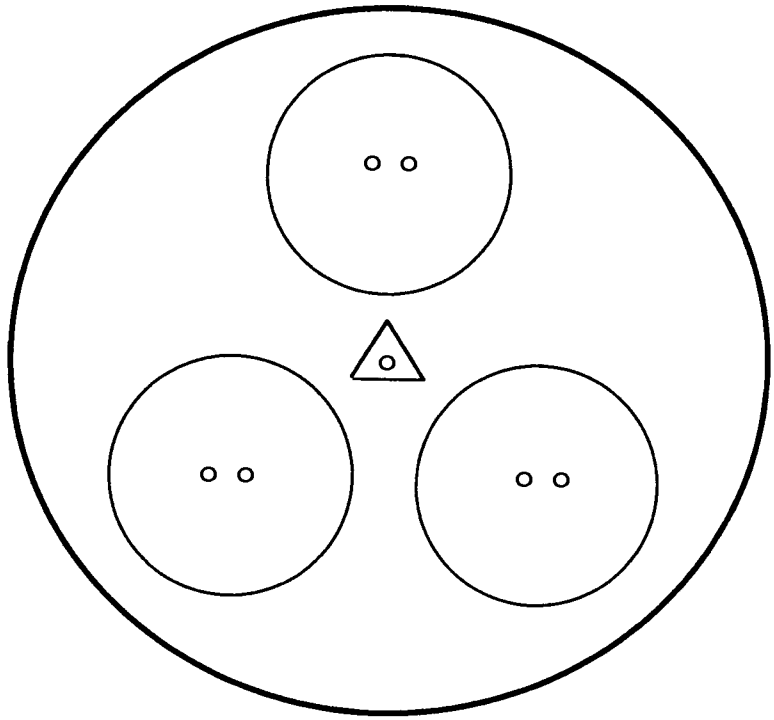


mummies were placed into coded gelatin capsules and stored at 20 ± 1°C until offspring eclosed, which I counted and sexed.

Experiment 6: Re-encounters with Batches of Marked Hosts

I was motivated by the results of Experiment 5 to determine if a female's sex ratio response is influenced by re-encounters with batches of *A. ervi* mummies that had been previously exploited. A *D carpenteri* female (N= 20) was placed near a single mummy on an equilateral triangle of filter paper (1 cm per side) in the center of a large plastic petri-dish (14.0 cm in diam by 2.5 cm in ht) which contained three small plastic petri-dishes (5.5 cm in diam by 0.9 cm in ht) equally spaced (Fig. 9). Each of the small dishes contained a pair of mummies on a circular piece (4.2 cm diam) of filter paper. At the beginning of the experiment, all of the mummies in the small dishes were covered with one half of a gelatin capsule. After the female wasp had examined and probed the single mummy on the triangle of filter paper, she was allowed to forage freely in the arena. I timed the removal of capsules so that the wasp foraged for 10-15 min. before encountering a pair of new unmarked hosts and also allowed her to re-encounter marked hosts in dishes that she had previously foraged in, but otherwise followed the same protocol as in Experiment 2. The female wasp was removed after it had attacked the third pair of mummies and held inside a gelatin capsule (size 00). After 5-6 min, the wasp was released into another large dish that contained a single mummy on a paper triangle and a small dish with six mummies arranged in a 2 x 3 matrix, with a space of approximately 1 cm between mummies. I allowed the wasp to attack the single mummy first and then directly observed the wasp's foraging behaviour. A trial was terminated when the wasp had attacked and left the group of six mummies in the second arena. All mummies were placed into coded gelatin capsules and stored at 20 ± 1°C until offspring eclosed, which I counted and sexed.

Figure 9. Arrangement of three small petri-dishes (5.5 cm in diam by 0.9 cm in ht) around a single *Aphidius ervi* mummy on a triangle of filter paper. Each of the small dishes contained a pair of *A. ervi* mummies. All the small dishes were arranged within a large petri-dish (14.0 cm in diam by 2.5 cm in ht).



Statistical Analysis

I estimated the sex ratio as the proportion ($*p$) of daugthers among n offspring, with SE ($*p$) = $\sqrt{(*p*q / n - 1)}$, where $*q = 1 - *p$ is the proportion of sons. For ANOVA, I transformed the proportions to their arcsine values by equation 14.5 in Zar (1984, p. 186). I compared the observed and expected frequencies, using the log-likelihood ratio test with Williams' correction for continuity (Sokal and Rohlf 1981, p. 737). I corrected Spearman's coefficient of rank correlation for tied data by the procedure of Kendall (1962, p. 38).

The data from Experiment 3 was classified in the form of a 3-way contingency table, using the model LOGLIN in the BIOM package of statistical procedures (Rohlf 1987), with the conditioning host (*A. ervi* versus *E. californicus*), and the hyperparasitoid sexes (male versus female) as the three levels; the iteration for convergence in the estimation was terminated when the largest difference between F and FHAT was 0.0001. I followed Fleiss (1981, equations 5.14 and 5.17) to estimate the odds ratio "o" of an unfertilized egg being laid in a mummy containing *E. californicus*.

I used paired-difference t-tests to compare the number of hyperparasitized hosts from groups with the same number of hosts in Experiment 5. I estimated the sex ratio as the proportion of daughters among all offspring. Host mortality was estimated as the proportion of dead primary larvae among all mummies that hyperparasitoid offspring did not emerge from or develop in. For ANOVA, I transformed the proportions to their arcsine values by equation 14.5 in Zar (1984, p. 186). To evaluate the influence of encounter sequence on host mortality among the first and second groups of eight mummies, I used fully randomized one-way ANOVAs. The sex ratio of the progeny from patches with the same number of mummies were compared by Wilcoxon's signed-rank tests. I used the log-likelihood ratio test with Williams' correction for continuity (Sokal and Rohlf 1981, p. 707) to compare the observed frequencies of sexes among broods with two

offspring to expected frequencies from a 1:1 (daughters:sons) ratio with a binomial distribution.

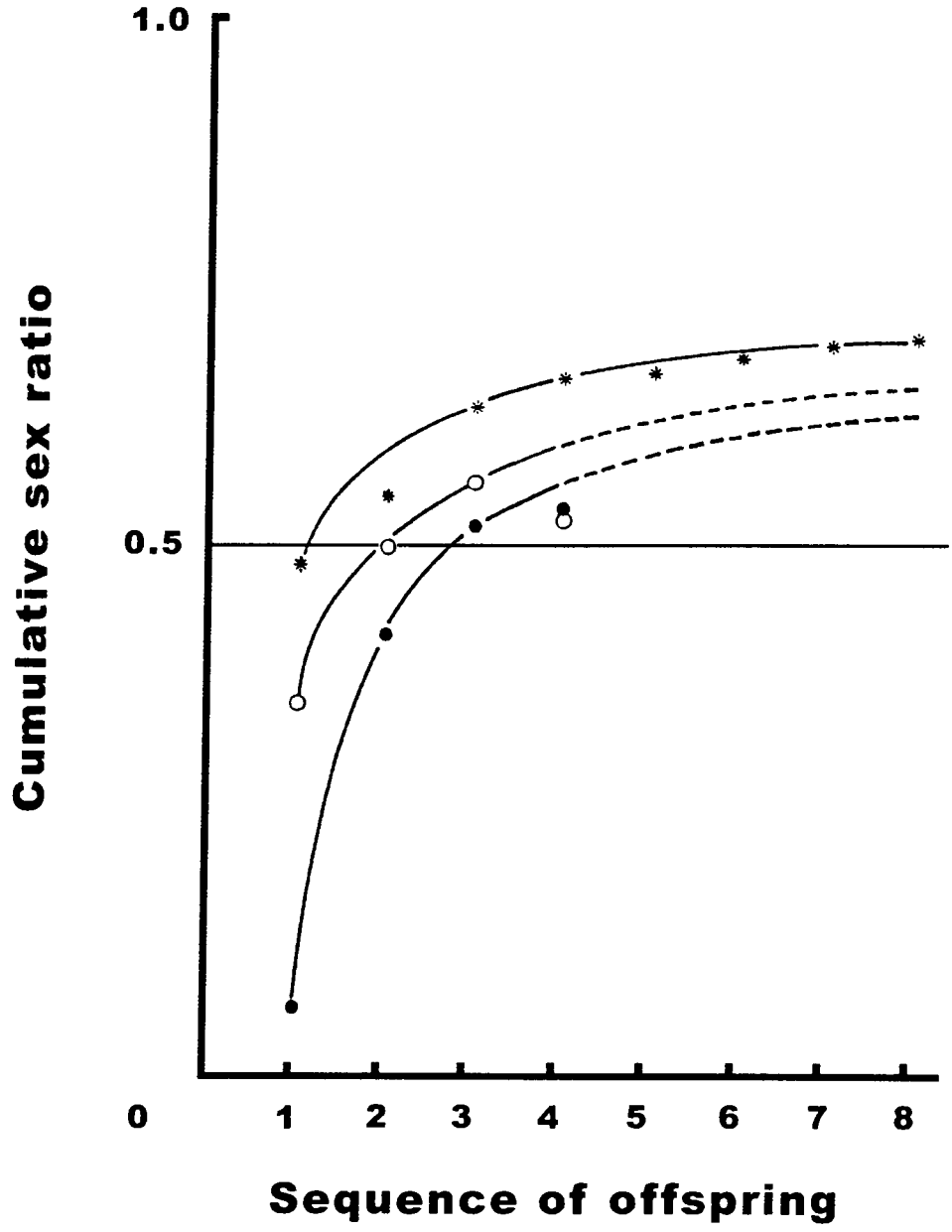
For Experiment 6, I evaluated the influence of encounter sequence on brood sex ratio by using linear regression to analyze the sex ratio of the progeny from the first, second, and third pairs of mummies of the first arena. I also used Wilcoxon's signed-rank tests to evaluate the influence of host spacing on brood sex ratio by comparing the cumulative sex ratio of the three pairs of mummies in the first arena and the group of six mummies from the second arena. To compare the observed offspring sex frequencies of individual broods from each of the three small dishes in the first arena to expected frequencies from a 1:1 (daughters:sons) ratio with a binomial distribution, I used the log-likelihood ratio test with Williams' correction for continuity.

RESULTS

Experiment 1: Influence Of Encounter Sequence

The overall offspring sex ratio was female-biased,with a mean of $*p = 0.695$ (SE = 0.015, $N = 1008$). Females alternated the sex of their first two offspring; those that had first laid a fertilized egg generally laid an unfertilized egg second, and vice versa. As a result, the overall sex ratio among the first two offspring produced by the 126 females was approximately 1:1 ($G_W = 2.677$, df = 1, P = 0.102). Sex was not randomly allocated, however. There were fewer single-sex broods, either sons ($N = 3$) or daughters ($N = 15$), than broods containing both one son and one daughter ($N = 108$); the difference between observed and expected binomial frequencies was significant ($G_W = 79.636$, df = 2, $P < 0.001$). On average, every third or fourth egg laid (after the first two eggs) was unfertilized, which resulted in a gradually more female-biased offspring sex ratio, approaching an equilibrium value of about 0.70 (Fig. 10). As the brood sex ratio approached its upper

Figure 10. Cumulative sex ratios produced by mated females of *Dendrocerus carpenteri* in relation to offspring sequence and host type. Hosts were mummified pea aphids that were parasitized by *Aphidius ervi* at different ages: ✳, second nymphal instar (N= 126 females); o, third nymphal instar ('large' mummies; N= 34 females); ●, first nymphal instar ('small' mummies; N= 45 females). The sex ratio is the proportion of daughters among offspring, summed over all females. Regression lines are eye-fitted.


1.0
0.5
0
Cumulative sex ratio
1
2
3
4
5
6
7
8
Sequence of offspring

asymptote, sex ratio variance declined, with fewer females producing single-sex broods (Fig. 11a).

Experiment 2: Influence Of Host Size

Females accepted both small and large mummies containing 9-day-old *A. ervi* immatures, but they differentially allocated offspring sexes to each mummy class. On small hosts, most females (93.3%, $N = 45$) laid first an unfertilized egg and fertilized the second egg (Fig. 10). In contrast, 35.3% ($N = 34$) of females provided with large mummies laid a fertilized egg on their first host and an unfertilized egg on the second (Fig. 10). In both sets of trials, the overall sex ratio after two offspring did not differ significantly from a 1:1 ratio (Table 18).

A female's perception of relative host quality was not dependent on her size in terms of dry mass. Females that laid a fertilized egg in the first (large) mummy they had encountered did not differ in dry mass (mean $\pm$SE = 0.133 $\pm$0.004 mg, $N = 12$) from those that laid first an unfertilized egg (0.127 $\pm$0.003 mg, $N = 22$) ($F_{1,32} = 1.295$, $P = 0.264$). Because females provided with small mummies varied little in their sex ratio response (see above), I did not test for a correlation between a female's dry mass and her decision to fertilize the first egg.

Experiment 3: Influence Of Host Species and Immature Experience

I found no evidence of partial preference by *D. carpenteri* for mummies containing immatures of *A. ervi* or *E. californicus*, both kinds of mummies were accepted at the same rate (Kolmogorov-Smirnov 2-sample test, $d_{max} = 0.100$, $P > 0.05$). Also, acceptance was independent of the host species on which the hyperparasitoid had developed (log-linear analysis, $G_W = 0.648$, df = 2, $P = 0.723$). *Dendrocerus carpenteri* females allocated offspring sexes differently to the two kinds of hosts, however, producing a sex ratio that was more female-biased on mummies that contained *A. ervi* ($*p = 0.800$, SE = 0.032, $N = 160$) than

Figure 11. Cumulative brood sex ratios in relation to offspring sequence in *Dendrocerus carpenteri*. Proportions of broods are plotted against the cumulative brood sex ratio, estimated as the proportion of daughters among offspring, summed over all females. (a) Wasps ($N = 126$) were provided with eight mummies containing immature *Aphidius ervi*; (b) wasps ($N = 40$) were provided with eight mummies, four each containing *A. ervi* and *Ephedrus californicus*.

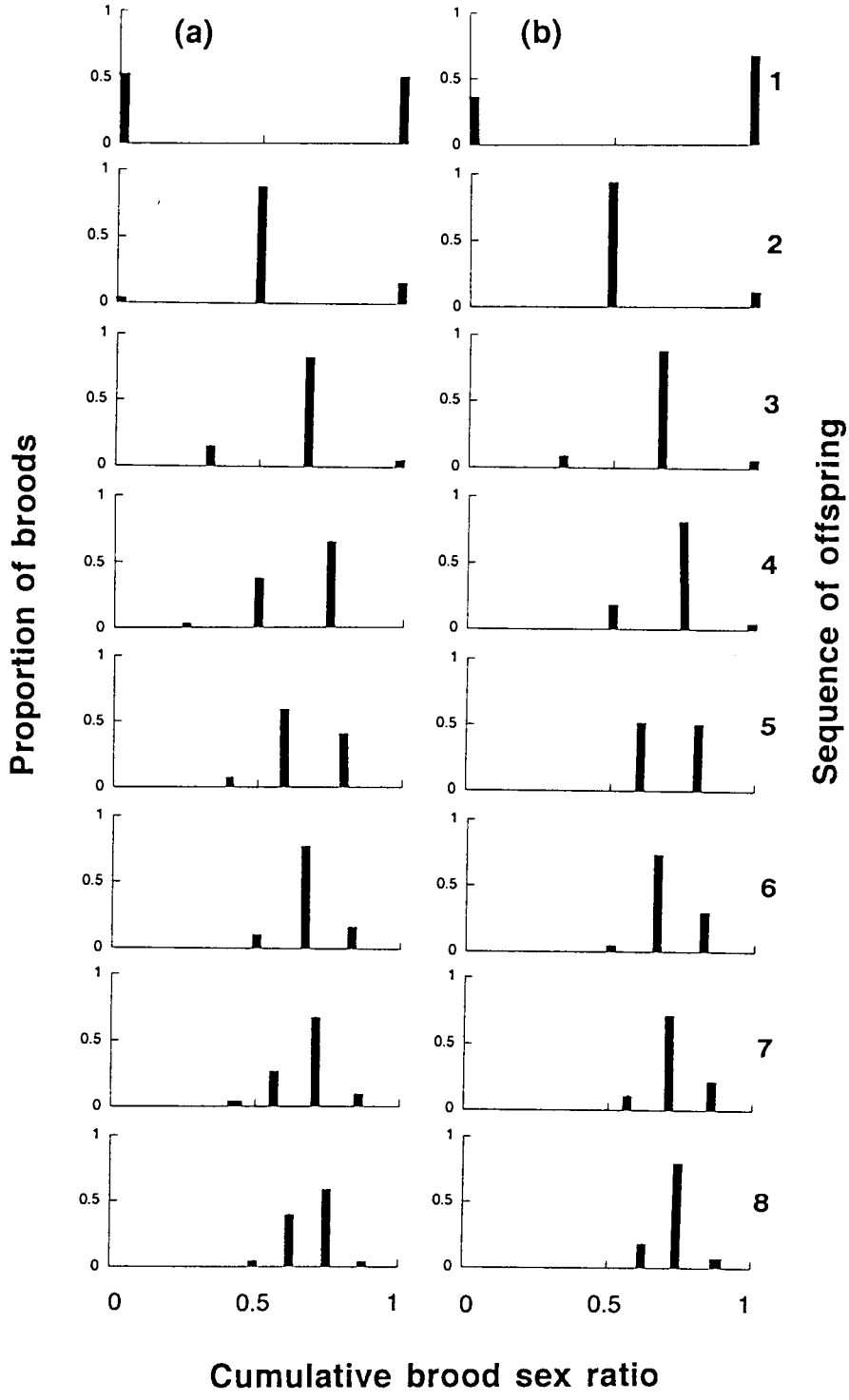

(a)
(b)
1
2
3
4
5
6
7
8
0
0.5
1
Proportion of broods
Sequence of offspring
Cumulative brood sex ratio

Table 18. Mean sex ratio and distribution of daughters between the first two offspring produced by mated females of *Dendrocerus carpenteri*, a hyperparasitoid of *Aphidius ervi* and *Ephedrus californicus* in mummified pea aphids[1].

			Frequencies of broods						
Expt	*p1*	(SE)	0	1	2	*N*	*GW*	*B*	(95% CI)
1	0.484	(0.045)	3	108	15	126	79.636***	0.857	(0.791-0.913)
2 (SH)	0.067	(0.038)	7	29	1	37	17.408***	0.784	(0.639-0.900)
2 (LH)	0.353	(0.083)	2	28	2	32	19.835***	0.875	(0.740-0.965)
3	0.650	(0.088)	0	36	4	40	34.417***	0.900	(0.789-0.972)
4 (Day 1)	0.406	(0.088)	3	26	3	32	13.201***	0.813	(0.661-0.926)
4 (Day 2)	0.281	(0.081)	4	26	2	32	13.867***	0.813	(0.661-0.926)

[1]Abbreviations: SH, small host; LH, large host; *p1* (SE), overall proportion (and standard error) of daughters among first offspring; *N*, total number of broods with 0, 1 and 2 daughters between first two offspring; *GW*, fit of observed frequencies with their binomial expectations (by log-likelihood ratio test with William's correction; ** $p \leq 0.01$; *** $p \leq 0.001$); *B*, proportion of broods among *N* with one son and one daughter between first two offspring, with 95% confidence interval of the arcsine-transformed proportion.

on those that contained *E. californicus* (*p = 0.669, SE = 0.037, N = 160). These proportions differed (log-linear analysis, G_W = 7.561, df = 2, p = 0.023), with the odds of a female laying an unfertilized egg being 1.4 times higher for a mummy containing *E. californicus* than *A. ervi* (odds ratio, $o \pm$SE = 1.386 $\pm$0.359).

Sex allocation and sequential selection of host types were not independent. For their first host, females apparently did not distinguish between mummies containing *A. ervi* and *E. californicus* (G_W = 0.049, df = 1, P = 0.825; Fig. 12). They produced an overall sex ratio of 0.650 (SE = 0.076), which did not differ from an expected 1:1 ratio (G_W = 1.814, df = 1, P = 0.178). In subsequent selections, however, the ratio of *E. californicus* to *A. ervi* mummies accepted was correlated with the ratio of fertilized to unfertilized eggs laid in these mummy classes (Spearman's coefficient of rank correlation, r_S = 0.735; critical $r_{S\ 0.05\ (2),\ 8}$ = 0.738). The overall sex ratio gradually approached an equilibrium value near *p = 0.73. Females varied little in their sex ratio response to the two mummy classes, all producing mixed sex-broods in a pattern that departed significantly from an expected binomial distribution (Fig. 11b).

Experiment 4: Resetting The Oviposition Clock

On day 1, the mean sex ratio increased from *p_1 = 0.406 (N = 32 females) for the first offspring to *$p_{1\text{-}4}$ = 0.578 after four offspring. When females were deprived of hosts for 24 h after an initial oviposition bout and then provided with mummies of the same quality, they again laid more unfertilized than fertilized eggs on the first host encountered (Table 18). The pattern of offspring sex allocation was nearly identical on both days, however (Fig. 13).

Figure 12. Offspring sexes produced by mated females of *Dendrocerus carpentri* ($N = 40$) in successive selections from among eight mummies, four of which contained immature *Aphidius ervi* and four immature *Ephedrus californicus* (solid bars = daughters; open bars = sons).

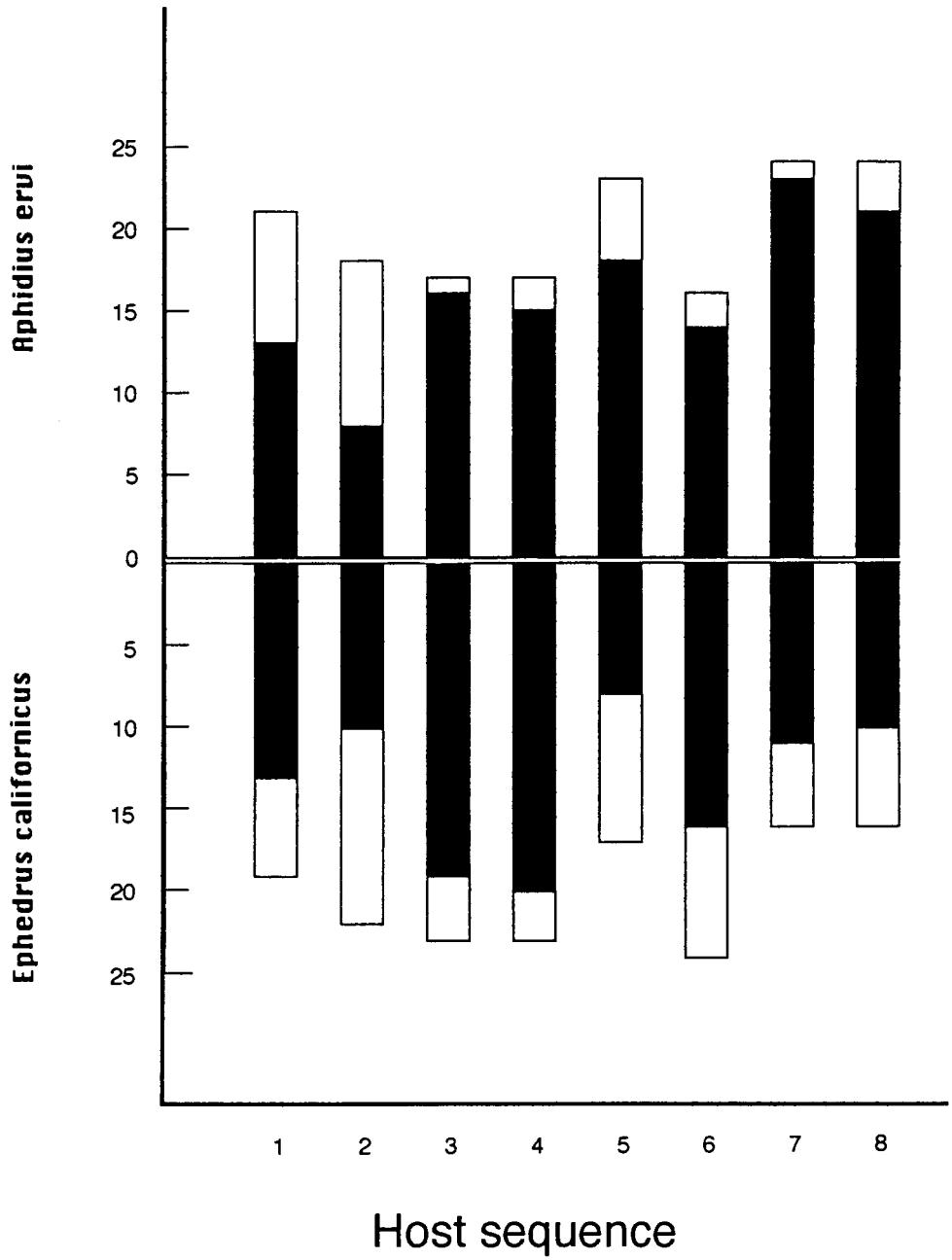
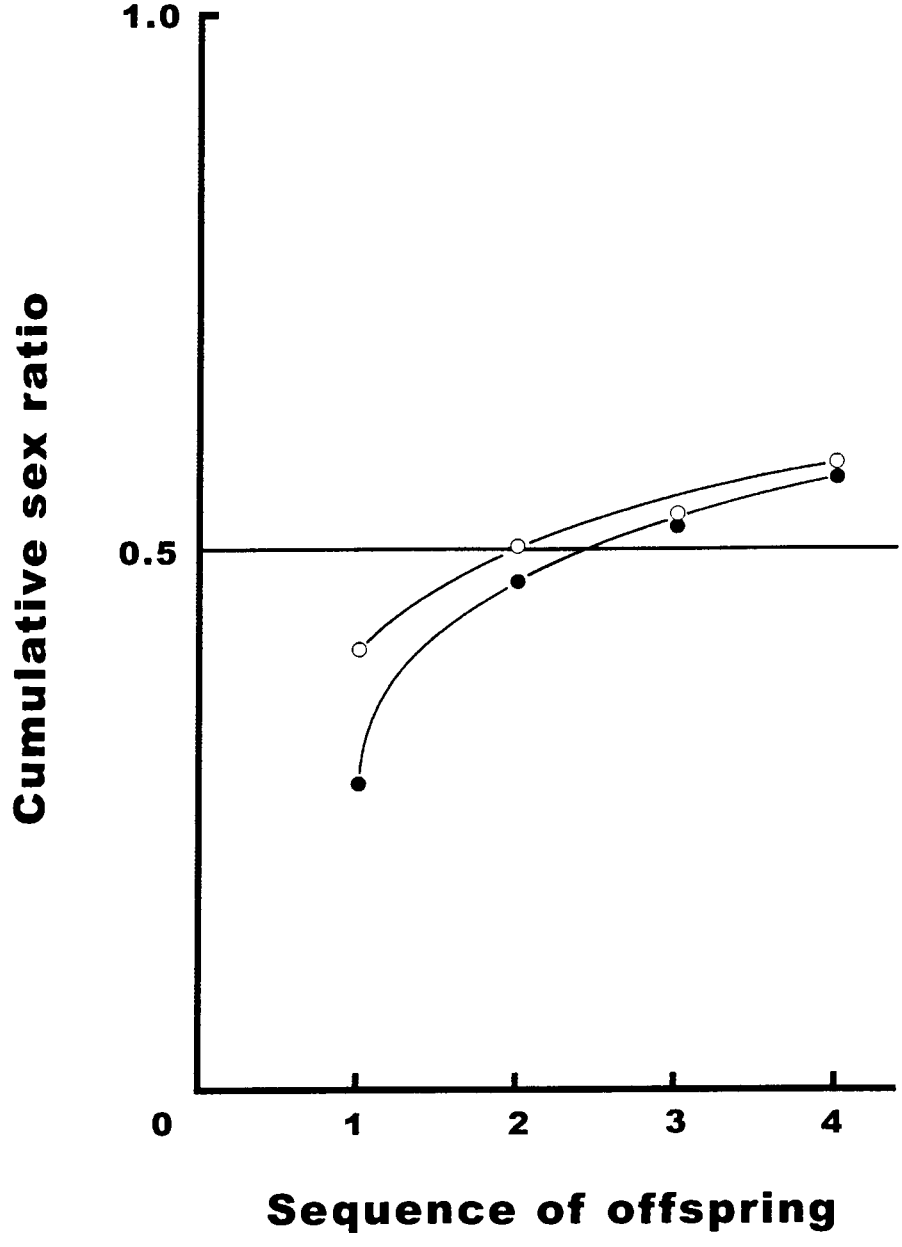

Aphidius erui
Ephedrus californicus
25
20
15
10
5
0
5
10
15
20
25
1
2
3
4
5
6
7
8
Host sequence

Figure 13. Cumulative sex ratios produced by mated females of *Dendrocerus carpentri* ($N = 32$) in relation to offspring sequence. Females were provided with four mummies containing *Aphidius ervi* in each of two oviposition bouts, the second after a 24-h rest period (o, day; ●, day 2). The sex ratio is the proportion of daughters among offspring, summed over all females. Regression lines are eye-fitted.


1.0
0.5
0
1
2
3
4
Cumulative sex ratio
Sequence of offspring

Experiment 5: Sequences of Small and Large Broods

Female *D. carpenteri* parasitized most of the hosts in each group before they left to search for a new group of hosts. The numbers of hosts parasitized by females, among groups with similar numbers of mummies, did not vary with encounter sequence. All the hosts in groups with two mummies were successfully parasitized. The mean number of hosts parasitized in the first and second groups with eight mummies were respectively 7.43 $\pm$0.14 and 6.90 $\pm$0.20 ($\pm$SE, N= 20) and not significantly different (t = 2.006, df = 29, P = 0.054). The mean number of hosts attacked by *D. carpenteri* in each trial was 19.33 $\pm$ 0.22 ($\pm$SE, N= 20). Mean mortality of hosts in the first and second groups with eight mummies were respectively 7.5 $\pm$1.7% and 5.0 $\pm$1.7% ($\pm$SE) and not signficantly different (F = 0.724, df = 58, P = 0.398). Female wasps would usually examine but rarely attacked hosts that they had previously probed and marked.

Most females (66.7 %, N= 30) laid a son on their first host, but they usually laid one son and one daughter among groups with two mummies. The sex ratios from the first and second groups with two mummies were not significantly different (t = 22, n = 10, P > 0.05). I pooled the sex ratio from all broods with two offspring (N= 60) and found that the pooled value was not significantly different from an expected 1:1 ratio (G_W = 3.335, df = 1, P > 0.05). The mean sex ratio of broods with two offspring was 0.583 $\pm$0.038 ($\pm$SE). There were fewer single-sex broods, either sons (N= 1) or daughters (N= 10), than broods containing both one son and one daughter (N= 49); the difference between observed and expected binomial frequencies was highly significant (G_W = 34.176, df = 2, P < 0.001). *Dendrocerus carpenteri* laid two to three times as many daughters as sons among groups with eight mummies. Although females laid statistically fewer daughters among large broods at the end than near the beginning of a trial (T^- = 78.5, n = 23, P < 0.05), the difference was very slight. The mean sex ratio from the first and second groups with eight mummies were respectively 0.776 $\pm$0.020 and 0.701 $\pm$0.025 ($\pm$SE, N= 30).

Experiment 6: Re-encounters with Batches of Marked Hosts

Female *D. carpenteri* parasitized both hosts in each dish of the first arena before moving onto a different dish. As in previous experiments, female wasps would examine but rarely attacked hosts that they had previously probed and marked. In the first arena, the offspring sex ratio of broods was influenced by the sequence in which they were laid. The proportion of daughters among broods increased linearly with each subsequent pair of mummies that females encountered ($Y = 0.433 + 0.100(\pm 0.0362)X$; $F = 7.648$; $df = 1, 58$; $P = 0.008$). Females produced more broods, with only daughters, among pairs of mummies that were attacked latter during the trial. Differences between observed and expected binomial frequencies of broods were highly significant among all three dishes in the first arena (Table 19). *Dendrocerus carpenteri* appeared to also lay more daughters among broods if the mummies were grouped rather than dispersed. Offspring sex ratio of *D. carpenteri* was less female-biased among dispersed pairs of mummies in the first arena than among groups of six mummies in the second arena ($t^{+} = 12.50$, $n = 16$, $P > 0.01$). The mean sex ratio of broods from the first and second arena were respectively, 0.633 ± 0.023 and 0.747 ± 0.025 ($\pm$SE, $N = 20$). Most of the hosts in the groups of six mummies from the second arena were successfully parasitized (mean + SE = 5.80 ± 0.09; $N = 20$). The proportion of females ($N = 20$) that laid a son on their first host were respectively 45% and 55% in the first and second arenas.

Table 19. Mean sex ratio and distribution of daughters among broods of two offspring produced by mated females of *Dendrocerus carpenteri*. A single female was allowed to forage freely in a large arena with three dishes. Each dish contained two hosts and the female was allowed to re-encounter previously attacked hosts. Hosts were 8-day old *Aphidius ervi* in mummified pea aphids[1].

Brood	Female Sex Ratio[2] (Mean ± SE)	Frequencies of broods				
		0	1	2	n	G_W
Dish 1	0.525±0.044	1	17	2	20	10.798**
Dish 2	0.650±0.053	0	14	6	20	11.235**
Dish 3	0.725±0.057	0	11	9	20	12.268**

[1]Abbreviations: n, total number of broods with 0, 1, and 2 daughters among two offspring; G_W, fit of observed frequencies with their binomial expectations (by log-likelihood ratio test with Williams' correction); **, $P < 0.01$.

DISCUSSION

Many parasitoid species produce 'precise' offspring sex ratios by laying male and female eggs in a more or less fixed sequence. The production of males late or last in an oviposition bout has been observed in several gregarious parasitoids (Mertins 1980, 1985b; Putters and van den Assem 1985; Dijkstra 1986; Rojas-Rousse *et al.* 1988). In these species, the females lays many eggs in a single host and presumably can estimate host size and adjust the total number of offspring accordingly. Other gregarious and most soliatry species for which information is available, however, tend to lay male eggs early, but not necessarily first, in an oviposition bout (Hokyo *et al.* 1966; Waage 1982; Waage and Lane 1984; Suzuki *et al.* 1984; Strand 1988; Braman and Yeargan 1989; Noda and Hirose 1989; Wajnberg 1993). For example, females of the egg parasitoid *Trichogramma evanescens* allocate two daughters per host egg and add a son in the first one or two hosts per oviposition bout (Waage and Ng 1984). Wajnberg (1993) showed that such patterns are under genetic control. Although a fixed sequence of male and female eggs can account for 'precise' sex ratios, it cannot account for a flexible sex ratio response to variations in, for example, host size (van den Assem 1971; Charnov *et al.* 1981; Cloutier *et al.* 1991; King 1988; Werren and Simbolotti 1989; Seidl and King 1993) and host quality (van Alphen and Thunnissen 1983; Werren 1984; King 1989, 1993b; King *et al.* 1995; Ode and Strand 1995). Wasps that produce a fixed sequence of male and female offspring may change the sex ratio in the presence of other foundresses searching the same patch (Waage and Lane 1984; Strand 1988). This change is the result of wasps laying fewer eggs to avoid superparasitism, rather than of an altered production schedule, however.

A 'male first' strategy ensures that each mated female produces a sufficient number of sons to fertilize her daughters, regardless of variations in host quality, patch size, or the reproductive contributions of other females competing for the same host resources. The strategy may be adaptive in parasitoids that have incomplete information about available host resources, or encounter hosts which vary in quality, or

can be easily disturbed during an oviposition bout. For example, *D. carpenteri* females require an average of 400 sec to lay an egg (refer to Chapter 3) and, if disturbed may leave the patch before all suitable hosts are parasitized. Mated females of *D. carpenteri* produce males early during an ovipositon bout, but they do not strictly follow a 'males first' strategy. Wasps alternate the sex of their first two offspring and lay an unfertilized egg into the first or the second host accepted.

If fitness gains from increased size are more important for females than for males of solitary species of parasitoids, 'host quality' models (Charnov *et al.* 1981; Charnov 1982) predict that mothers allocate sons and daughters to lower- and higher-quality hosts, respectively. According to this theory, a female should lay an unfertilized egg in all hosts below a critical threshold size and a fertilized egg in those above the threshold; this threshold may shift with the experience and physiological state of the wasp (Charnov *et al.* 1981) Much empirical evidence supports these model predictions (King 1988; Cloutier *et al.* 1991; Godfray 1994). Host quality can affect a female's sex ratio response in two different ways, however. A female may either reject a lower quality host and continue searching for one of higher quality, or she may accept lower- and higher-quality hosts equally but adjust her sex ratio response accordingly. Mackauer and Lardner (1995) reported that *D. carpenteri* females accepted large and small pea-aphid mummies equally but laid more fertilized eggs in large rather than small, mummies that they had accepted. Also, significantly more females eclosed from field-collected pea-aphid mummies that had died in the adult stage and were large (75.7%) than from those that had died in the fourth nymphal instar and were small (47.3%) (Mackauer and Lardner 1995). The results of Experiments 2 and 3 are consistent with these values. When host quality was uniform, wasps provided with small *A. ervi* mummies typically produced a son as their first offspring, but those provided with large mummies often produced a daughter (Table 18). With additional ovipositions, however, the cumulative brood sex ratio became more female-biased in both groups. A female's immature experience did not affect her host acceptance behaviour, and her perception of mummy size (in terms of egg

fertilization) was not dependent on her size. In contrast, *D. carpenteri* females offered a choice between two kinds of mummies, *A. ervi* and *E. californicus*, accepted both equally as their first host, but they allocated a higher proportion of unfertilized eggs to mummies containing *E. californicus* in subsequent selections, which suggests that they assessed the latter as having less value than *A. ervi.*

I did not directly evaluate the manner in which the encounter rate influenced a female's sex ratio response. The results of Experiments four and five suggest, however, that females of *D. carpenteri* also reset their sequence of son and daughter production after an extended period of rest or a short period of searching without encountering hosts. Noda and Hirose (1989) found that females of *Gyron japonicum* reset their sequence of son and daughter production after a delay of only 3 h between oviposition. I found that resting *D. carpenteri* females for 24 h, without hosts, or allowing females to actively search for 15-25 min, without encountering hosts, caused them to re-set their sequence of son and daughter production.

Most species of aphids form colonies. Because aphidiid wasps typically parasitize relatively few individuals in each colony (Mackauer and Völkl 1993), mummified aphids that are susceptible to hyperparasitism by *D. carpenteri* tend to be loosely aggregated. I have no information about the foraging behaviour of *D. carpenteri* under natural conditons. Even widely dispersed hosts may be perceived as belonging to the same patch if the patch's physical boundaries are either very large or not clearly defined by a foraging wasp (Ayal 1987). A wasp may perceive a single foraging bout to include all hosts encountered before she has depleted her egg supply, or was disturbed, or did not find new hosts during a lengthy period of searching. As long as environmental conditions do not change, a female need remember only short sequences of oviposition decisions and adjust the mean sex ratio according to current information on host quality and availability. After a *D. carpenteri* female has laid the first two eggs in an oviposition bout, her sex ratio response to up to six additional hosts, encountered later in the sequence, remains more or less the same (Fig. 10).

The decision matrix for sex ratio adjustment in *D. carpenteri* is more complex than a simple mechanism for counting eggs, as suggested by Putters and van den Assem (1985). To determine whether an egg will be fertilized, females apparently use a set of 'rules' that depend on host quality and oviposition sequence. These rules can be expressed as follows: (1) If the quality of the first host encountered in a patch is relatively high, the first egg should always be fertilized. An unfertilized egg should be laid, however, if host quality is relatively low. (2) If a second egg is laid, its sex should be opposite to that of the first, independent of host quality. These two rules together ensure that the first two offspring per oviposition bout from *D. carpenteri* females will typically consist of one son and one daughter (Table 18) and, consequently, that daughters will normally find a mate. (3) If the female encounters and accepts more than two hosts during a single oviposition bout, she should use any additional eggs to adjust the brood sex ratio, one step at a time, towards an equilibrium value. This value varies with host quality; she should fertilize a larger proportion of eggs laid on 'good' hosts and a smaller proportion on 'bad' hosts.

When hosts are found in defined areas of the environment, it is obviously in the parasitoid's interest to recognize the boundary of the host patch and to adjust its foraging or reproductive strategies accordingly when the boundary is crossed. A number of studies have examined the behaviours associated with detecting the edge of the patch and whether parasitoids turn back when they cross boundaries. Parasitoids may respond to either chemicals associated with host feeding (Waage 1978; Strand and Vinson 1982), chemicals directly associated with the host (i.e. kairomone) (Vet and van der Hoeven, 1984), or parts of the host left in the habitat (i.e. scales) (Gardner and van Lenteren 1986). In addition, parasitoids may also respond to feeding damage or structural modification of the host plant due to feeding. Casas (1988) found that two parasitoids of leaf mining insects turn back when they encounter the margin of mines. If such cues are not available, parasitoids may rely on direct encounters with hosts to determine search patterns in the habitat. In these situations, it may be

difficult for parasitoids to determine patch boundaries and search efficiently for hosts.

Little information is available on the host cues that hyperparasitoids may respond to. Vater (1971) found that the searching behaviour of several hyperparasitoid parasitoids of *Diaeretiella rape* on cabbage aphid, *Brevicoryne brassicae*, were not apparently influenced by host habitat or the olfactory substances of host plants. Female *D. carpenteri* are strongly arrested when they contact mummified remains of aphids with their antennae (Sullivan 1988). Both male and female *D. carpenteri* have been reported to be attracted to the honeydew of aphids (Bundenberg 1990). However, honeydew may also comprise a food source and orientation to its odour may therefore occur for reasons other than host location. Interestingly, *D. carpenteri* does not stay and search longer after contact with adult primary parasitoids in the vicinity of aphid colonies and tends to only search the periphery of aphid colonies (Völkl *et al.* 1995). Evaluation of the importance of sensory cues for host location by *D. carpenteri* would improve our understanding of its foraging behaviour and warrants future study.

Some parasitoids leave chemical markers in the enviroment that they and other parsitoids recognize. Chemicals may be left as a parasitoid leaves a patch or moves through the habitat and this often enables a parasitoid to avoid previously searched areas. Patch marking has been thoroughly investigated and recorded in many primary parasitoid species (Godfray, 1994), but this phenomenon has not been well studied among hyperparasitoids. Höller and Hörmann (1993) claim that *D. carpenteri* females with low egg loads continuously apply a marking phermone while walking. However, their interpretation of *D. carpenteri* foraging behaviour is questionable because of the extremely small areas within which they tested females. In addition, similar compounds have been identified as chemicals released by other hyperparasitoid species as a defense against attack by predators (Völkl *et al.* 1994) and it is unlikely that they are also used as patch markers.

Host marking is more common among parasitoids than patch marking (Godfray 1994, Hoffmeister and Roitberg 1996). Godfray (1994) presented three explanations for host marking: (1) Marking may improve the efficiency of the parasitoid population by increasing the evenness of host attack, (2) Marking may allow the female to avoid a host she had just attacked, (3) Marking may alert other females that the host has been attacked. I suggest that *D. carpenteri* females may mark hosts not only for the above reasons, but to also provide means to determine if they are still in the same patch of hosts. Female wasps that come across self-marked hosts, after a period of searching, will be alerted that they are still among previously attacked hosts. *Dendrocerus carpenteri* appears to have a limited memory for host encounters and it may be adaptive for females to behave as if they are still in the same patch as long they encounter self-marked hosts. This behaviour may especially be adaptive when the features or boundaries of the habitat are uniform and not clearly defined. I propose that the density and distribution of hosts does not affect the sex allocation behaviour of *D. carpenteri* females as long as the encounter time between marked hosts is short.

The use of host marking in *D. carpenteri* is similar to patch marking in other parasitoids because both behaviours tell females that they are in a previously searched area. Patch marking usually gives a parasitoid information that tells her to avoid searching for hosts in a given area. Similarly, a continuous sequence of encounters with self-marked hosts will also eventually cause a female *D. carpenteri* to 'give-up' host searching and disperse to a different area. The actual number of encounters required will probably vary with the physiological state and experience of the female wasp. However when a female *D. carpenteri* has been (1) searching for some time without encountering any hosts or (2) encountering unmarked hosts frequently enough to continue a foraging bout, host marking may provides information about the degree to which the patch has been exploited.

Recent theories on patch definition for parasitoids have promoted the concept of patches being discrete units of the environment that

have distinct boundaries or borders defined by the behaviour of foraging females (Hassell and Southwood 1978, Waage 1979, van Alphen and Vet 1986). Although interpretations of the elementary unit of foraging have ranged from a single leaf (Nelson and Roitberg 1995) or fruit (Reeve 1987) to an entire plant (Ayal 1987) coupled with host-derived cues, the scale by which the patch is measured has never been questioned as being other than 'spatial'. I have investigated predictions concerning patch perception and optimal allocation of progeny under conditions of uncertain host distribution and quality. The sex allocation patterns of *D. carpenteri* females suggest that the borders of a 'patch' are defined by a 'temporal' and not a 'spatial' scale. I argue that this form of patch perception may be adaptive if host-derived cues are absent after female hyperparasitoids search beyond the broadest hierarchical levels of the host habitat.

Parasitoid foraging behaviour is often viewed as consisting of a series of steps: habitat location, host food-plant location, host location, and host acceptance (Salt 1935, Doutt 1959, Vinson 1976). During the foraging process, female parasitoids use specific cues which arrest and confine it to a level of the environment where they search for cues at the next level down the hierarchy of host searching (Waage 1979). However, it is unlikely that immature aphidiids produce either chemical or physical cues that are detectable by foraging *D. carpenteri* females. Hyperparasitoids probably use the same cues as aphidiid wasps to find plants that are currently or had previously been colonized by aphids. But it is unlikely that *D. carpenteri* females use additional cues or information to determine their search parameters for mummies within individual plants. Neither the size of an aphid colony, number of foraging aphidiid adults, or the quantity of honeydew on the foliage are reliable indicators of the number or distribution of mummies within a plant. As discussed in Chapter 4, *D. carpenteri* females appear to search plants systematically and the time invested will depend on their encounter rates with self-marked and unmarked mummies.

In conclusion, *D. carpenteri* females apparently use relatively simple decision rules to determine the sequential allocation of offspring sexes to hosts in a patch. Although sons and daughters are produced in a non-random sequence, the exact schedule varies with host quality. Females ensure that daughters find mates by producing one son and one daughter as the first two offspring per oviposition bout. Females differentially allocate sons and daughters to higher- and lower-quality hosts that are encountered later in an oviposition bout, however, which results in departures from a 1:1 sex ratio. Female wasps also seem to adjust their foraging and sex allocation behaviour in response to re-encounters with self-marked hosts. Patch perception appears to be defined according to temporal rather than spatial scales. Further investigations into the effects of conspecifics or other hyperparasitoids species on offspring and offspring sex allocation are needed for a more complete understanding of the reproductive strategy of *D. carpenteri*. However, such studies lie beyond the scope and objectives of this thesis.

CHAPTER 6

General Discussion and Conclusions

Studies of pathogens and their animal hosts have provided some of the best cases of genetic variation and adaptation between populations of two species (reviewed in Holmes 1983, Lively 1989). Due to the intimate relationship between insect parasitoids and their often specific range of hosts, I would also expect selective interactions and accompanying genetic changes to occur between them. A few studies provide some evidence for physiological coevolution between insect hosts and their parasitoids (reviewed in Godfray 1994, Henter 1995, Henter and Via 1995). In comparison, evidence for ecological coevolution among parasitoids and their hosts may come from hosts with adaptations that seem to have evolved under selection pressure of parasitoids, but where a parasitoid has managed to keep up with the host. Godfray (1994) suggested that the torymid wasp *Apocryptaphagus* sp. evolved from a parasitoid or inquiline of pollinating fig wasps to become a true gall former in order to avoid hyperparasitism or parasitism by parasitoids of the pollinating agaonid. Yet, *Apocryptaphagus* appears ultimately unsuccessful because it is heavily parasitized by another torymid, *Apocrypta mega*, which is specific to it. *Apocrypta mega* apparently evolved a long ovipositor that enables it to reach *Apocryptaphagus* larvae within their large galls which jut out into the interior of figs (Godfray 1988).

Many coevolutionary models predict that interactions between hosts and their parasites can have several outcomes (Futuyma and Slatkin 1983). Thompson (1994) proposed that when a host evolves defenses against a parasite, it may result in one of three evolutionary outcomes: local extinction of the parasite, the evolution of counterdefenses in the parasite, or a shift onto a new and less-defended host. The first outcome ends the interaction, the second results in an arms race between the two species, and the third sets the stage for coevolutionary alternation. In the third case, the parasite shifts to a new poorly-defended host and selection for defense will decrease on the original host. Over evolutionary time, the original host may become less-well defended while the new host becomes better defended, and natural selection will favor those parasites that attack either the original host or yet another host. In this manner, a parasite

may come to alternate among several hosts and coevolve with all of them. Evolution and loss of defenses and counterdefenses may produce a range of specialization in the parasite populations and a complex distribution of defenses among hosts. The diversity in strategies by aphidiid wasps to reduce mortality among their progeny and both the foraging behaviour and reproductive strategies of some of their hyperparasitoids are consistent with the second and third outcomes of coevolutionary association.

D. carpenteri is a common hyperparasitoid over a broad range of aphids and host plants on several continents (Takada 1973, Stary 1977). The degree of specialization among different populations of this species has not been well studied, but *D. carpenteri* is generally perceived as a generalist that does well in a variety of hosts and habitats. In this thesis, results from host choice (Chapter 3) and foraging (Chapter 4) experiments confirm this perception and support the hypothesis that the foraging behaviour of *D. carpenteri* may have evolved to counter the defensive strategies of its hosts.

Fritz (1982) predicted that selection pressure by natural enemies is instrumental in the evolution of host behaviour modification in insect parasitoids. Aphidiid wasps have apparently evolved an extensive range of strategies to reduce the risk of hyperparasitism among their immature progeny (reviewed in Mackauer and Völkl 1993). However, it is difficult to determine the relative role of *D. carpenteri* and other hyperparasitoid species in the evolution of these strategies among aphid parasitoids. Aphidiid wasps must often contend simultaneously with multiple enemies, whether hyperparasitoids or predators.

In collections of *A. ervi* mummies from alfalfa in the southern interior of British Columbia, I found that *D. carpenteri* was consistently more abundant than species of either *Asaphes* or *Alloxysta. Aspahes lucens* can be more abundant than *D. carpenteri* among *A. ervi* populations in other regions of North America (Mertins 1985, Schon *et al.*1996), but the latter was usually the second most common species of hyperparasitoid. Although *D. carpenteri* is a prolific species of

hyperparasitoid, its ecological success may not be a useful indicator of its importance as a selection agent among past or present aphidiid populations.

Brodeur and McNeil (1992) suggested that there may be no significant advantage to modified host behaviour if levels of predation or hyperparasitism among aphidiid populations are either very low or very high. Selection intensity may rely on relative rather than absolute survival of aphidiid progeny among different habitats. Further investigation into the adaptiveness of behaviour by parasitized aphids, relative to both predation and hyperparasitism, is needed. A thorough understanding of the interaction between aphids and their primary parasitoids may require studies on all possible agents of selection in the habitat. Studies on the tritrophic interactions between golden rods, stem gall-making flies, and the parasitoids and birds that attack the gall-feeding larvae provide evidence for the view that interactions vary in outcome and selection among environments (Weis *et al.* 1985, Weis and Gorman 1990, Abrahamson *et al.* 1989, Weis *et al.* 1992).

When the behaviour of parasitized insects is modified such that their distributions differ from those of nonparasitized individuals, it is commonly assumed to result from movement by the former that is both directional and intentional (Stamp 1981, Brodeur and McNeil 1989, 1990, 1992). Evidence from my studies (Chapter 2) suggest that pathology-induced disorientation is an equally viable explanation for the re-distribution of aphids following parasitism by some species of aphidiid wasps. Modified behaviour in aphids parasitized by *E. californicus* was consistent with predictions from pathology or trauma hypotheses (Thompson 1983, Beckage 1985) and yet may still have adaptive value for this aphidiid species. Field experiments with a plant-aphid-*E. californicus* system would enable us to examine adaptive predictions over a range of biotic and abiotic factors and should be pursued in the future.

Changes in the behaviour of parasitized pea aphids in my studies were the result of the aphids' interaction with immature *E. californicus* (Chapter 2). Under certain conditions, these changes may benefit *E. californicus* (Chapter 4). There are a number of cases in the literature where parasitized insects experience lower mortality compared to unparasitized hosts, and the differences have been attributed to behavioural changes induced by the parasitoid (Fritiz 1982, Stamp 1981, Brodeur and McNeil 1992). Altered host behaviour that results in density-dependent dispersal of parasitized aphids may be adaptive for the primary parasitoid, if the risk of hyperparasitism increases with the density of parasitized aphids or mummies on a plant (Ayal and Green 1993; Weisser *et al.* 1994). However, parasitoids will not always be selected to change host behaviour to reduce predation or hyperparasitism. Godfray (1994) suggested that there are trade-offs between the risks of predation and other components of both host and parasitoid fitness; if the trade-offs are similar in magnitude, then behavioural manipulation by the parasitoid may not be favored. If the risk of mortality for parasitized aphids is greater if they move away from colonies or plants, parasitoids may benefit from minimizing traumatic effects on the host.

Most of the aphidiid species in my studies did not apparently alter the behaviour of their host aphid (Chapter 2). It is possible for altered behaviour to not benefit either partner and still not be strictly neutral. Altered behaviour may be neutral for the aphid, which is already dead and whose clone-mates may be uninfluenced by the behaviour, but deleterious for the parasitoid. In this case, natural selection would favor evolutionary changes in the parasitoid that would allow it to suppress host behaviour. Trade-offs or alternative strategies may explain why some aphidiid species do not apparently alter the behaviour of their hosts. Development may be faster if the parasitoid pupates within a host that dies on a plant site exposed to direct sunlight and thereby benefits from more rapid development because of higher temperatures. A reduction in pupal development time would reduce exposure time to predators or hyperparasitoids. Alternatively, some aphidiid species concentrate their attacks on aphid

colonies attended by ants (Mackauer and Völkl 1993). Since mutalistic ants protect the colonies from intruders, the immature aphidiids within these colonies may benefit from hyperparasitoid-free space created by these mutualistic associations. Factors such as the developmental time within the mummy, availability of refuges, and both the type and abundance of natural enemies will influence selection for modification of host behaviour by primary parasitoids.

Unique challenges may result in unique solutions by animals. Because of the obvious link between successful searching and the production of offspring, optimization hypotheses have been extensively used to explain the foraging and reproductive decisions of female parasitoids (van Alphen and Vet 1986). However, to determine the adaptive nature of parasitoid behaviour, we must first understand the context in which they forage. Optimization is context dependent and the most adaptive solution which the parasitoid can realize may be both unique and unexpected. Sex allocation and foraging behaviour of *D. carpenteri* are apparently influenced by this species' perception of a patch primarily on a 'temporal' and not a 'spatial' scale (Chapter 4, 5). The behaviour of this hyperparasitod evidently resulted from the unusal foraging challenges presented by its hosts.

Reduced antagonism or 'attenuation' can evolve in some associations either through increased defenses or through the evolution of traits that have less of a harmful effect on the fitness of the other species (Futuyma and Slatkin 1983, Thompson 1994). It is possible that through the process of reciprocal co-adaption, many associations of aphidiid wasps and their hyperparasitoids have reached a stable equilibrium. Some primary parasitoids may have undergone selection to alter the behaviour of their hosts or forage in a manner that reduces the risk of hyperparasitism to their progeny. In turn, *D. carpenteri* has apparently adopted foraging and reproductive strategies that enable it to 'keep up' with its hosts. Although hyperparasitoids can cause considerable mortality among aphidiids, their impact at the metapopulation level is probably limited by the low fecundity and long generation times of the former (Mackauer and Völkl 1993). Several

examples of successful biological control by aphidiid species in spite of significant hyperparasitism (van den Bosch *et al.* 1979; Hughes *et al.* 1987, Farrell and Stufkens 1990) provide evidence that hyperparasitoids have limited impact on aphid-aphidiid population dynamics. Further pursuit of tri-trophic level approaches in the study of interactions between insect parasitoids and their hosts should enhance our understanding of both behavioural and evolutionary ecology.

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