Static and Dynamic Criteria in Host Evaluation by Aphid Parasitoids (Hymenoptera: Aphidiidae)

by

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STATIC AND DYNAMIC CRITERIA IN HOST EVALUATION BY APHID PARASITOIDS (HYMENOPTERA: APHIDIIDAE)

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ABSTRACT

The host selection and foraging behaviour of seven species of aphid parasitoid (Hymenoptera: Aphidiidae) was studied in the laboratory (number of species varied among experiments): *Aphidius ervi*, *A. pisivorus*, *A. smithi*, *Ephedrus californicus*, *Lysiphlebus testaceipes*, *Monoctonus paulensis*, and *Praon pequodorum*. When provided with choices between two aphid species (*Acyrthosiphum pisum*, *Macrosiphum creelii*) and two colour forms (green and pink *M. creelii*), five of six parasitoid species distinguished between hosts on the basis of both colour and species. In darkness, host preference was unchanged in *A. ervi* and *M. paulensis*, disappeared in *A. pisivorus*, and was reversed in *P. pequodorum*. Host movement elicited attack by *Aphidius* species and *P. pequodorum*. *E. californicus* did not orient visually to hosts; rate of parasitization varied with aphid defensive behaviour. Antennal contact with aphid cuticle appeared to confirm host recognition in all species.

Rates of parasitism and superparasitism by *A. ervi* and *M. paulensis* varied with individual experiences. *A. ervi* females parasitized more preferred hosts (*A. pisum*) after encounters with a less-preferred host (*M. creelii*), whereas *M. paulensis* females accepted fewer *M. creelii* after encounters with *A. pisum*. Self superparasitism by *M. paulensis* females declined with egg load, but increased with mating and exposure to conspecifics. Patch residence time and number of hosts parasitized by virgin *M. paulensis* females increased with age and following encounters with parasitized hosts. Mating increased patch residence time and/or number of aphids parasitized by females of *A. smithi*, *E. californicus*, *M. paulensis* and *P. pequodorum*, but had no effect on *L testaceipes*.

DEDICATION

This work is dedicated to my parents, Roland and Barbara Michaud, for their lifelong support, and to my wife, Anastasia, for her patience and encouragement over the last four years.

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I wish to thank my supervisor, Dr. M. Mackauer, for his valued advice and guidance, for financial assistance, and most of all, for providing me with the opportunity to pursue my own ideas. I am also indebted to Drs. Peter Belton and Bernie Roitberg for their editorial suggestions and critiques which have helped improve this manuscript, and to Liping Wang who assisted me with many of the experiments in Chapter III. Finally, I must thank the various colleagues who have shared our laboratory over the past five years and provided many valuable and spirited discussions: Barry Bai, Amanda Chau, Andrew Chow, Troy Danyk, Ross Lardner, Richard Sequeira, and Wolfgang Völkl.

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Preface

This thesis has two main objectives: to determine the nature of the sensory information used by female aphidiids to recognize and assess host quality, and to identify factors which influence patterns of reproductive allocation by individual females to hosts and host patches.

Evolutionary ecology seeks to explain variation in behaviour among living organisms in the context of natural selection. We can often observe that individuals modify their behaviour so as to maximize their individual fitness (number of surviving offspring) in particular situations. Explanations of behaviour can be sought at either the proximate or ultimate level of causation (Tinbergen 1963). On the one hand, we may seek to identify which (unconditioned) stimuli elicit a particular response, and on the other, we may assess the potential fitness consequences or adaptive value of the resulting behaviour. It has been suggested that ethological analyses should proceed in mechanistic and adaptive contexts simultaneously (Smith 1993). Investigations of proximate and ultimate causation in ethology require the empiricist to ask 'How?' and 'Why?' questions, respectively. Traditionally, the comparative approach has studied variation in behaviour by comparing species or groups of individuals in the context of their phylogeny (Ratner 1980, Rosenheim 1993). For example, we might compare a number of species of parasitoid with respect to their average responses to a particular set of stimuli if we are interested in comparing species-specific attributes such as host preference. A comparison of average responses among populations or species may be useful for identifying phylogenetic or mechanistic constraints on behaviour, but a different approach is required to account for variation in foraging tactics among individuals. Instead of averaging out the 'noise' within groups to detect mean differences among them, we are now interested in the causes of differences in behaviour among individuals. Empirically, a careful manipulation of

environment or physiological state is required to observe possible effects on behaviour, a so-called 'state-variable' approach (Mangel 1989). The development of a true understanding of behaviour will require investigations at both the level of the individual and the species. I endeavour to examine the foraging behaviour of aphid parasitoids at both levels in this thesis.

Solitary parasitic wasps of the family Aphidiidae that occur in North America include indigenous and introduced species that are important biological control agents of aphids on a large variety of agricultural crops and ornamentals (Stary 1970). The efficacy of these species for biological control is determined by many factors. These include physical factors such as climate (Messenger 1969, Cohen & Mackauer 1987), population factors such as the functional and numerical responses of the parasitoid to its host (Huffaker *et al.* 1968, Huffaker 1969), and ecological factors such as plant architecture (Gardner & Dixon 1985, Andow & Prokrym 1990), ant-aphid mutualisms (Völkl & Mackauer 1993) and hyperparasitism (Ayal & Green 1993). However, it is the foraging behaviour of the female wasp as she seeks hosts for her offspring that determines local patterns of host utilization, *i.e.* rates of host discovery, which species are attacked and accepted, and the proportion of available hosts parasitized. Aside from the importance of this behaviour in biocontrol contexts, it provides an ideal opportunity to test theories of reproductive investment and ask questions such as "How many offspring should a female allocate to a particular host, or clump of hosts, in order to maximize her fitness?"

The classical approach considers host selection as a step-wise process that proximately determines how many available hosts are parasitized and, ultimately, the host range of a parasitoid species. This approach poses questions of a mechanistic nature about species-specific behaviours, *i.e.* "How do parasitoid females recognize and evaluate their hosts and what sensory information is used?" In the first chapter I examine the sensory

criteria used by various parasitoids to assess host quality and compare mechanisms of host recognition and acceptance across species. I refer to these sensory criteria as 'static', not because they do not change over evolutionary time, but because they are relatively invariant over the lifetime of individuals. In subsequent chapters I examine the effects of individual experiences and certain physiological variables as sources of dynamic variation in the foraging behaviour of individuals.

Modern approaches to foraging behaviour have postulated 'rules of thumb' which a female might use to make context-specific decisions. One such approach is to construct a stochastic-dynamic model of alternative behaviours, evaluate the fitness consequences, and solve backwards for the adaptive behaviour set in various circumstances (*e.g.* Mangel & Ludwig 1992). In such an *a priori* model the probability of a decision is assumed to be context-dependent and may vary with a female's recent experience, age, or egg load (Mangel & Roitberg 1989). Experiments are often carried out to test female responses across a range of physiological states or following different experiences. While the model and its assumptions can be refined following repeated comparisons with empirical data, the validity of this approach rests somewhat tenuously on the assumption that each individual acts to maximize its own fitness, *i.e.* the number of offspring surviving to reproductive age. My objective in this thesis is not to construct such a model, but rather to identify various extrinsic and intrinsic factors which may have dynamic influence on the foraging behaviour of female wasps, some of which may not have been previously recognized. This behaviour can be represented as a series of decisions, each of which is to some extent contingent on those preceding it. The effects of various dynamic factors on the probable outcomes of these decisions can then be examined.

Another way to analyze parasitoid behaviour is to test for covariance of events in continuous observations and infer causal

relationships *a posteriori*, *e.g.* Haccou *et al.* (1991). However, both this approach and the previous one require conceptual constructions based solely on biological inferences pertaining to ultimate causation. From an empirical perspective, the assumption that individuals behave in an adaptive manner (*i.e.* fitness-maximizing) may not be true for all individuals in a test group. While it is possible to test for the validity of particular inferences in a stochastic-dynamic model and refine it, there is always the risk of false positives, *i.e.* obtaining the right results for the wrong reasons. The same applies to *a posteriori* inferences based on analyses of covariance; spurious correlations may occur within the data that suggest causality where none exists.

The proximate- and ultimate-causation approaches can be complementary and together provide a more complete understanding of behaviour. In this thesis I draw on both of these approaches to test the how and why of parasitoid foraging behaviour, and to compare and contrast the dynamics of female decision-making among several species. I begin with the assumption that a female makes five important and discrete decisions: (1) To search for hosts, (2) To attack them, (3) To accept (oviposit), (4) To lay one or more eggs per host (superparasitism) and, (5) To leave the host patch. I examine various intrinsic and extrinsic factors that influence one or more of these decisions.

In the first chapter I examine factors influencing decisions (2) and (3) for several aphidiid species which share a common host, the pea aphid, *Acyrthosiphum pisum* Harris, but which are also capable of development in an alternate host, the alfalfa aphid, *Macrosiphum creelii* Davis. Besides sharing host species, these parasitoids were readily available to me and were easily reared in the laboratory. I ask the question "What sensory cues influence host recognition and the probability of attack and oviposition?". Reviews of the literature on parasitoid host selection (Vinson 1976, 1985) reveal a research emphasis on the role of chemical cues in mediating this process. It is clear that odours of the host

complex, particularily honeydew, are involved in host location by the Aphidiidae (Bouchard & Cloutier 1984, Powell & Zhang 1984, Ayal 1987, Cloutier & Bauduin 1990). I will suggest that, following an encounter with a host, female aphidiids frequently evaluate its visual characteristics first, and that appropriate visual stimuli may be neccessary to elicit an attack and initiate the chain of events that culminates in oviposition.

At a local level, patterns of host utilization are largely determined by the behaviour of female parasitoids once they encounter a plant infested with aphids. However, many aphidiid females frequently leave host patches before all hosts have been parasitized (Mackauer & Völkl 1993). In Chapter Two, I examine some factors influencing decisions (1), (4) and (5) and ask the questions "Do female aphidiids evaluate hosts and host patches relative to those they have previously encountered?" and "Do females quantitatively adjust their reproductive investment in individual hosts, or host patches, as a result of experience?" For these experiments I selected *Aphidius ervi* Haliday and *Monoctonus paulensis* (Ashmead). Preliminary experiments revealed that these two species express a consistent preference for pea aphid over alfalfa aphid and are capable of discriminating conspecifically parasitized hosts. I observe the oviposition behaviour of females as they forage sequentially in patches of high and low quality which I create using preferred *vs.* less-preferred host species and unparasitized *vs.* previously-parasitized aphids.

In Chapter Three I ask the question "What state variables and/or adult experiences influence the oviposition tactics of *M. paulensis*?" A preliminary experiment revealed that this species was unusual in that females self-superparasitized many hosts during a single attack. Previous work has shown the potential importance of factors such as female age (Weisser 1993), egg load (Rosenheim & Rosen 1991) and conspecific encounter (Visser *et al.* 1992b) in influencing parasitoid foraging behaviour and patterns

of reproductive allocation. I test the influence of age, egg load, host density, mating status, and selected adult experiences (contact with conspecific females and the hosts they have parasitized) on patterns of progeny allocation by this parasitoid (decisions 4 & 6). I argue that mating increases the relative value of hosts and host patches to female parasitoids because it enables them to produce daughters. I test the generality of this hypothesis in Chapter Four by examining five of the available species for differences in foraging behaviour between virgin and mated females. I conclude with a summary of the decisions made by females within host patches in which I identify the various sensory cues used to assess host quality (static criteria), and the various state-variables that influence host value and patch value (dynamic criteria).

Chapter I

The Role of Visual Cues in Host Evaluation by Aphidiid Wasps

INTRODUCTION

The process whereby female parasitoids encounter and parasitize their hosts has been termed 'Host Selection' (Salt 1935, 1937, Doutt 1959, Vinson 1976) and subdivided into a series of discrete steps that are thought to occur in a specific sequence. A typical parasitoid is presumed to seek the habitat of its host first (Host Habitat Location) and then the host itself (Host Location). Following an encounter with the host, an egg may or may not be laid (Host Acceptance). The egg, in turn, may or may not survive and develop to produce a viable adult (Host Suitability) (Salt 1938).

From a behavioral perspective, the term 'host selection' is perhaps misleading as it could imply that a host is selected from an array of simultaneously available alternatives, whereas females encounter hosts one at a time and make independent decisions to accept or reject them. Patterns of host utilization by female parasitoids are generated by differential rates of encounter, recognition, acceptance, and survival among available host species. I prefer the term 'Host Recognition' to 'Host Location' since the former refers explicitly to a threshold neurological event that results in a change in behaviour. Empirically, host recognition can be determined as the point where searching behaviour ceases and other behaviours are initiated which function to assess host quality. I use the term 'Host Evaluation' to refer to the series of behaviours which begin with host recognition and culminate in either acceptance or rejection.

Members of the family Aphidiidae are exclusively solitary parasitoids of aphids (Mackauer & Stary 1967). Most aphidiid species are recorded from a small number of host species in related genera, or species sharing a particular habitat or host plant. Mackauer (1965) suggested that a phylogenetic history of association with particular aphid species may be one factor determining the observed selectivity of aphidiid wasps. Host

location by these wasps, at the scale of orientation to infested plants, appears to be guided primarily by the odour of aphid honeydew (Bouchard & Cloutier 1985, Cloutier & Bauduin 1990). However, it seems unlikely that host specificity is determined at this level; the honeydew of non-host aphids, or those reared on artificial diets, may be equally attractive to foraging aphidiids (Budenburg 1990). Honeydew may also comprise a food source and orientation to its odour may therefore occur for reasons other than host location. Nevertheless, the possibility remains that the olfactory profile of some host complexes may be more attractive than that of others. Once an infested plant is discovered, aphidiid females probably search for hosts at random (Hafez 1961, Li *et al.* 1992) although they sometimes follow trails of honeydew (Ayal 1987). It therefore seems likely that host specificity is largely a function of female responses to potential hosts once they have been encountered. In this chapter I test the hypothesis that differential patterns of host utilization may arise from species-specific responses to host stimuli evaluated at close range, *i.e.* following recognition of the host.

Host evaluation has various components and may involve assessment of visual and chemical cues, both before and during attack. There are three distinguishable stages in this process: recognition, attack, and acceptance. Aphids may be recognized visually or by antennal contact, whereupon female searching behaviour ceases. Other behaviours are then initiated to assess the quality of the host. I define an 'attack' as a strike, or probe, with the ovipositor that makes contact with the host. An attack may result in either host acceptance (oviposition) or rejection. Visual cues may be evaluated prior to attack, external chemical cues during antennation of the host cuticle, and internal chemical cues during ovipositor probing.

Salt (1937) suggested that sight was involved in host recognition by *Trichogramma* spp. that parasitize the eggs of Lepidoptera. Griffiths (1960) provides a detailed description of

visual orientation to hosts by *Monoctonus paludum* Marshall (= *M. crepidis*) from which he infers visual perception of size and shape in this genus. Preferences for particular host instars are common among aphidiid wasps (Mackauer 1973, Liu *et al.* 1984, Sequeira & Mackauer 1987, Völkl 1991) and suggest perception of size and shape is widespread in this family. However, research on host recognition by aphidiid wasps has emphasized the role of chemical cues emanating from the host (Singh & Sinha 1982a, Powell & Zhang 1983, Srivastiva & Singh 1988, Hardie *et al.* 1991) or the host complex (plant plus honeydew) (Read *et al.* 1971, Vater 1971, Tamaki *et al.* 1981, Bouchard & Cloutier 1984). However, host location may be partly guided by visual cues (Mackauer 1965, Vater 1971, Goff & Nault 1984) and vision may also play a role in host recognition and evaluation. Manipulation or handling of the host during an attack may incur costs in terms of time, energy, and even risk of mortality, whereas a preliminary assessment of visual cues can be accomplished without these risks (Gerling *et al.* 1990).

I examined the importance of visual cues in host evaluation using six species of wasps from four aphidiid genera and three kinds of hosts reared on the same plant species: pea aphid, *Acyrthosiphum pisum* (Harris), and two colour morphs of the alfalfa aphid, *Macrosiphum creelii* Davis. These aphids represented both a phenotypic contrast (colour), and a genotypic contrast (relatedness). Relative rates of host examination and attack were observed in choice situations and the influence of host colour and movement on the probability of attack and oviposition was assessed. Ideally, one would wish to construct a visual model of the host containing all stimuli neccessary to elicit an attack response, then subtract sensory elements of the model individually to determine their relative importance. However, practical constraints forced me to adopt more indirect approaches, such as using darkness and carbon dioxide to selectively limit the sensory perception of parasitoids.

A classical comparative approach would have included a phylogenetic analysis of species relatedness (Ratner 1980), so that homologies with respect to host evaluation traits could be compared with the phylogeny of the family (Rosenheim 1993). A phylogeny would enable one to distinguish ancestral traits from derived ones and possibly detect convergence with respect to the sensory information utilized in host selection in particular ecological contexts. However, there is still considerable disagreement among taxonomists with regard to the organization of this family (*e.g.* Mackauer 1961, Mackauer & Stary 1967, Capek 1970, Finlayson 1985) and a reliable phylogeny is not yet available. My comparisons of host selection behaviour are therefore interpreted primarily in the contexts of life history and ecology, rather than phylogeny.

MATERIALS AND METHODS

Insect Colonies

Colonies of the various parasitoid species (Hymenoptera: Aphidiidae) were established from material collected in coastal British Columbia and the southern interior of the province. Colonies of *A. ervi*, *A. pisivorus* Smith, and *A. smithi* Sharma & Subba Rao, were started from material collected from pea aphid, *A. pisum*, on alfalfa, *Medicago sativa* (L), at various locations in the interior of British Columbia. *Ephedrus californicus* Baker was collected from *Macrosiphum albifrons* Essig on lupine, *Lupinus sp.* in Burnaby, B. C.; *M. paulensis* from individuals parasitizing pea aphid on broad bean, *Vicia faba* L., in Burnaby, B.C.; and *Praon pequodorum* Viereck from individuals parasitizing pea aphid on alfalfa near Kamloops, B.C. All parasitoids were reared on pea aphid.

A. pisivorus is indigenous to North America, whereas *A. ervi* and *A. smithi* were introduced as biological control agents from Europe and India, respectively (Mackauer & Stary 1967, Mackauer 1971, Gonzalez *et al.* 1978). Both *A. ervi* and *A. pisivorus* have been recorded as parasitoids of the alfalfa aphid in parts of North America (Halfhill *et al.* 1972, A. Chow unpublished). However, laboratory tests indicated that these parasitoids 'prefer' the pea aphid over the pink form of alfalfa aphid; *i.e.* more of the former are parasitized when both are equally available (Chow & Mackauer 1991, 1992). In contrast, *A. smithi* is not known to parasitize the alfalfa aphid in the field, although it may accept it in the laboratory (Chow & Mackauer 1992). *P. pequodorum* is a common indigenous parasitoid of pea aphid, whereas *E. californicus* and *M. paulensis* are rarely found on this host in British Columbia but readily accept it for oviposition in the laboratory.

All parasitoid species were reared in growth chambers at 20 ± 1° C, 50-60% relative humidity, and under continuous light. Although a diurnal cycle of light-dark might have served to synchronize the endogenous activity rhythms of individual females, it is impossible to test all females at the same time in a given experiment. In the absence of information on the effects of various daylengths on the activity of females, it was decided to control for any effect of daylength by rearing under continuous light, despite the fact that free-running endogenous cycles might increase variation in responses among females. All aphid colonies were maintained on potted broad bean, *Vicia faba* L. cv. 'Broad Windsor' under the same conditions as the parasitoids. In addition to pea aphid, colonies of pink and green colour morphs of *Macrosiphum creelii* Davis were established. The pink morph was collected on alfalfa near Kamloops, British Columbia, and the green morph on vetch, *Lathyrus japonicus* Willd. var. glaber (Ser.) Fern., in Vancouver, British Columbia. Both forms have been reared successfully on broad bean for several years.

All aphids used as hosts in experiments were in the late second instar (72 ± 4 h old at 20° C). Second nymphal instars of pea and alfalfa aphid are approximately equally susceptible to attack by all three *Aphidius* species (Chow & Mackauer 1991). Parasitoids emerged in synchronous mixed colonies and females were used in experiments when they were 3 to 5 days old without previous exposure to aphids.

Host Examination and Attack

The behaviour of individual parasitoid females was observed as they examined and attacked aphids a plastic petri dish (4.0 cm in diameter x 1.0 cm in height). Single females were placed in a dish containing 10 aphids, five of each kind (pea aphid *vs.* green alfalfa aphid; pea aphid *vs.* pink alfalfa aphid; and green *vs.* pink alfalfa aphid), and observed continuously as they encountered and attacked aphids. A petri dish was selected as the experimental arena in lieu of a plant in order to facilitate

manipulation of the insects and to control for plant effects and differences in aphid dropping behaviour (Chow 1989). This was done because I was more interested in observing the intrinsic preference of females for a particular aphid than I was in replicating the natural situation. Aphids of all three types tend to wander around in a petri dish and are presumably encountered with equal probability by a foraging female. An examination was counted as any encounter with an aphid that resulted in a change in parasitoid behaviour, such as arrested movement, apparent visual inspection, or antennation. An examined aphid was immediately removed and replaced with another of the same kind, whether or not it was attacked. While it is possible that some rejected aphids would have been attacked during subsequent encounters, it was deemed more important to prevent the possible accumulation of rejected aphids within the arena and to maintain equal numbers of the two host types available at all times. The number of examined aphids of each kind that were attacked, *i.e.* probed with the ovipositor, was also tallied. There was no discernable indication that the behaviour of females was in any way influenced by manipulation of hosts within the arena. Each female was permitted a total of 20 attacks within a period of 40 min; replicates in which the female failed to complete 20 strikes within this period were excluded from the analysis. Twelve replicates were performed in each test for all species except *E. californicus* where the number was 10.

Host Acceptance

Because an attack by a female may or may not result in oviposition, I designed another series of experiments to assess species-specific patterns of host acceptance among parasitoids. Experiments were performed under both light and dark conditions. A different pattern of host acceptance in the dark compared to in the light would indicate that host preference is some function of responses to visual cues. Under lighted conditions (Phillips "Cool White" fluorescent bulbs), each female

was placed in a petri dish (6.0 cm x 1.5 cm) containing 15 of each of two kinds of hosts for 40 min (*A. ervi*, *A. pisivorus*, *A. smithi* and *E. californicus*) or 60 min (*M. paulensis* and *P. pequodorum*); attacked aphids were not replaced. Under dark conditions, the same procedure as above was used except that the petri dishes (4.0 cm x 1.0 cm) were placed in complete darkness for 3 h. Since the activity of parasitoid females is reduced in the dark, the same number of hosts were presented in a smaller arena for a longer period in order to obtain adequate levels of parasitism. No dark experiments were performed with *E. californicus* as this species gave no indication of visual orientation to hosts. The time intervals and numbers of hosts were selected to provide optimum resolution of preference under the particular conditions; in all replicates (30 in each test) sufficient hosts were parasitized to resolve any preference, but the preferred host type was never exhausted (Mackauer 1983). Aphids from each replicate were caged on a bean shoot in a screened plastic mini-cage. After four days I selected 20 aphids (10 of each kind of host) from each replicate and dissected them to count the number of parasitoid eggs and larvae they contained. This enabled me to dissect a standardized sample from each replicate and exclude the occasional dead aphid. Replicates that did not contain any parasitized aphids were excluded from the analysis.

Host Movement

Aphid behaviour may influence the success of a parasitoid's attack (Gardner *et al.* 1984, Gerling *et al.* 1990) and host acceptability (Mackauer & Chow, 1990, Kouamé & Mackauer, 1991). I therefore evaluated the importance of aphid movement in eliciting attack by parasitoid females and its influence on oviposition rates and host preferences. To determine if parasitoid females would examine and attack immobile and 'normal' pea aphids at similar rates, 'no-choice' experiments were carried out using females of *A. ervi*, *A. pisivorus*, *A. smithi*, and *E. californicus*. One group of females was each provided with 10 normal pea

aphids in a petri dish (4.0 cm x 1.0 cm) whereas another group each received 10 aphids anaesthetized with a 5 min exposure to carbon dioxide. An exposure of this duration is sufficient to render second-instar pea aphids immobile for 30-40 min. I observed parasitoids continuously and counted the number of examinations and attacks; each examined aphid was replaced immediately with another of the same kind, whether or not it was attacked. Females were permitted to forage for a fixed length of time: *A. ervi* and *A. smithi* for 15 min (10 and 12 replicates, respectively), and *A. pisivorus* and *E. californicus* for 20 min (15 and 10 replicates respectively).

I reasoned that if immobile aphids were less acceptable than moving aphids, fewer of them would be parasitized in a fixed period of foraging. Alternatively, if aphid defensive behaviour was a factor limiting foraging efficiency, more immobile than moving aphids would be parasitized. Therefore females of *A. ervi*, *A. smithi*, *E. californicus*, *M. paulensis* and *P. pequodorum* were divided into two groups of 20 females for each species to determine rates of oviposition into anaesthetized pea aphids. Females of one group were confined individually with 16 normal pea aphids in a petri dish (6.0 x 1.5 cm), while their counterparts received 16 anaesthetized aphids. Females of *E. californicus* were confined for 20 min, all other species, for 30 min. After 4 days of rearing I dissected a sample of 10 aphids from each replicate.

To test whether host preference was influenced by differences in behaviour between aphid species, I gave *A. ervi*, *E. californicus*, *M. paulensis* and *P. pequodorum* females a choice between pea aphids and pink alfalfa aphids that had been anaesthetized. I reasoned that if host preference were a function of differences in behaviour among the various aphid species, parasitoid preference would disappear when aphids were unable to move or defend themselves. Females were each provided with 8 pea aphids and 8 pink alfalfa aphids in a petri dish (6.0 cm x 1.5

cm) for 20 min. After four days of rearing 10 aphids (5 of each kind) were dissected from each replicate.

Since *M. paulensis* preferred green alfalfa aphid over pink, even in the dark, I designed a separate experiment to determine whether females of this species were responding to chemical differences between green and pink alfalfa aphid. Alternatively, preference for the former strain might be a result of differences in defensive behaviour which would disappear if hosts were immobilized. In order to control for the difference in coloration between the two forms, the experiment had to be performed in the dark. I therefore performed a dark choice test using CO_2-treated aphids to eliminate responses to both colour and aphid behaviour at the same time. Individual parasitoid females were confined in plastic petri dishes (4.0 cm x 1 cm) containing 8 green *M. creelii* and 8 pink *M. creelii* that had received a five minute treatment with CO_2. Upon introduction of the female, each dish was immediately placed in complete darkness. After 30 min, the female was removed so that the aphids could receive an additional 5 min treatment with CO_2 to keep them immobile. Following this, each female was returned to her respective arena for another 30 min in darkness. Subsamples of 10 aphids from each replicate (5 of each type) were dissected after rearing for four days.

Of all the species examined, *P. pequodorum* appeared most reticent to attack aphids which did not move. However, host acceptance may be influenced by the visual perception of host movement, or the tactile perception of the host struggling during an attack. A 'no-choice' oviposition test was performed under dark conditions with *P. pequodorum* using CO_2-treated pea aphid versus normal pea aphid to answer this question. Forty females were divided randomly into 2 groups of 20; individuals of one group were each confined with 16 CO_2-treated pea aphids, while those of the other group received 16 normal pea aphids. All dishes were immediately placed in the dark. Each replicate was

reared for 4 days after which 10 aphids were selected randomly from each and dissected.

A further oviposition experiment was performed with *P. pequodorum* in order to test whether visual cues other than host coloration were influencing host preference. Individual females were confined with 30 aphids each (15 green alfalfa aphid and 15 pink alfalfa aphid) for a period of 90 min under red light produced by a 100 watt incandescent bulb. These conditions were selected in an attempt to negate colour perception while permitting females access to other visual cues such as size and shape. Each replicate was reared for 4 days on an individual bean shoot after 20 aphids (10 of each type) were dissected.

Statistical Analysis

A *t*-test for paired comparisons (Sokal & Rohlf 1981, p. 356) was used to determine the statistical significance of differences in the numbers of aphids examined and parasitized. Pooling over all replicates within an experiment, the conditional probabilities of attack given examination was compared among the different kinds of hosts using the *G*-test of independence with Williams' correction (Sokal & Rohlf 1981, p. 735). All other data were analyzed by one-way ANOVA.

RESULTS

Host Examination and Attack

The mean numbers of hosts examined and attacked (± SE) by females of *A. ervi*, *A. pisivorus*, and *A. smithi* are shown in Figs 1.0, 1.1, and 1.2 respectively. All three species examined similar numbers of pea aphid and green alfalfa aphid (*A. ervi*: $t = 1.383$, $P = 0.197$; *A. pisivorus*: $t = 0.541$, $P = 0.603$; *A. smithi*: $t = 0.852$, $P = 0.411$). However, in all cases more pea aphids were examined than pink alfalfa aphids (*A. ervi*: $t = 7.059$, $P = 0.001$; *A. pisivorus*: $t = 4.662$, $P < 0.001$; *A. smithi*: $t = 7.621$, $P < 0.001$) and more green than pink alfalfa aphids (*A. ervi*: $t = 7.761$, $P < 0.001$; *A. pisivorus*: $t = 6.188$, $P < 0.001$; *A. smithi*: $t = 4.665$, $P = 0.001$, $n = 10$).

Females of *A. ervi* and *A. pisivorus* attacked pea aphids and green alfalfa aphids with equal probability (*A. ervi*: $G_W = 0.029$, $P = 0.862$; *A. pisivorus*: $G_W = 0.001$, $P = 0.969$), but *A. smithi* females attacked fewer green alfalfa aphids than pea aphids following examination ($G_W = 31.21$, $P < 0.001$). All three parasitoids attacked more examined pea aphids than pink alfalfa aphids (*A. ervi*: $G_W = 86.33$, $P < 0.001$; *A. pisivorus*: $G_W = 80.79$, $P < 0.001$; *A. smithi*: $G_W = 111.86$, $P < 0.001$) and a larger proportion of examined green than pink alfalfa aphids (*A. ervi*: $G_W = 123.81$, $P < 0.001$; *A. pisivorus*: $G_W = 62.81$, $P < 0.001$; *A. smithi*: $G_W = 115.86$, $P < 0.001$).

The mean numbers of hosts examined and attacked by *E. californicus* are shown in Fig 1.3. Females of this species examined similar numbers of pea and green alfalfa aphids ($t = 0.100$, $P = 0.917$), pea and pink alfalfa aphids ($t = 0.171$, $P = 0.867$), and green and pink alfalfa aphid ($t = 0.332$, $P = 0.747$). There were no differences among host types in the probability of attack following examination.

Figure 1.0. Mean number of aphids examined and attacked (+ SE) by *A. ervi* females when offered equal numbers of two kinds of hosts simultaneously in plastic petri dishes. (a) pea aphid (Ap) vs green alfalfa aphid (gM), (b) pea aphid vs pink alfalfa aphid (pM), and (c) green vs pink alfalfa aphid.

Figure 1.0: *Aphidius ervi*

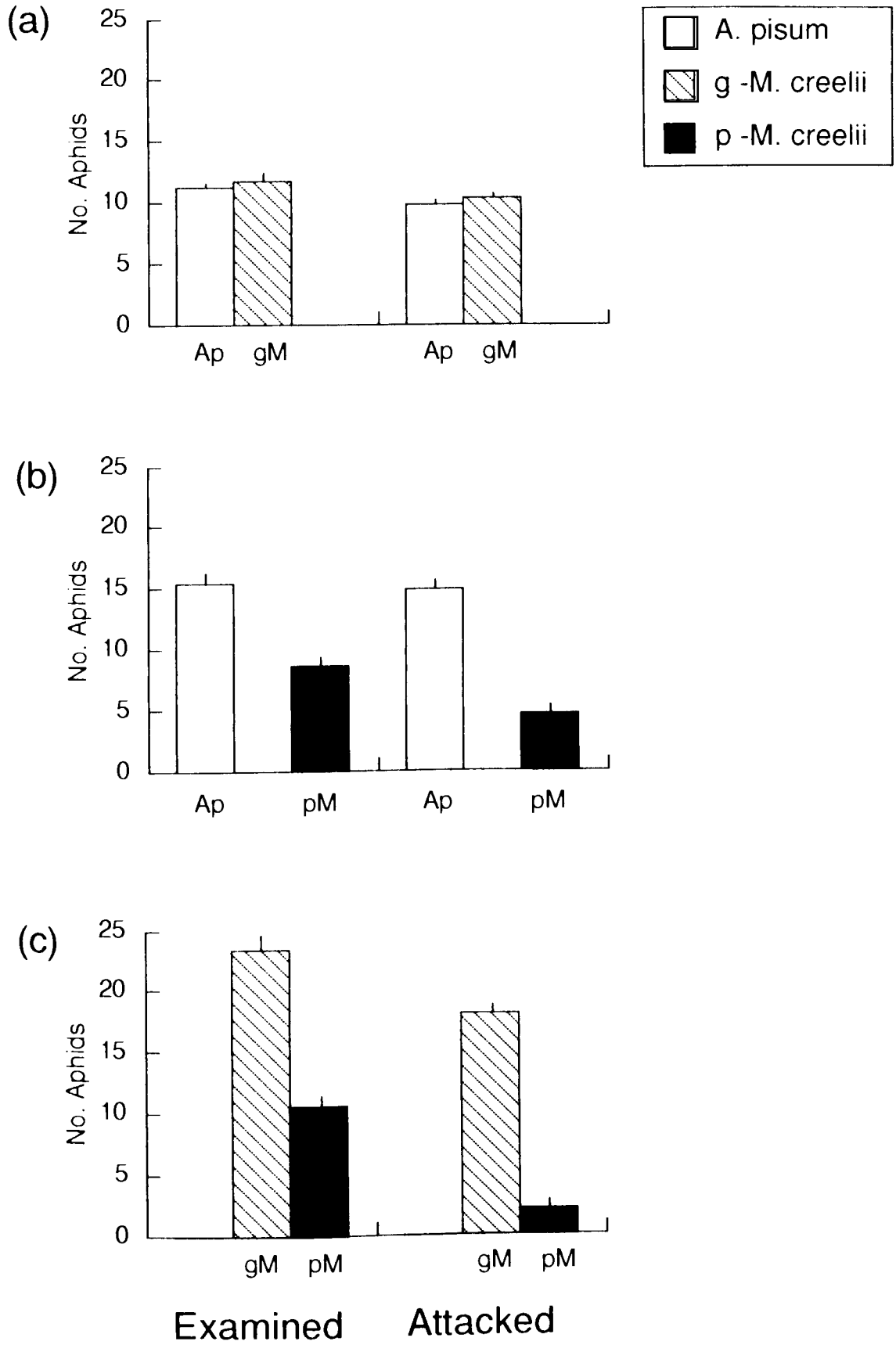


Figure 1.1. Mean number of aphids examined and attacked (+ SE) by *A. pisivorus* females when offered equal numbers of two kinds of hosts simultaneously in plastic petri dishes. (a) pea aphid (Ap) vs green alfalfa aphid (gM), (b) pea aphid vs pink alfalfa aphid (pM), and (c) green vs pink alfalfa aphid.

Figure 1.1: *Aphidius pisivorus*

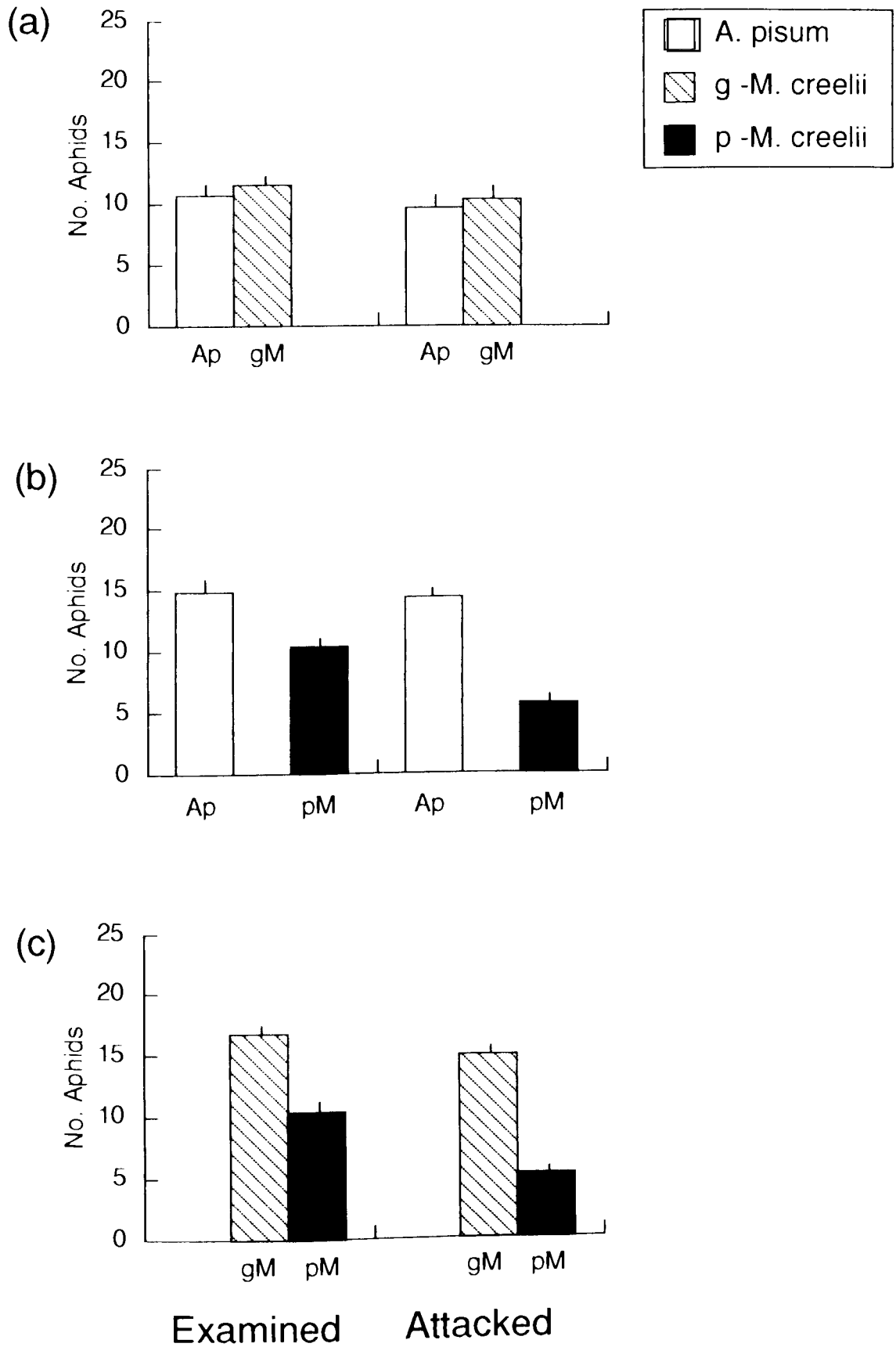


Figure 1.2. Mean number of aphids examined and attacked (+ SE) by *A. smithi* females when offered equal numbers of two kinds of hosts simultaneously in plastic petri dishes. (a) pea aphid (Ap) vs green alfalfa aphid (gM), (b) pea aphid vs pink alfalfa aphid (pM), and (c) green vs pink alfalfa aphid.

Figure 1.2: *Aphidius smithi*

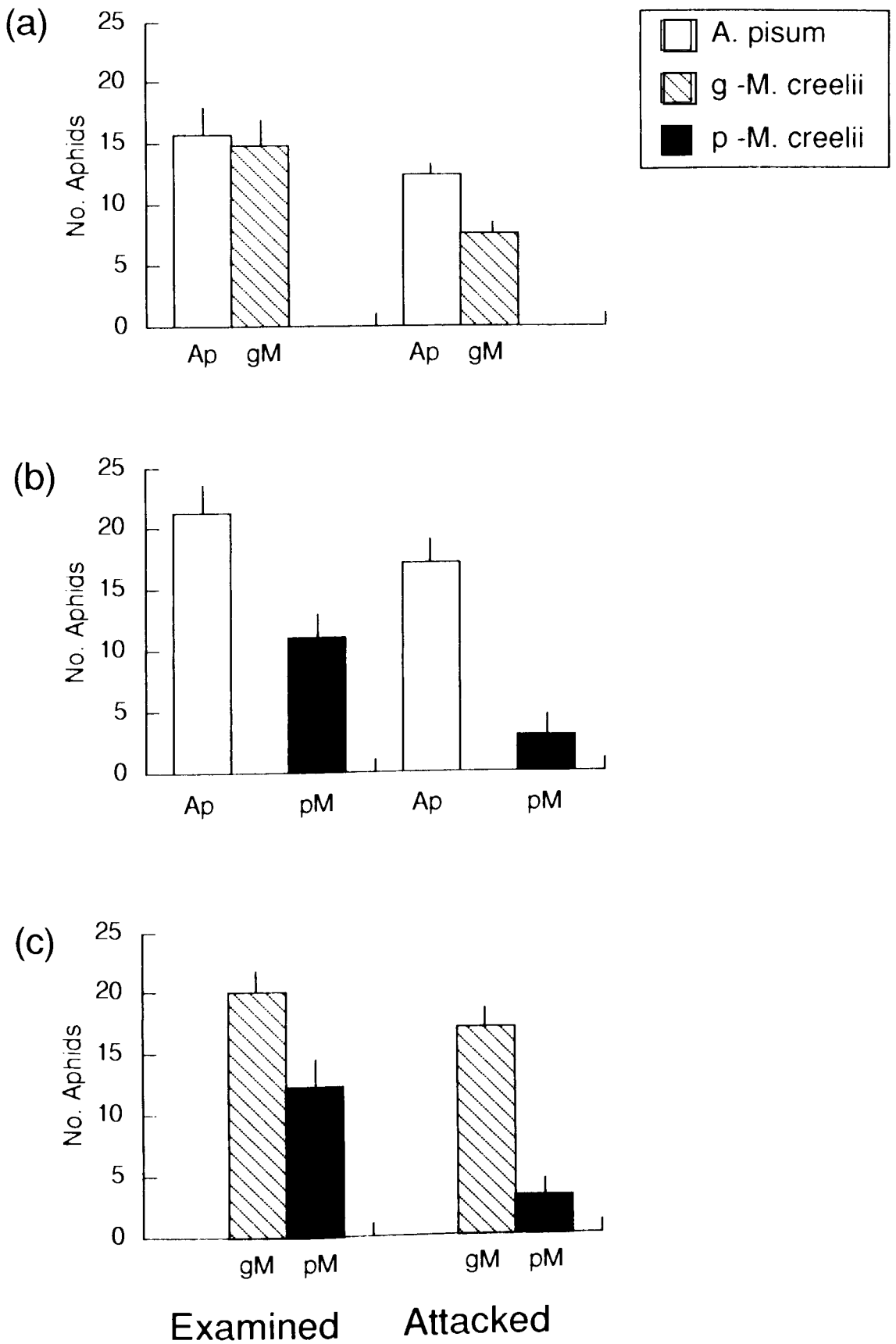
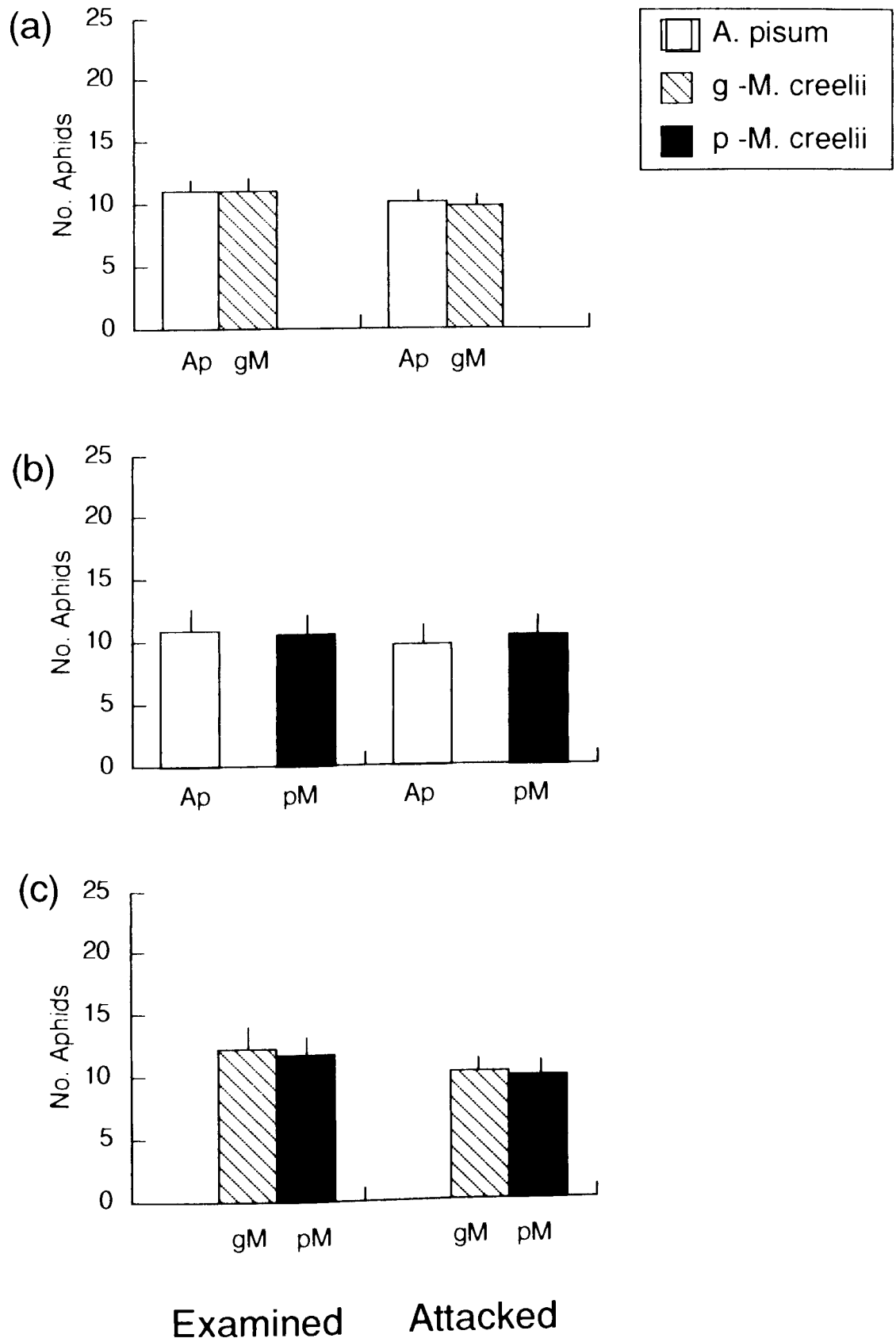


Figure 1.3. Mean number of aphids examined and attacked (+ SE) by *E. californicus* females when offered equal numbers of two kinds of hosts simultaneously in plastic petri dishes. (a) pea aphid (Ap) vs green alfalfa aphid (gM), (b) pea aphid vs pink alfalfa aphid (pM), and (c) green vs pink alfalfa aphid.

Figure 1.3: *Ephedrus californicus*



The mean numbers of aphids examined and attacked by *M. paulensis* are depicted in Fig 1.4. *M. paulensis* females examined more pea than pink alfalfa aphids (t = 10.200, P < 0.001) and more green than pink alfalfa aphids (t = 21.655, P < 0.001), with no apparent preference for examining either type of green aphid (t = 0.484, P = 0.638). Females attacked a smaller proportion of pink alfalfa aphids examined than either pea (G_W = 71.065, P < 0.001) or green alfalfa aphids (G_W = 132.831, P < 0.001). Pea and green alfalfa aphids were attacked with equal probability after examination (G_W = 2.274, P = 0.132).

The mean numbers of aphids examined and attacked by *P. pequodorum* are depicted in Fig 1.5. When given a choice between pea aphid and pink alfalfa aphid, *P. pequodorum* females examined more pea aphids (t = 4.780, P = 0.001), but did not distinguish between pea aphid and green alfalfa aphid (t = 0.732, P = 0.480). Females attacked green alfalfa aphids more often than pink (t = 4.643, P = 0.001), but the difference in number of examinations was not significant (t = 2.015, P = 0.069). A larger proportion of examined pea aphids and green alfalfa aphids were attacked than were pink alfalfa aphids (G_W = 43.166, P < 0.001 and G_W = 46.241, P < 0.001 respectively), with no difference between pea and green alfalfa aphids (G_W = 0.598, P = 0.439).

Host Acceptance

When foraging in the light, females of *A. ervi* (n = 26) parasitized more pea than pink alfalfa aphids (Fig 1.6b) (t = 12.776, P < 0.001) and more green than pink alfalfa aphids (Fig 1.6c) (t = 6.758, P < 0.001). The same pattern was evident in *A. pisivorus* (Fig 1.7b, 1.7c) (n = 21, t = 7.262, P < 0.001 and n = 25, t = 4.961, P < 0.001, respectively). *A. ervi* females (n = 25) also parasitized more pea than green alfalfa aphids (Fig 1.6a) (t = 7.709, P < 0.001), whereas *A. pisivorus* females (n = 25) parasitized similar numbers of each (Fig 1.7a) (t = 0.458, ns).

Figure 1.4. Mean number of aphids examined and attacked (+ SE) by *M. paulensis* females when offered equal numbers of two kinds of hosts simultaneously in plastic petri dishes. (a) pea aphid (Ap) vs green alfalfa aphid (gM), (b) pea aphid vs pink alfalfa aphid (pM), and (c) green vs pink alfalfa aphid.

Figure 1.4: *Monoctonus paulensis*

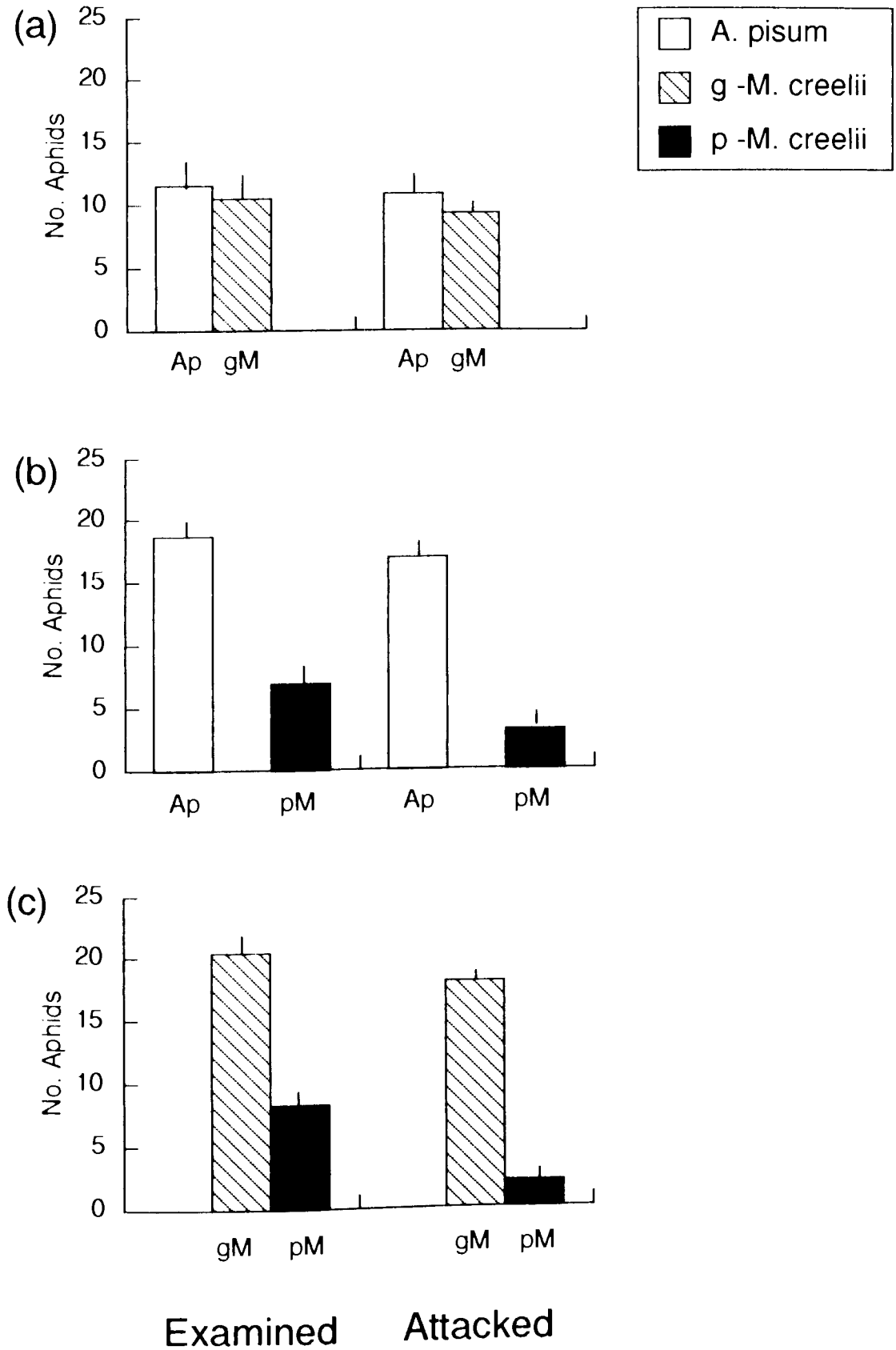


Figure 1.5. Mean number of aphids examined and attacked (+ SE) by *P. pequodorum* females when offered equal numbers of two kinds of hosts simultaneously in plastic petri dishes. (a) pea aphid (Ap) vs green alfalfa aphid (gM), (b) pea aphid vs pink alfalfa aphid (pM), and (c) green vs pink alfalfa aphid.

Figure 1.5: *Praon pequodorum*

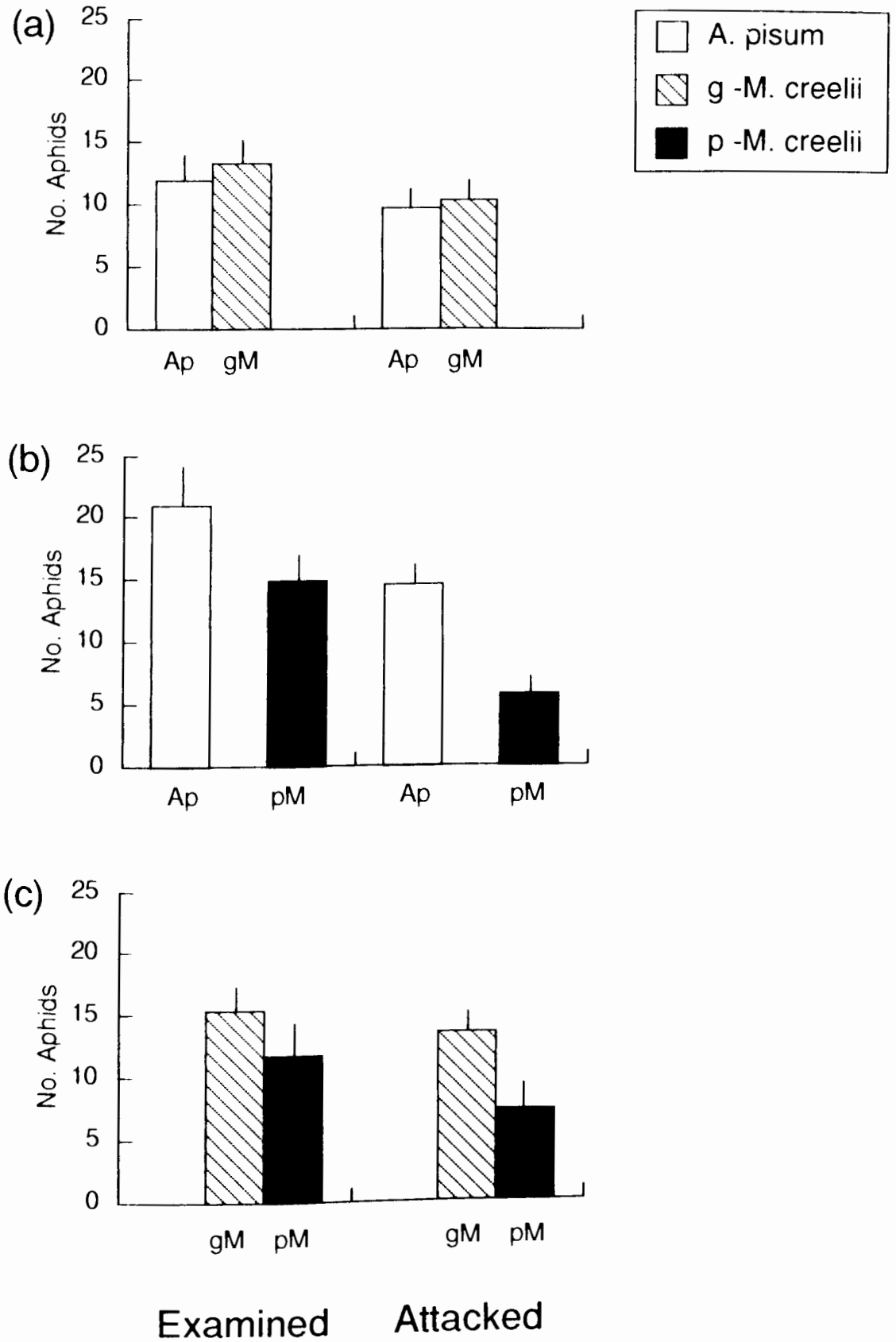


Figure 1.6. Mean numbers of aphids parasitized (+ SE) by females of *A. ervi* caged with 15 of each of two kinds of hosts in both light and dark conditions. (a) pea aphid (A. pisum) vs green alfalfa aphid (g -M. creelii), (b) pea aphid vs pink alfalfa aphid (p -M. creelii), and (c) green vs pink alfalfa aphid.

Figure 1.6: *Aphidius ervi*

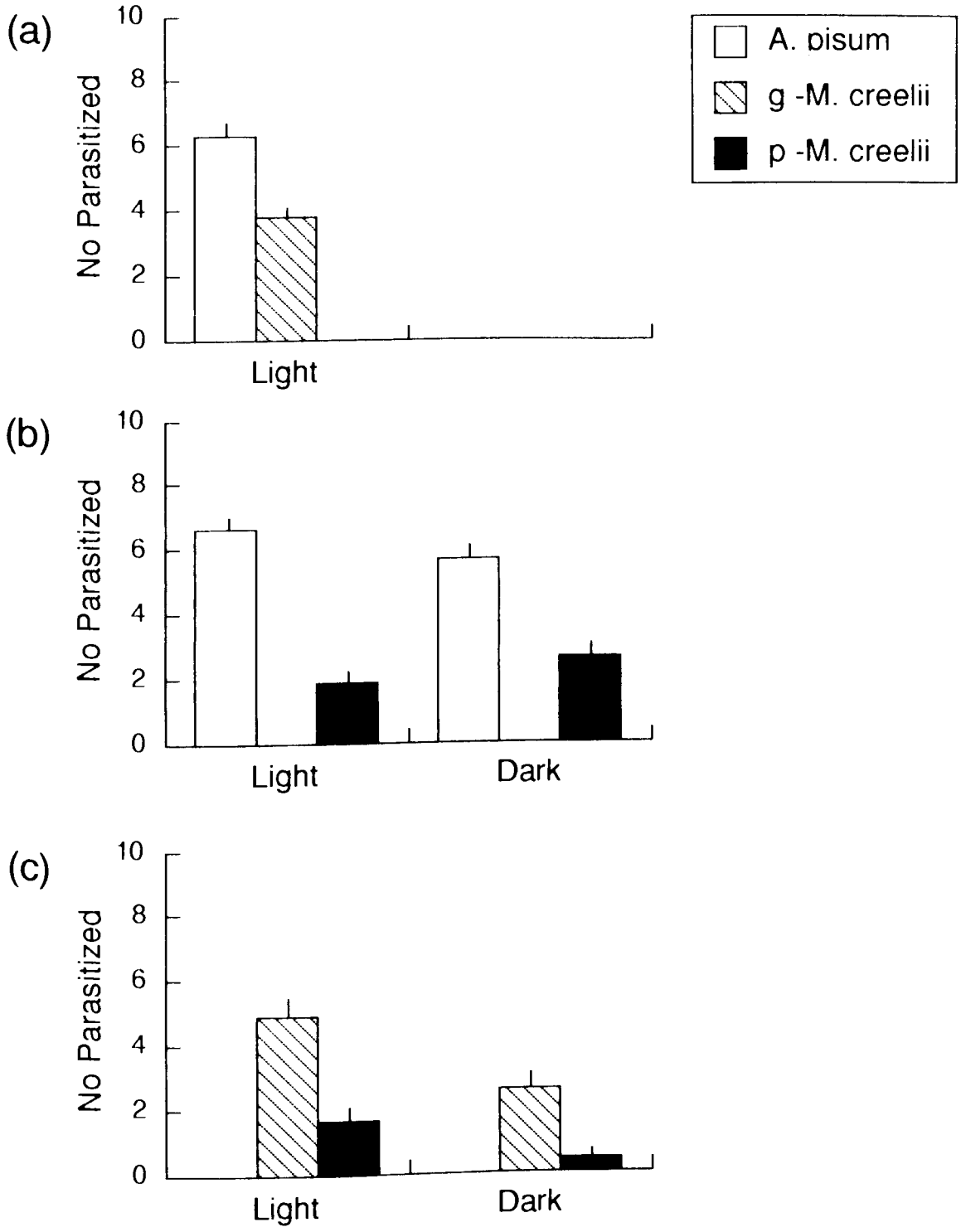
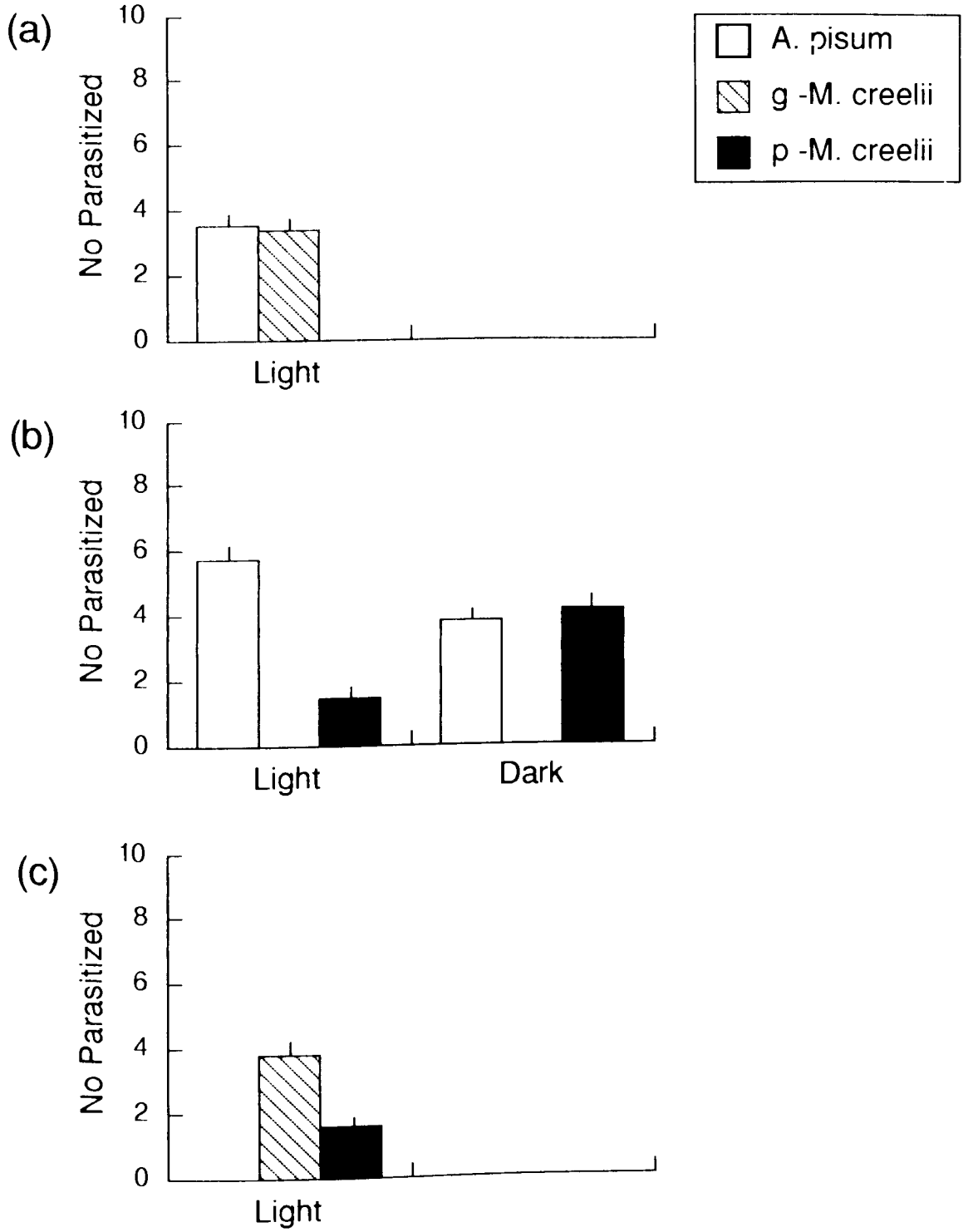


Figure 1.7. Mean numbers of aphids parasitized (+ SE) by females of *A. pisivorus* caged with 15 of each of two kinds of hosts in both light and dark conditions. (a) pea aphid (A. pisum) vs green alfalfa aphid (g -M. creelii), (b) pea aphid vs pink alfalfa aphid (p -M. creelii), and (c) green vs pink alfalfa aphid (dark experiment not performed).

Figure 1.7: *Aphidius pisivorus*



Dissection of pea (n = 198) and pink alfalfa aphids (n = 87) attacked by *A. pisivorus* females in choice tests revealed that alfalfa aphids were less likely to be accepted following attack than pea aphids (69.7% *vs* 54.0%; G_W = 6.352, P = 0.010). The total numbers of aphids parasitized by *A. ervi* was higher in the two tests that included pea aphids than in the one without them (F = 6.287, df = 2,70, P = 0.003).

In the dark, *A. ervi* also parasitized more pea than pink alfalfa aphids (Fig 1.6b) (n = 28, t = 8.752, P < 0.001) and more green than pink alfalfa aphids (Fig 1.6c) (n = 13, t = 4.328, P < 0.001). Only 13 of the 30 *A. ervi* females tested laid any eggs when host choice was restricted to the two colour morphs of the alfalfa aphid and the 17 non-responders were excluded from the analysis. Females of *A. pisivorus* parasitized similar numbers of pea and pink alfalfa aphids in the dark (Fig 1.7b) (n = 22, t = 1.053, P = 0.304).

Females of *A. smithi* parasitized only pea aphids; no eggs or larvae of this parasitoid were found in either colour morph of alfalfa aphid in either light or dark experiments (Fig 1.8a & b). Furthermore, *A. smithi* parasitized significantly fewer pea aphids when these were presented together with green, rather than pink, alfalfa aphids (F = 11.043, df = 1, 50, P = 0.002). Under photophase conditions, females of *E. californicus* (n = 29) parasitized more pea than green or pink alfalfa aphids (Fig 1.9a & b respectively; t = 8.718 P < 0.001 and t = 5.683, P < 0.001) and more green than pink alfalfa aphid (Fig 1.9c) (n = 29, t = 4.589, P < 0.001).

In the light, females of *M. paulensis* parasitized significantly more pea than either green or pink alfalfa aphids (Fig 1.10a & b respectively; n = 19, t = 2.673, P = 0.016 and n = 21, t = 7.164, P < 0.001) and more green than pink alfalfa aphids (Fig 1.10c) (n = 21, t = 4.804, P < 0.001). Similarily, in the dark, *M. paulensis* females

Figure 1.8. Mean numbers of aphids parasitized (+ SE) by females of *A. smithi* caged with 15 of each of two kinds of hosts in light and dark conditions. (a) pea aphid (A. pisum) vs green alfalfa aphid (g -M. creelii), and (b) pea aphid vs pink alfalfa aphid (p -M. creelii).

Figure 1.8: *Aphidius smithi*

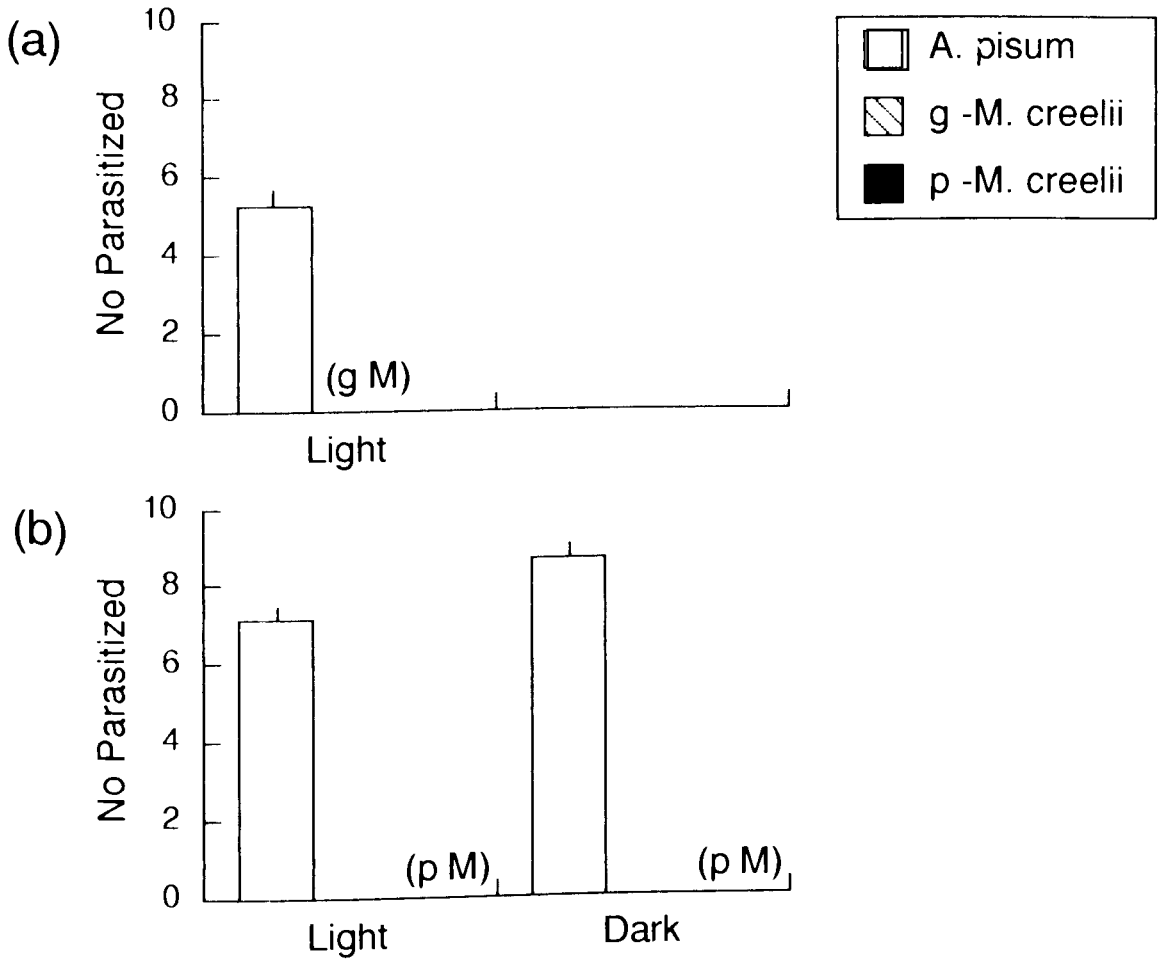


Figure 1.9. Mean numbers of aphids parasitized (+ SE) by females of *E. californicus* caged with 15 of each of two kinds of hosts in the light. (a) pea aphid (A. pisum) vs green alfalfa aphid (g -M. creelii), (b) pea aphid vs pink alfalfa aphid (p -M. creelii), and (c) green vs pink alfalfa aphid.

Figure 1.9: *Ephedrus californicus*

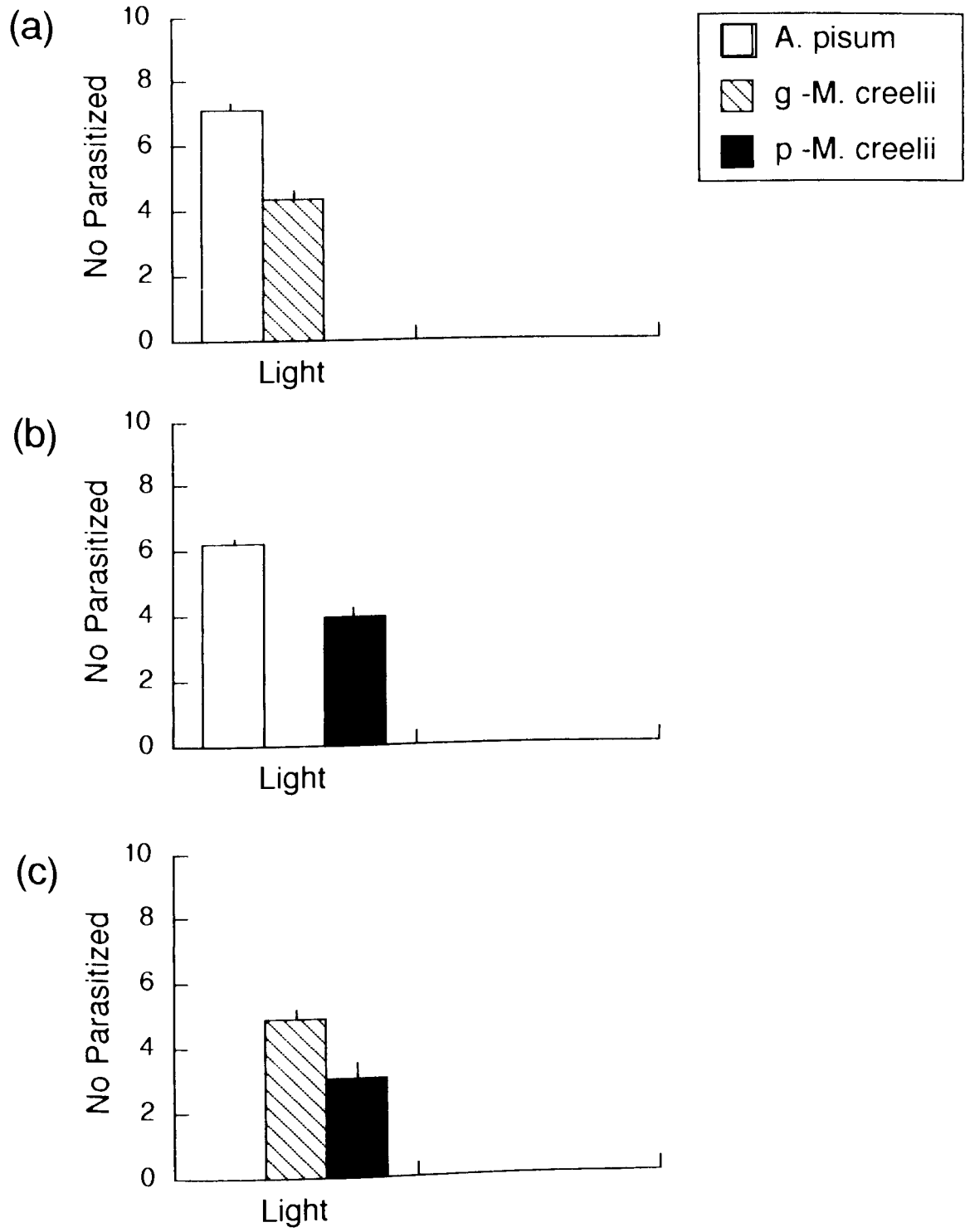
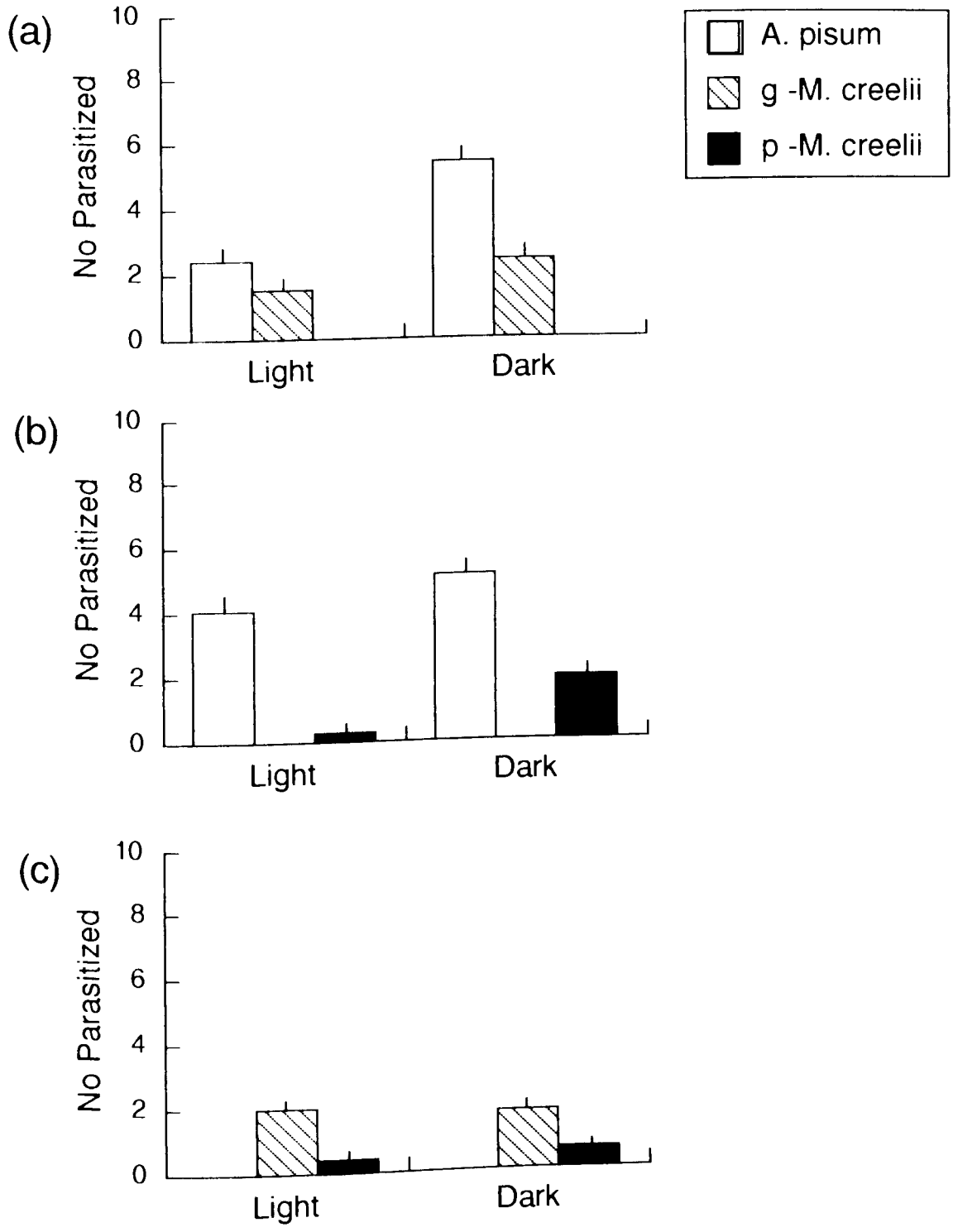


Figure 1.10. Mean numbers of aphids parasitized (+ SE) by females of *M. paulensis* caged with 15 of each of two kinds of hosts in both light and dark conditions. (a) pea aphid (A. pisum) vs green alfalfa aphid (g -M. creelii), (b) pea aphid vs pink alfalfa aphid (p -M. creelii), and (c) green vs pink alfalfa aphid.

Figure 1.10: *Monoctonus paulensis*



parasitized significantly more pea aphids than green or pink alfalfa aphids (Fig 1.10a & b respectively; n = 29, t = 8.136, P < 0.001 and n = 28, t = 7.296, P < 0.001), and more green than pink alfalfa aphids (Fig 1.10c) (n = 19, t = 3.489, P < 0.010).

Females of *P. pequodorum* foraging in the light parasitized significantly more pea than green or pink alfalfa aphids (Fig 1.11a & b respectively; n = 24, t = 2.600, P = 0.016 and n = 28, t = 10.846, P < 0.001) and more green than pink alfalfa aphids (Fig 1.11c) (n = 26, t = 8.292, P < 0.001). However, in the dark females parasitized significantly fewer pea aphids than green or pink alfalfa aphids (Fig 1.11a & b respectively; n = 29, t = 7.185, P < 0.001 and n = 28, t = 2.500, P = 0.022) and similar numbers of pink and green alfalfa aphids (Fig 1.11c; n = 19, t = 0.397, P = 0.697). Under red light, *P. pequodorum* females (n = 31) also parasitized similar numbers of pink and green alfalfa aphids (mean ± SE = 5.97 ± 0.42 and 6.23 ± 0.44, respectively; t = 0.556, P = 0.583).

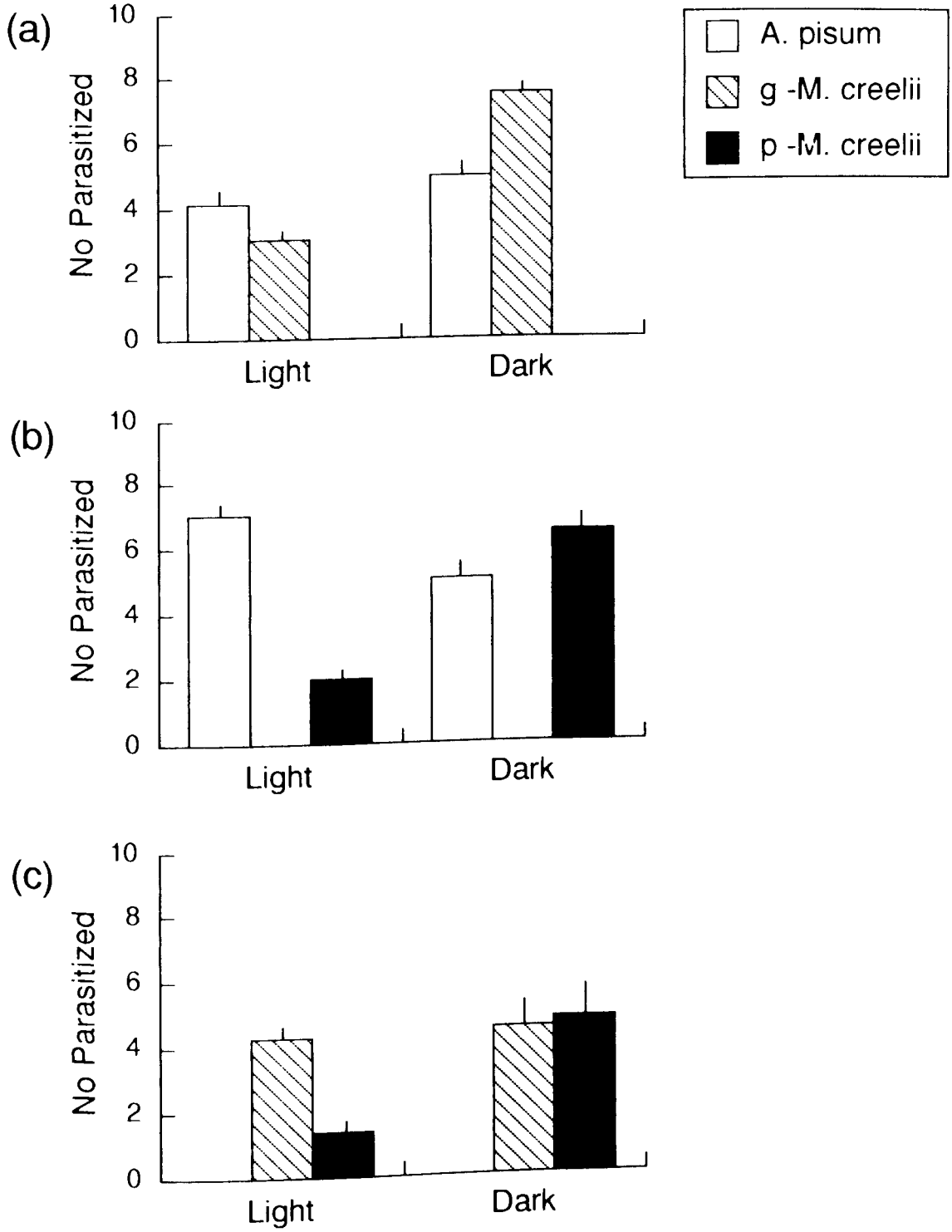
Host Movement

There was no significant difference in the mean number of anaesthetized aphids examined compared with unanaesthetized controls for all three *Aphidius* species (Table 1.0). *A. ervi* females attacked both kinds of host with equal probability (G_W = 3.400, P = 0.073), whereas *A. pisivorus* (G_W = 100.757, P < 0.001) and *A. smithi* (G_W = 38.511, P < 0.001) were less likely to attack an aphid that did not move. *A. pisivorus* oviposited in a larger proportion of attacked aphids that were anaesthetized than in those that were not (76.7% vs 67.6%, G_W = 4.913, P = 0.030), but *A. smithi* made no such distinction (73.9% vs. 82.5% G_W = 3.150, P = 0.082).

When confined with either anaesthetized or moving aphids, *A. ervi* females parasitized more moving aphids (Table 1.1). Superparasitism was slightly higher among anaesthetized than control aphids (2.01 eggs *vs.* 1.77 eggs per aphid parasitized),

Figure 1.11. Mean numbers of aphids parasitized (+ SE) by females of *P. pequodorum* caged with 15 of each of two kinds of hosts in both light and dark conditions. (a) pea aphid (A. pisum) vs green alfalfa aphid (g -M. creelii), (b) pea aphid vs pink alfalfa aphid (p -M. creelii), and (c) green vs pink alfalfa aphid.

Figure 1.11: *Praon pequodorum*



Parasitoid Species	n	No. Aphids Examined			% Aphids Attacked	
		CO_2	Control	*F*	CO_2	Control
A. ervi	10	38.8 ± 14.8	31.9 ± 6.9	1.79^{ns}	86.9	91.2^{ns}
A. pisivorus	15	52.1 ± 8.8	35.8 ± 3.6	2.96^{ns}	46.3	73.7***
A. smithi	12	23.3 ± 10.9	27.6 ± 4.7	1.60^{ns}	63.1	84.9***
E. californicus	10	52.7 ± 2.6	39.3 ± 3.1	10.67**	96.4	96.6^{ns}

Table 1.0. Mean numbers of 'normal' and anaesthetized pea aphids examined (± SE) and percentages attacked by *A. ervi*, *A. pisivorus*, *A. smithi* and *E. californicus*. Each female was provided with 10 pea aphids (*A. ervi* and *A. smithi* for 15 min, *A. pisivorus* and *E. californicus* for 20 min) that were either anaesthetized with CO_2 or 'normal' controls. Asterisks denote levels of significance in ANOVA (ns, $P > 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

	Anaesthetized		Control			
	Parasitized	Eggs/A	Parasitized	Eggs/A	n	*F*
A. ervi	4.03 ± 0.57	2.01	5.84 ± 0.52	1.77	79	4.955*
A. smithi	3.75 ± 0.73	1.51	3.60 ± 0.70	2.17	40	0.022^{ns}
E. californicus	5.90 ± 0.72	1.29	3.95 ± 0.56	1.11	40	4.562*
M. paulensis	4.85 ± 1.50	1.78	6.45 ± 1.10	1.52	40	0.723^{ns}
P. pequodorum	3.00 ± 0.79	1.13	6.43 ± 0.85	1.16	22	6.620**

Table 1.1. Mean numbers of 'normal' and anaesthetized pea aphids parasitized (± SE) and mean numbers of eggs laid per aphid parasitized (= Eggs/A) by *A. ervi, A. smithi, E. californicus, M. paulensis*, and *P. pequodorum*. Females were confined for 30 min (*E. californicus* for 20 min) with either 16 'normal' pea aphids or 16 which had been anaesthetized by a 5 min exposure to CO_2. A subsample of 10 aphids of each type was dissected after rearing for 4 days. Asterisks denote levels of significance between numbers of aphids parasitized in ANOVA (ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$).

although the mean number of eggs laid per female did not differ significantly between the two groups of parasitoids (F = 1.138, df = 1, 77, P = 0.291). In contrast, *A. smithi*, parasitized both kinds of hosts to the same extent, but superparasitism was higher among controls than anaesthetized aphids (2.17 *vs.* 1.51 eggs per host parasitized; Table 1.1).

Females of *E. californicus* (n = 10) given only anaesthetized aphids attacked 50.8 ± 2.6 aphids (mean ± SE) in a 20 min period, compared with 38.1 ± 3.3 for those (n = 10) provided with control aphids (F = 9.716, df = 1, 17, P = 0.006, Table 1.0). Females of this species confined with anaesthetized pea aphids parasitized significantly more hosts than did females provided with an equal number of normal pea aphids, with no difference in the numbers of eggs laid per aphid parasitized (Table 1.1). Females of *M. paulensis* given anaesthetized pea aphids parasitized the same number as females given normal aphids, with no difference in numbers of eggs per aphid. Only 8 out of 21 *P. pequodorum* females parasitized anaesthetized pea aphids, compared with 14 out of 21 provided normal pea aphids. Among females that accepted aphids (n = 22), those given anaesthetized pea aphids parasitized fewer compared with those given normal pea aphids. However, *P. pequodorum* females caged with anaesthetized pea aphids in the dark parasitized the same number as did females confined with normal pea aphids (mean ± SE = 7.86 ± 0.91 and 7.25 ± 0.2.14 respectively; F = 0.094, df = 1, 10, P = 0.766).

When given a choice of anaesthetized pea and pink alfalfa aphids, *A. ervi* and *M. paulensis* females parasitized more pea aphids (Table 1.2), whereas *E. californicus* parasitized similar numbers of each kind of host. When the choice was between anaesthetized green and pink alfalfa aphids in the dark, *M. paulensis* females (n = 22) parasitized more of the former (mean ± SE = 2.96 ± 0.31 and 1.68 ± 0.33 respectively; t = 6.384, P < 0.001). Only 5 out of 31 *P. pequodorum* females parasitized any hosts when given a choice of anaesthetized pea aphids and pink alfalfa

	A. pisum (green)		*M. creelii* (pink)			
	Parasitized	Eggs/A	Parasitized	Eggs/A	n	*t*
A. ervi	3.15 ± 0.22	2.17	1.58 ± 0.32	1.61	26	4.811***
E. californicus	4.18 ± 0.22	1.11	4.39 ± 0.19	1.14	28	1.0^{ns}
M. paulensis	3.32 ± 0.28	1.69	1.40 ± 0.25	1.40	25	5.331***
P. pequodorum	2.40 ± 0.85	1.00	1.2 ± 0.56	1.00	5	-

Table 1.2. Mean numbers of pea aphids and pink alfalfa aphids parasitized (± SE) and mean numbers of eggs laid per aphid parasitized (= Eggs/A) by females of various species provided with a choice of anaesthetized hosts under white fluorescent light. Individual females were placed for 20 min into a plastic petri dish containing 8 pea aphids and 8 pink alfalfa aphids immobilized by a 5 min exposure to CO_2. A subsample of 5 aphids of each type were dissected after rearing for 4 days. Asterisks denote levels of significance between numbers of hosts parasitized in a paired *t*-test (ns, $P > 0.05$; *** = $P < 0.001$).

aphids in the light and these females parasitized twice as many pea aphids (Table 1.2).

DISCUSSION

Patterns of resource exploitation vary among species, with generalist parasitoids and predators often showing a ranked order of preference for different kinds of hosts or prey (Courtney *et al.* 1989, Courtney & Kibota 1990, Chow & Mackauer 1992). This differential exploitation of a particular resource type may result from behavioral differences between species in response to environmental and host cues. For example, chemical cues and aphid defensive behaviours are most commonly implicated as the basis for host preference (Read *et al.* 1971, Gerling *et al.* 1990, Völkl 1991, Völkl & Mackauer 1993). However, visual cues can be assessed without the risks of handling the host (Gerling *et al.* 1990) and may influence a female's decision to attack or handle a host. In the following discussion I will attempt to delineate the respective roles of visual and chemical cues in the process of host evaluation as it occurs in the aphidiid species I have studied and suggest possible reasons for some of the observed differences among them.

Size and Shape

Antennal contact with an aphid appears to result in host recognition, either through contact chemoreception or tactile recognition of surface texture, notably during initial encounters. However, experienced females of all species except *E. californicus* frequently attack aphids without preliminary antennation, although antennal contact often occurs during attack and may provide confirmation of host identity.

The compound eyes of insects are very complex structures but afford only fixed-focus vision and little depth of field (Prokopy & Owens 1983). As a result of this 'myopic' condition, contrasting outlines may be perceived at a distance but pattern recognition is only possible at very close range. In aphidiids, a change in behaviour suggestive of visual host recognition occurs at

distances of 5 - 8 mm, depending on the species. Ovipositor thrusts were sometimes observed directed at aphids on the other side of a glass or plastic barrier. *A. smithi* females could discriminate between pea aphids and green alfalfa aphids without contact, despite their similarity in coloration (Fig. 1.2a). I conclude that all the aphidiid species I have studied, with the exception of *E. californicus*, form some sort of search image during the first few host encounters and, thereafter, orient visually to hosts.

Colour

Many studies have demonstrated responses by hymenopterous insects to particular wavelengths of light, *e.g.* Wardle (1990). Honey bees learn to associate particular colours with food sources (von Frisch 1971, Menzel 1985, Giurfa & Núñez 1989, Gould 1993). Colour discrimination has been implicated in host micro-habitat selection by the parasitoid *Itoplectis conquisitor* (Say) (Arthur 1966), and in host selection by *Nasonia vitripennis* Walker (Takahashi & Pimentel 1967). Wardle (1990) showed that the ichneumonid parasitoid *Exeristes roborator* (F.) preferred to forage for hosts in artificial microhabitats that were similar in colour to those previously experienced. Ankersmit *et al.* (1986) reported a preference for green over brown colour morphs of *Sitobion avenae* (F.) by *Aphidius ropalosiphi* De Stephani Perez, although they did not directly observe attack behaviour. Evidence of colour vision was reported for *Diaeretiella rapae* (M'Intosh) (Vater 1971) and *A. ervi* (Goff & Nault 1984). However, in both of these studies colour preferences were interpreted only in the context of parasitoid orientation to plants.

These data suggest an important role for host coloration in determining the probability of attack by various species of aphidiid. A visual model which one could use to manipulate colour independently of all other sensory features of the host would have been the ideal approach for unambiguously demonstrating the role of colour in host evaluation. The following

inferences with repect to the role of colour must therefore be tempered by the consideration that darkness eliminates visual cues in addition to colour and, furthermore, that it may in some way affect the behaviour of the parasitoids themselves. The apparent preference for 'green' aphids over 'pink' was shared by all the visually-orienting aphidiid species I examined and appears to be intrinsic in that it is consistent across generations and does not appear to be influenced by experience (Chow & Mackauer 1992). Since it is virtually impossible to determine when host recognition actually occurs, I could not distinguish whether the lower examination rates of pink aphids were the result of their being recognized less often, or rejected more often, at an early stage of host evaluation. However, female *A. smithi* parasitized significantly fewer pea aphids when these were present with green as opposed to pink alfalfa aphids (Fig. 1.8), presumably because they lost time examining green alfalfa aphids. These observations suggest that females took longer to distinguish a less preferred host that was similar in colour to the preferred host, whereas one of contrasting colour was more easily recognized and avoided.

Females of all species, except *E. californicus,* expressed consistent preferences for particular host phenotypes (*i.e.* aphids that were green as opposed to pink) which were not neccessarily associated with host suitability. Pink aphids were apparently perceived as less acceptable hosts, despite proving to be of equivalent acceptability in the dark in some cases, *e.g. A. pisivorus*. *P. pequodorum* females preferentially attacked the two types of green aphid more often than the pink one (Fig. 1.5), indicating that colour influenced host preference. Females of this species did not distinguish between the two strains of alfalfa aphid either in the dark or under red light illumination, indicating that the preference for green alfalfa aphid observed under normal illumination was strictly a response to coloration. Furthermore, the preference of *P. pequodorum* for pea aphid over pink alfalfa aphid was reversed when females foraged in darkness, suggesting

that the importance of visual cues overrides that of other, presumably chemical, cues in determining preference under normal conditions. If many potentially suitable hosts escape contact examinations as a result of unusual appearance or coloration, or if preferences with respect to host physiology are subordinate to visual preferences, it is possible that the host ranges of some species are restricted, in part, by 'visual specialization' in the sense of Prokopy & Owens (1978; 1983). These authors observed that many herbivorous insects appear to respond to "a specific predetermined template of stimulus perception, and... ignore stimuli that do not conform".

Movement

Host movement has proved to be an important releasing stimulus for attack in a number of parasitoids (Vinson 1976). For example, Monteith (1956) showed that moving feathers in an olfactometer elicited attacks by the tachinid fly *Drino bohemica*, but only in the presence of host odors. Aphid movement increased the probability of attack by females of *A. pisivorus, A. smithi* and *P. pequodorum*. Females of *A. pisivorus* and *A. smithi* that examined anaesthetized aphids attacked them less often than females examining their normal counterparts. Tactile perception of host struggling appears to increase host acceptability in *A. ervi*, but not in closely related species. Females of this species also parasitized moving aphids at a faster rate than immobilized aphids in the no-choice confinement experiment.

M. paulensis was the only species that found moving and non-moving hosts equally acceptable, perhaps because this species paralyzes its hosts (for *ca.* 15 min) with injected venom (Calvert & van den Bosch 1972b), a capability which may have evolved to reduce resistance in the host during attack. Movement of the host may therefore not act as a releasing stimulus for attack behaviour in this species. *M. paulensis* females use their forelegs to clutch and grapple with aphids at close quarters, often re-inserting the ovipositor several times during an attack.

Nevertheless, attacks appear guided by visual cues and aphids are attacked in a preferred orientation, usually from the side.

A majority of *P. pequodorum* females did not attack any immobilized aphids under lighted conditions despite extensive examination of them. Anaesthetized aphids were acceptable for oviposition when presented in the dark, presumably because females used non-visual cues to evaluate hosts. I conclude that the visual perception of host movement is an important releasing stimulus for attack behaviour in this species under normal lighted conditions, whereas the tactile perception of host struggling is not.

Host Defenses

The strong and consistent preference for pea aphid over alfalfa aphid by *A. ervi* and *M. paulensis* did not change when hosts were anaesthetized, a result indicating that preference was not because of differences in aphid behaviour. The behaviour of aphids did not appear to influence the success of attacks by *Aphidius* females that are very fast, but there may have been an effect similar to aversion learning (Dethier 1980, Jermy 1987) that caused females to avoid pink aphids following contact with them. All parasitoids seemed to find the cornicle secretion of alfalfa aphid particularily deterrent compared to that of pea aphid (Chow 1989), and alfalfa aphids appeared more effective in smearing their attacker with it. The alfalfa aphids were also more active than pea aphids, a fact which may have increased their probability of escape from slower parasitoids.

The response to 'smearing' with cornicle secretions was remarkably similar for all parasitoid species: the female would quickly take a number of steps backward, often shaking her head and grasping at her antennae. This would inevitably be followed by a period of grooming during which the antennae, and sometimes the ovipositor, were carefully wiped clean with the fore tarsi and mouthparts. In most species, the experience of being smeared with alfalfa cornicle secretions seemed to deter

females from further attacks on these aphids and reinforce their preference for pea aphid. This could be tested experimentally by giving one group of females experience with pink alfalfa aphid until they contacted cornicle secretions, and another group experience only with pea aphid. In a subsequent choice situation, females of the former group would be expected to display a stronger preference for pea aphid (aversion for alfalfa aphid) than those of the latter. Although *E. californicus* females were comparatively inept attackers and were frequently smeared with cornicle secretions, they seemed less deterred by the experience and resumed their search for hosts much earlier than did females of other species.

Kouamé & Mackauer (1991) showed that hosts immobilized with carbon dioxide were more susceptable to parasitization by *E. californicus*. My observations indicate that aphids immobilized by carbon dioxide are parasitized at higher rates by *E. californicus* than normal aphids capable of evasive behaviour. *E. californicus* was the only species examined that did not appear to utilize visual information in host recognition or evaluation. Potential hosts are not recognized until contacted with the antennae, whereupon females thrust with the ovipositor in the general direction of an aphid that has been contacted. Aphids moving in response to antennal contacts frequently escape and the different rates of parasitism obtained in oviposition choice tests (Fig. 1.9) probably reflect differences in the defensive behaviour of the various aphid biotypes. Pink alfalfa aphid appeared to be the most agile and the fastest at responding to antennation, with green alfalfa aphid second, and pea aphids the slowest. No difference in rates of examination or attack were observed for any pairwise combination of hosts presented to *E. californicus* (Fig. 1.3), nor in the probability of attack following examination. Pink alfalfa aphids were parasitized by *E. californicus* at the same rate as pea aphids when immobilized, indicating they are assessed as equal in quality.

Chemical Recognition

Although the odor of honeydew is probably involved in the location of infested plants by these aphidiid species, none of my observations leads me to believe that odor is involved in the location or recognition of individual hosts, or in the host evaluation process. Dark experiments with *A. pisivorus* in which host preference disappeared, and with *P. pequodorum* in which it was reversed, provide indirect evidence that hosts were not being distinguished on the basis of their odor.

The tactile or contact-chemosensory event that occurs during antennation was the only stimulus that elicited attack by *E. californicus*. Attacks by the other species are often, though not always, preceded by a brief antennation. Hays & Vinson (1971) reported that, in the parasitoid *Cardiochiles nigricepes* Viereck, ovipositor thrusting was elicited by antennal contact with chemical factors in the cuticle of the host, *Heliothis virescens* (F.). Similarly, antennal contact with the shed skins of both host and non-host aphids results in reflexive probing by aphidiid females of almost all species. Cuticles of non-aphid insects caused no such response in the few tests I performed with *A. smithi* and *E. californicus*. Skins of pea aphid that were extracted with mixtures of methanol, methyl chloride and hexane retained their activity, suggesting that the recognition factor(s) is not a cuticular hydrocarbon, but a stable component of the aphid cuticle. These cuticular factors appear to provide confirmation of host identity as 'aphid', rather than specific criteria for host evaluation.

Host Acceptance

All three *Aphidius* species responded to apparent chemical differences between pea and alfalfa aphid, although the extent to which this influenced preference varied from absolute (*A. smithi*) to partial (*A. ervi*) to very little (*A. pisivorus*). The complete rejection of alfalfa aphids by *A. smithi* was unexpected and is at variance with previous work in our laboratory which showed that

this aphid may be accepted as a host (Chow & Mackauer 1991, 1992). *A. pisivorus* females were more likely to oviposit in pea aphids they attacked than in pink alfalfa aphids. Although females of *A. ervi* attacked equal numbers of pea aphid and green alfalfa aphid, they parasitized a greater proportion of the former. Furthermore, *A. ervi* females discriminated between the two colour morphs of alfalfa aphid in the dark (Fig 1.6), a result indicating that these aphids differed in attributes other than pigmentation.

Host evaluation by *E. californicus* begins during ovipositor insertion and appears based solely on internal chemcial cues associated with host physiology (Chow & Mackauer 1986). Although the hosts provided in this study were all equally acceptable to *E. californicus*, this species can discriminate among parasitized and unparasitized hosts (Völkl & Mackauer 1990).

Calvert (1973) studied the host selection behaviour of *M. paulensis* and found it accepted a wide range of aphid species, although demonstrating preferences in some cases. *M. paulensis* responded to chemical differences between host species in these experiments, fewer alfalfa than pea aphids were parasitized by this species in the dark (Fig 1.10). Note also that pea aphid and green alfalfa aphid were attacked and parasitized at equal rates in the light (Figs 1.4 & 1.10, respectively) whereas the former species was preferred in the dark (Fig 1.10). Apparently, *M. paulensis* does not distinguish between these aphids prior to contact due to their similar coloration and therefore attacks them with equal frequency. There was a preference for pea aphids in the dark when females selected hosts solely on the basis of chemical cues. Furthermore, *M. paulensis* detected and responded to chemical differences between the two *M. creelii* colour morphs when these were anaesthetized to control for aphid behaviour and presented in the dark to control for the difference in coloration.

No preference for pea aphid over green alfalfa aphid by *P. pequodorum* was evident in experiments carried out under

illumination (Figs. 1.5 & 1.11), but when hosts were evaluated in the dark, both pink and green alfalfa aphid were preferred for oviposition over pea aphid (Fig. 1.11), evidently a response to species-specific chemistry. These results indicate that the host preferences of this species may result from reponses to visual criteria alone, and that different host preferences may emerge when females are denied access to visual cues.

Summary

The six parasitoid species I examined appeared to utilize similar visual and chemosensory cues in host evaluation, but interpreted sensory information in different ways. With the exception of *E. californicus*, all species apparently used visual information such as colour and movement to screen potential hosts under the conditions of my experiments. These species may therefore choose hosts on the basis of phenotypic appearance, although acceptance of an aphid remains contingent on an evaluation of the internal chemistry of the host during ovipositor probing. Preferences for particular host species based on physiological differences are most likely expressed at this final stage in host selection.

Host evaluation by aphidiid wasps can be subdivided into three distinct stages, recognition, attack, and acceptance. These distinctions are very similar to those Schmidt (1974) described for *Campoletis sonorensis*, although I do not distinguish between "thrusting" and "inserting" of the ovipositor. This distinction might be meaningful for *E. californicus* in which thrusting seems to be part of search behaviour, but in the other species a thrust is invariably a directed attack. Aphids are recognized either by visual cues prior to contact, or by antennal contact with the host cuticle. In *E. californicus*, antennal contact with host cuticle appears to elicit reflexive ovipositor probing, but in *M. paulensis*, *P. pequodorum* and the three *Aphidius* species antennation only confirms host identity; some, but not all, attacks are preceded by antennation. In all species except *E. californicus*, the probability

of attack hinges on an evaluation of visual cues. Parasitoid females then assess the chemical suitability of the host during ovipositor insertion, and it is at this level that preferences based on genotypic criteria are likely to be expressed.

If host selection criteria are compared across the six aphidiid species (Table 1.3), *E. californicus* appears the most atypical of the group. An absence of pre-strike evaluation may represent the primitive condition in this family; more complex (visual) criteria probably evolved later. Although host records for most of these species are probably incomplete, *E. californicus* has been recorded from a relatively large number of aphid species, as has *M. paulensis* (Calvert & van den Bosch 1972a) and *A. ervi* (Mackauer & Stary 1967, Pungerl 1984). *A. smithi*, on the other hand, appears to be the most specialized of the group, parasitizing only pea aphid. This species expressed an absolute preference for pea aphid, rejecting alfalfa aphids in which it can develop (Chow & Mackauer 1991). In contrast, *M. paulensis* oviposits in aphids in which it cannot complete development (Calvert 1973), a behaviour also observed in *M. crepidis* (Griffiths 1960).

Given the large fecundities of these wasps, the costs of attacking unsuitable hosts probably arise from risk of injury or loss of search time, rather than a waste of eggs. Among the less specialized aphidiids, host range may be limited by requirements for larval development, rather than by oviposition behaviour. The payoffs for early rejection of unsuitable hosts may be greater for specialized parasitoids that reject many potential hosts, and therefore benefit from an assessment of visual cues prior to attack. On the other hand, polyphagous species with broad host ranges attack hosts that vary greatly in phenotypic appearance and might gain less by avoiding hosts on the basis of stringent visual criteria. Host acceptance by polyphagous species is more likely to depend solely on an assessment of chemical cues detected during attack, since these can be expected to provide a more reliable indication of suitability.

Effect on Host Evaluation	Colour	Movement	Physiology*	Host Defenses**
E. californicus	–	–	(+)	++
M. paulensis	+	–	+	(+)
P. pequodorum	+++	+++	+	(+)
A. ervi	++	+	+	(+)
A. pisivorus	++	+	+	(+)
A. smithi	++	–	++	(+)

Table 1.3. Summary of effects of various sensory cues on host selection by six species of aphid parasitoid. Plus (+) and minus (–) signs indicate whether or not particular factors had observable effects on host evaluation behaviour. Parentheses denote effects reported in other studies (see text for references). *Discrimination among host types was evident following ovipositor insertion. **Probability of parasitism was influenced by host behaviour.

Chapter II

Variation in Oviposition Tactics with Female Experience in *Aphidius ervi* and *Monoctonus paulensis*

INTRODUCTION

In the previous chapter I examined host evaluation criteria that I referred to as 'static' because they were characteristic of parasitoid species. In this chapter I address the question of whether or not oviposition tactics vary in response to dynamic criteria at the level of the individual wasp, and whether variation in reproductive investment is evident at the level of the host patch, and the individual host. Aphids are neither randomly nor evenly distributed in the environment, but usually occur in clumps or 'patches'. One might consider a patch to be an infested leaf, plant, or clump of plants, but in order to be an elemental unit of foraging, *sensu* Ayal (1987), it must have a finite size and possess recognizable boundaries. Thus a group of aphids in a plastic petri dish may also comprise a patch of hosts to a searching female parasitoid, albeit an artificial one.

Female aphidiids make six important decisions within each host patch: 1. to search for aphids (as opposed to grooming or feeding), 2. to attack an aphid, 3. to accept (oviposit) or reject it, 4. to fertilize the egg or not (sex determination), 5. to lay additional eggs (superparasitism) or not, and 6. to continue searching or leave the patch (emigration). In the previous chapter I demonstrated that the decisions to attack and oviposit hinge on responses to sensory cues which are obtained and interpreted in a manner specific to each parasitoid species. The fourth decision relates to brood sex ratio strategies, a topic which I do not address in this thesis. Decisions five and six relate to patterns of reproductive investment in individual hosts and host patches respectively. In this chapter I examine how experience in one patch of hosts may influence a female's reproductive investment in a subsequent patch containing hosts of the same or different quality.

Recently, a new approach to modelling foraging behaviour has sought to analyze the fitness consequences of various behavioral responses that arise from differences in physiological

and motivational states (Mangel & Clark 1986, Roitberg 1990). Such models generally hinge on two assumptions; firstly that individuals behave optimally to maximize their fitness, and secondly that individuals can assess their own physiological state (age, egg load, *etc.*) and sample their environment. For example, female parasitoids might estimate average host quality and availability based on their previous encounters with hosts. This information, although imperfect, might be used by a female to adjust her reproductive tactics, *i.e.* the number of eggs she lays in each host, and the number of hosts she parasitizes in each patch. Foraging by expectation could improve a female's fitness, *i.e.* the number of her offspring surviving to reproductive age, provided that the information acquired by sampling generates a reasonably reliable estimate of local host quality and availability.

Variation among insect populations with respect to host preference may represent either heritable variation in the way host quality is assessed, or the effects of different environmental influences (Rausher 1985). It is perhaps meaningful to distinguish between host 'quality' in an absolute sense, and host 'value' in a relative sense. Host quality can be considered a static property that is assessed according to sensory criteria interpreted by females in a parasitoid-specific manner. On the other hand, the value of a host, or host patch, to a female will depend on the relative fitness returns of laying one or more eggs in a host, as opposed to rejecting it and seeking other hosts or patches. This is analogous to the marginal value theorem as applied to models of optimal foraging (Charnov 1976). A rate-maximizing forager should select a per-host and per-patch investment so that the marginal rate of fitness gain equals the long term average rate of fitness gain. If a female has access to information regarding her physiological state and current ecological conditions, this information might affect her dynamic assessment of host value at a particular point in time, which can be inferred from the number of eggs laid per host. I hypothesized that the relative value of a host to a female parasitoid will be influenced by (1) the number

of eggs she has available, (2) the quality of the host relative to those previously encountered, and (3) the number of hosts already parasitized by the female in that patch. Thus, hosts should be worth more to a female when eggs are abundant than when they are in short supply. Assuming a finite optimum brood size, even high quality hosts should decline in value within a patch following a series of ovipositions. Furthermore, the value of low quality hosts might increase over time if higher quality hosts are not encountered, or decrease if they are.

The decision of how many eggs a solitary parasitoid should lay in a host was not considered important until relatively recently. It seemed obvious that a female should lay only one egg per host since only a single offspring could complete development. Superparasitism was originally thought to result from ovipositional mistakes or a failure to discriminate (Salt 1961) and yet there are various circumstances under which both conspecific and self superparasitism may be adaptive strategies for improving offspring survival (van Alphen & Visser 1990). These will be examined in more detail in the following chapter, but at this point I wish to consider the number of eggs laid in a host as an index of reproductive allocation to individual hosts. The self-superparasitized host represents a larger maternal reproductive investment compared to the singly parasitized host. By laying additional eggs in a host she has already parasitized, a female may improve the survival of one offspring by overwhelming host immune responses (Streams 1971, Puttler 1974) or increase her reproductive success when her offspring face competition from conspecific larvae (Visser 1993). For aphidiid wasps, there is evidence that the probability of securing a host for one's own progeny increases as a function of the number of eggs laid into a multiply-parasitized host (Mackauer *et al.* 1992).

Differential reproductive investment among host patches that vary in quality might also be expected. The relative value of a patch to a foraging female can be estimated by her residence

time and the number of hosts she attacks, factors which together will determine local brood size and levels of patch exploitation. Rates of superparasitism provide an estimate of reproductive investment in individual hosts and this behaviour can also be examined in the larger context of patch investment strategies. In this chapter I investigate the relationship between host quality and host value in *A. ervi* and *M. paulensis*. Both of these species consistently oviposited in more pea than alfalfa aphids in the experiments reported in Chapter One and this host preference permitted a manipulation of patch quality that was independent of host density. Furthermore, females of these species begin attacking hosts immediately upon exposure to them, and parasitize hosts of a given species at a relatively predictable rate. This facilitates resolution of differences in oviposition behaviour that result from female responses to relatively fine-grained differences in host quality, provided a suitable time interval is selected.

In these experiments I compare the behaviour of females across pairs of patches that vary in quality (*i.e.* the species of host) in order to test whether host value and patch value are assessed by females relative to their experience in previous patches. There are four permutations of two host species possible in two sequential patches. I predicted that exposure to a patch of 'preferred' hosts (pea aphids) would reduce the value of a subsequently encountered patch of 'less-preferred' hosts (alfalfa aphids), and the value of individual hosts within the patch. In addition, I hypothesized that acceptance of the less-preferred host would increase if preferred hosts were not previously encountered, leading to parasitization of a greater number of alfalfa aphids in the second patch than in the first. I expected no difference between patches in numbers of pea aphids parasitized, or numbers of eggs laid per pea aphid.

Previous work on other parasitoids has demonstrated effects of conspecific encounter on female oviposition tactics (Visser *et al.*

1992b). If conspecific encounter is reliable evidence of a threat of competition, females that encounter others prior to foraging might improve their rate of host exploitation and, hence their reproductive success, by self-superparasitizing hosts. I hypothesized that females encountering conspecifics prior to foraging would self-superparasitize more aphids than those that had not.

There is also evidence to suggest that parasitoid oviposition behaviour can be influenced by encounters with hosts parasitized by other females (van Alphen *et al.* 1987). I hypothesized that encounters with conspecific females, and previously parasitized aphids, might result in higher rates of self superparasitism than conspecific encounter alone. I further hypothesized that effects of exposure to previously parasitized hosts might raise the value of a subsequently encountered patch of unparasitized hosts, *i.e.* that females encountering aphids previously parasitized by conspecifics would subesquently parasitize a larger number of aphids in a given period relative to females that encountered only unparasitized aphids.

MATERIALS AND METHODS

All parasitoids and aphids were reared in synchronous cultures under the same conditions described in Chapter One. Mummies of *A. ervi* and *M. paulensis* were removed from the plants on which they had matured, placed in wax paper cups, and adults fed diluted honey upon emergence. Näive mated females 24 - 48 h old were used in experiments. Late second instars of pea aphid, *A. pisum*, and green alfalfa aphid *M. creelii*, were used as hosts (72 ± 4 h old at 20° C).

The first experiment consisted of four treatments in which females foraged in two successive patches containing either the same or different host types which are detailed in Table 2.0 along with the predicted results. Individual females were removed from the colony and placed into empty plastic petri dishes on a lab bench for either 20 min (*A. ervi*) or 30 min (*M. paulensis*) prior to the experiment. Each female was then transferred to a plastic petri dish (6 cm dia x 1.5 cm ht) containing 15 aphids, and left undisturbed for 20 min and 30 min, respectively. Each female was then transferred to an empty dish on the lab bench for 20 min and 30 min, respectively, before being introduced to a second dish of 15 aphids for the same period. Twenty replicates were performed for each treatment. Following exposure to wasps, aphids from each dish were reared separately on a bean shoot for 4 days, after which a subsample of 10 was dissected to count the eggs and larvae they contained. The data for numbers of aphids parasitized and numbers of eggs laid per aphid parasitized were analyzed within treatments (patch one *vs* patch two) using ANOVA for repeated measures and across treatments using ANOVA followed by Fisher's LSD test for significant differences among means. Cases in which no eggs were laid in either patch were excluded from the analyses, and cases in which no

Treatment	Number of aphids parasitized in second *vs* first patches	Number of eggs laid per aphid in second *vs* first patches
1. Ap -> Ap	=	=
2. Ap -> Mc	<	<
3. Mc -> Mc	>	=
4. Mc -> Ap	>	>

Table 2.0. Predicted effects of host sequence on the oviposition behaviour of *A. ervi* and *M. paulensis* females. The primary hypotheses are: 1) fewer alfalfa aphids will be parasitized in second patches (and fewer eggs laid per alfalfa aphid parasitized) when the first patch contains pea aphid as opposed to alfalfa aphid; 2) more alfalfa aphids will be parasitized in the second patch than in the first patch in treatment 3.

eggs were laid in one patch were excluded from analysis of eggs laid per aphid parasitized.

In the second experiment, females received one of three treatments. Females of the first group (gp 1) emerged alone in gelatin capsules and were transferred to a wax paper cup with a bean stem and diluted honey and two males within 16 h of eclosion - they encountered no conspecific females. Females of the other two groups (gps 2 & 3) emerged and mated in mixed colonies provisioned with a bean stem and diluted honey for their first day of adult life. Individual females were removed from the colony and placed into empty plastic petri dishes (6 cm x 1.5 cm) on a lab bench for 20 min (*A. ervi*) or 30 min (*M. paulensis*) prior to the experiment. Females of gp 1 and gp 2 were conditioned by placing them individually into a petri dish containing 10 unparasitized pea aphids, while those of gp 3 received 10 pea aphids that had been attacked by a conspecific female 24 h earlier. After foraging for 20 min and 30 min, respectively, females were transferred to an empty dish for a rest period of 20 or 30 min. Each was then introduced to a second dish containing 15 unparasitized pea aphids for either 20 min or 30 min. Aphids from the second dish of each replicate were reared separately and a subsample of 10 was dissected after four days of rearing. The data for numbers of aphids parasitized and numbers of eggs laid per aphid parasitized were analyzed across treatments using ANOVA followed by Fisher's LSD test of significance among means. Replicates in which no aphids were parasitized were excluded from analysis.

RESULTS

Experiment 1.

Aphidius ervi - Comparison Across Patches. Significantly fewer pea aphids and alfalfa aphids were parasitized in the second patch than in the first when the first patch contained pea aphid (Table 2.1). Conversely, more pea aphids were parasitized in the second patch than in the first when the first patch contained alfalfa aphid. When alfalfa aphids were present in both patches no significant difference was observed. Significantly fewer eggs were laid per aphid parasitized in the second patch than in the first when alfalfa aphid occurred in both patches, whereas more eggs were laid per aphid parasitized in the second patch than in the first when alfalfa aphid was followed by pea aphid (Table 2.2). There were no significant differences between first and second patches in the other two treatments.

Comparison Among Treatments. There were significant differences in the total number of aphids parasitized among treatments (F = 11.162; df = 3, 74; P < 0.001). A larger total number of aphids were parasitized when pea aphid occurred in both patches than when alfalfa aphid occurred in the first patch (Fisher's LSD, P = 0.014), in the second patch (Fisher's LSD, P = 0.011), or in both patches (Fisher's LSD, P < 0.001). Fewer total aphids were parasitized when both patches contained alfalfa aphids compared to when the first patch (Fisher's LSD, P = 0.004), or the second patch (Fisher's LSD, P = 0.002) contained pea aphids. There was no difference in total aphids parasitized between pea aphid followed by alfalfa aphid, and alfalfa aphid followed by pea aphid (Fisher's LSD, P = 0.890).

There were also significant differences in the number of eggs laid per aphid parasitized among treatments (F = 3.323; df = 3, 74; P = 0.024). Significantly fewer eggs were laid per aphid parasitized (total) when alfalfa aphid occurred in both patches compared to when pea aphid occurred in either the first (Fisher's

Treatment	n	No. of aphids parasitized: Patch No.1	Patch No.2	Statistics	Total
1. A.p.-> A.p.	19	8.63 ± 0.46^{b}	6.53 ± 0.56^{b}	F = 25.352 P < 0.001	15.16 ± 0.95^{c}
2. A.p.-> M.c.	18	7.56 ± 0.64^{b}	4.11 ± 0.71^{a}	F = 17.251 P = 0.002	11.67 ± 1.06^{b}
3. M.c.-> M.c.	21	3.14 ± 0.57^{a}	4.57 ± 0.60^{a}	F = 3.049 P = 0.192	7.71 ± 0.83^{a}
4. M.c.-> A.p.	20	3.65 ± 0.65^{a}	8.20 ± 0.40^{c}	F = 52.816 P < 0.001	11.85 ± 0.87^{b}

Table 2.1. Mean numbers of aphids parasitized (± SE) by *A. ervi* females foraging in two sequential host patches. Mated females (24 - 48 h old) were each confined twice for 20 min in a plastic petri dish containing either 15 pea aphids (A.p.) or 15 alfalfa aphids (M.c.) with 20 min between patches. Means within columns bearing the same letter were not significantly different among treatments.

		No. eggs laid per aphid			
Treatment	n	Patch No.1	Patch No.2	Statistics	Total
1. A.p.-> A.p.	19	1.90 ± 0.20^{b}	1.41 ± 0.10^{a}	F = 5.727 P = 0.058	1.66 ± 0.13^{ab}
2. A.p.-> M.c.	18	2.02 ± 0.22^{b}	1.36 ± 0.08^{a}	F = 4.681 P = 0.100	1.79 ± 0.14^{b}
3. M.c.-> M.c.	21	1.32 ± 0.10^{a}	1.23 ± 0.11^{a}	F = 8.266 P = 0.026	1.33 ± 0.11^{a}
4. M.c.-> A.p.	20	1.40 ± 0.10^{a}	1.97 ± 0.17^{b}	F = 11.931 P = 0.008	1.79 ± 0.12^{b}

Table 2.2. Mean numbers of eggs laid per aphid parasitized (± SE) by *A. ervi* females foraging in two sequential host patches. Mated females (24 - 48 h old) were each confined twice for 20 min in a plastic petri dish containing either 15 pea aphids (A.p.) or 15 alfalfa aphids (M.c.) with 20 min between patches. Means within columns bearing the same letter were not significantly different among treatments.

LSD, P = 0.010) or second patch (Fisher's LSD, P = 0.008). No other differences in eggs laid per aphid parasitized (total) were significant.

Patch One. There were significant differences among treatments in the number of aphids parasitized in the first patch (F = 22.282; df = 3, 74; P < 0.001) and in the numbers of eggs laid per aphid parasitized (F = 3.987; df = 3, 63; P = 0.012). There was no difference between treatments one and two in the number of pea aphids parasitized in the first patch (Fisher's LSD, P = 0.209) (Table 2.1), or in the number of eggs laid per pea aphid parasitized (Fisher's LSD, P = 0.636) (Table 2.2). Similarily, there was no difference between treatments three and four in the number of alfalfa aphids parasitized in the first patch (Fisher's LSD, P = 0.532), or in the number of eggs laid per alfalfa aphid parasitized (Fisher's LSD, P = 0.769). Significantly more aphids were parasitized in the first patch when it contained pea aphid than when it contained alfalfa aphid (Fisher's LSD, P < 0.001 in all cases) and significantly more eggs were laid per aphid parasitized (treatment 1 *vs* treatment 3: P = 0.045; treatment 1 *vs* treatment 4: P = 0.020; treatment 2 *vs* treatment 3: P = 0.017; treatment 2 *vs* treatment 4: P = 0.007).

Patch Two. There were significant differences among treatments with respect to both the number of aphids parasitized in the second patch (F = 10.843; df = 3, 74; P < 0.001) and the number of eggs laid per aphid parasitized (F = 7.355; df = 3, 69; P < 0.001). However, valid comparisons between second patches can only be made among treatments in which either the first or second patch contained the same host type, but not among treatments in which a different host occurred in both patches. When the first patch contained alfalfa aphids, significantly more pea aphids were parasitized in the second patch than were alfalfa aphids (Fisher's LSD, P < 0.001) and more eggs were laid per aphid parasitized (Fisher's LSD, P = 0.001). When the first patch contained pea aphid, significantly fewer alfalfa aphids were

parasitized in the second patch than were pea aphids (Fisher's LSD, P = 0.005) but there was no significant difference in the number of eggs laid per aphid parasitized (Fisher's LSD, P = 0.779). Significantly more pea aphids were parasitized in the second patch when the first patch contained alfalfa aphids than when it contained pea aphids (Fisher's LSD, P = 0.042) and more eggs were laid per aphid parasitized (Fisher's LSD, P = 0.002). On the other hand, there was no significant difference in the number of alfalfa aphids parasitized in the second patch regardless of whether the first patch contained pea aphid or alfalfa aphid (Fisher's LSD, P = 0.572), and no significant difference in the number of eggs laid per aphid parasitized (Fisher's LSD, P = 0.489).

Monoctonus paulensis - Comparison Across Patches. Significantly more aphids of both species were parasitized in the second patch than in the first when the first patch contained alfalfa aphid (Table 2.3), but there was no difference in the number of eggs laid per aphid parasitized (Table 2.4). There was no difference in number of aphids parasitized between first and second patches when both contained pea aphids, but significantly fewer eggs were laid per aphid parasitized in the second patch. There were significantly fewer aphids parasitized in the second patch than in the first when pea aphids were followed by alfalfa aphids, and significantly fewer eggs laid per aphid parasitized in the second patch.

Comparison Among Treatments. There were significant differences among treatments in the total numbers of aphids parasitized (F = 8.930; df = 3, 66; P < 0.001). More aphids in total were parasitized when pea aphid were in both patches than when alfalfa aphid occurred in the first patch (Fisher's LSD, P < 0.001), in the second patch (P = 0.008), or in both patches (Fisher's LSD, P < 0.001). There was no significant difference in total aphids parasitized when both patches contained alfalfa aphid compared to when the first patch contained pea aphid (Fisher's LSD, P =

Treatment	n	No. of aphids parasitized: Patch No.1	Patch No.2	Statistics	Total
1. A.p.-> A.p.	21	7.86 ± 0.43[b]	7.43 ± 0.47[c]	F = 1.971 P = 0.352	15.24 ± 0.85[b]
2. A.p.-> M.c.	17	7.47 ± 0.58[b]	3.24 ± 0.39[a]	F = 72.758 P < 0.001	11.82 ± 1.07[a]
3. M.c.-> M.c.	14	3.29 ± 0.56[a]	5.86 ± 0.59[b]	F = 11.865 P = 0.008	9.14 ± 0.88[a]
4. M.c.-> A.p.	18	2.44 ± 0.51[a]	7.83 ± 0.47[c]	F = 96.299 P < 0.001	10.28 ± 0.82[a]

Table 2.3. Mean numbers of aphids parasitized (± SE) by *M. paulensis* females foraging in two sequential host patches. Mated females (24 - 48 h old) were each confined twice for 30 min in a plastic petri dish containing either 15 pea aphids (A.p.) or 15 alfalfa aphids (M.c.) with 30 min between patches. Means within columns bearing the same letter were not significantly different among treatments.

Treatment	n	No. eggs laid per aphid: Patch No.1	Patch No.2	Statistics	Total
1. A.p.-> A.p.	21	1.58 ± 0.07[a]	1.28 ± 0.05[a]	F = 12.117 P = 0.004	1.45 ± 0.04[a]
2. A.p.-> M.c.	17	1.70 ± 0.08[a]	1.35 ± 0.09[a]	F = 20.815 P < 0.001	1.60 ± 0.07[a]
3. M.c.-> M.c.	14	1.61 ± 0.10[a]	1.43 ± 0.09[a]	F = 1.480 P = 0.494	1.44 ± 0.08[a]
4. M.c.-> A.p.	18	1.46 ± 0.13[a]	1.29 ± 0.08[a]	F = 1.998 P = 0.366	1.43 ± 0.08[a]

Table 2.4. Mean numbers of eggs laid per aphid parasitized (± SE) by *M. paulensis* females foraging in two sequential host patches. Mated females (24 - 48 h old) were each confined twice for 30 min in a plastic petri dish containing either 15 pea aphids (A.p.) or 15 alfalfa aphids (M.c.) and with 30 min between patches. Means within columns bearing the same letter were not significantly different among treatments.

0.055), or the second patch contained pea aphid (Fisher's LSD, P = 0.406). There were no significant differences among treatments in the number of eggs laid per aphid parasitized (F = 1.509; df = 3, 66; P = 0.220).

Patch One. There were significant differences among treatments in the numbers of aphids parasitized in the first patch (F = 30.132; df = 3, 66; P < 0.001). There was no significant difference between treatments one and two in the number of pea aphids parasitized in the first patch (Fisher's LSD, P = 0.584), or between treatments three and four in the number of alfalfa aphids parasitized in the first patch (Fisher's LSD, P = 0.279). Significantly more aphids were parasitized in the first patch when it contained pea aphid than when it contained alfalfa aphid (Fisher's LSD, P < 0.001 in all cases). There were no significant differences among treatments in the number of eggs laid per aphid parasitized in the first patch (F = 1.108; df = 3, 66; P = 0.353).

Patch Two. There were significant differences among treatments in the numbers of aphids parasitized in the second patch (F = 19.202; df = 3, 66; P < 0.001). When the first patch contained alfalfa aphid, significantly more pea aphids were parasitized in the second patch than were alfalfa aphids (Fisher's LSD, P = 0.007). When the first patch contained pea aphid, significantly fewer alfalfa aphids were parasitized in the second patch than were pea aphids (Fisher's LSD, P < 0.001). There was no significant difference in the number of pea aphids parasitized in the second patch regardless of which host was encountered in the first patch (Fisher's LSD, P = 0.531). On the other hand, significantly fewer alfalfa aphids were parasitized in the second patch when the first patch contained pea aphid compared to when it contained alfalfa aphid (Fisher's LSD, P = 0.001). There was no significant difference among treatments in the number of eggs laid per aphid parasitized in the second patch (F = 0.862, df = 3, 66; P = 0.465).

Experiment 2.

Aphidius ervi. There were significant differences among treatments with respect to both the numbers of pea aphids parasitized (F = 10.003; df = 2, 57; P < 0.001), and the number of eggs laid per pea aphid parasitized (F = 9.998; df = 2, 57; P < 0.001) (Table 2.5). Females reared in groups and exposed to pea aphids previously attacked by conspecifics parasitized a significantly larger number of pea aphids than either females reared in groups and exposed to unparasitized aphids (Fisher's LSD, P < 0.001), or solitary females exposed to unparasitized aphids (Fisher's LSD, P = 0.001). The difference in number of pea aphids parasitized was not significant between the latter two groups (Fisher's LSD, P = 0.547).

Females reared in groups and conditioned with aphids previously attacked by conspecifics laid significantly more eggs per pea aphid parasitized than did either grouped females conditioned with unparasitized aphids (Fisher's LSD, P = 0.009), or solitary females conditioned with unparasitized aphids (Fisher's LSD, P < 0.001). Differences between the last two groups were not significant (Fisher's LSD, P = 0.092).

Monoctonus paulensis. There were no significant differences among treatments in the number of pea aphids parasitized (F = 0.353; df = 2, 59; P = 0.704) (Table 2.6), but differences in the number of eggs laid per pea aphid parasitized were significant (F = 3.194; df = 2, 59; P = 0.048). Females reared in groups and conditioned with parasitized aphids laid significantly more eggs per pea aphid parasitized than did solitary females conditioned with unparasitized aphids (Fisher's LSD, P = 0.015). However, the difference in number of eggs laid per aphid parasitized was not significant between solitary and grouped females conditioned with unparasitized aphids (Fisher's LSD, P = 0.165), nor between grouped females conditioned with parasitized versus unparasitized aphids (Fisher's LSD, P = 0.305).

Treatment	n	No. Hosts Parasitized	No. Eggs/Aphid
Solitary, Unparasitized Hosts	20	7.75 ± 0.39[a]	1.49 ± 0.08[a]
Grouped, Unparasitized Hosts	20	7.45 ± 0.42[a]	1.86 ± 0.11[a]
Grouped, "Parasitized" Hosts	20	9.50 ± 0.19[b]	2.44 ± 0.23[b]

Table 2.5. Mean number of pea aphids parasitized and number of eggs laid per aphid parasitized (± SE) by *A. ervi* females receiving one of three adult experiences. Solitary females emerged alone and were permitted to mate, but did not encounter conspecific females; grouped females were reared communally with males. Prior to the experiment, females (24 - 48 h old) were conditioned with a 20 min exposure to either 10 unparasitized pea aphids, or 10 pea aphids that had been attacked 24 hrs earlier by conspecific females (= "Parasitized Hosts"), and rested for 20 min before testing. Each female foraged for 20 min in a petri dish containing 15 pea aphids, 10 of which were dissected after four days of rearing. Means within columns bearing the same letter were not significantly different in ANOVA.

Treatment	n	No. Hosts Parasitized	No. Eggs/Aphid
Solitary, Unparasitized Hosts	22	8.50 ± 0.37[a]	1.55 ± 0.06[a]
Grouped, Unparasitized Hosts	19	8.74 ± 0.36[a]	1.68 ± 0.09[ab]
Grouped, "Parasitized" Hosts	21	8.91 ± 0.32[a]	1.79 ± 0.07[b]

Table 2.6. Mean number of pea aphids parasitized and mean number of eggs laid per aphid parasitized (± SE) by *M. paulensis* females receiving one of three adult experiences. Solitary females emerged alone and never came into contact with conspecific females; grouped females were reared communally with males. Prior to the experiment, females (24 - 48 h old) were conditioned with 30 min exposure to either 10 unparasitized pea aphids, or 10 pea aphids that had been attacked 24 hrs earlier by conspecific females (= "Parasitized Hosts"), and rested for 30 min before testing. Each female foraged for 30 min in a petri dish containing 15 pea aphids, 10 of which were dissected after four days of rearing. Means within columns bearing the same letter were not significantly different in ANOVA.

DISCUSSION

Females of both *A. ervi* and *M. paulensis* parasitized fewer aphids in patches containing their less preferred host, alfalfa aphid, than in patches containing pea aphid (Tables 2.1 & 2.3), indicating that both species responded to differences in host quality and quantitatively adjusted their reproductive investment accordingly. A summary of the results for *A. ervi* and *M. paulensis* are compared to the hypothesized effects in Tables 2.7 and 2.8 respectively. The first hypothesis was not supported by the results for *A. ervi* females; experience with pea aphids did not reduce the number of alfalfa aphids parasitized in a subsequent patch, nor the number of eggs laid per alfalfa aphid parasitized. The second hypothesis had to be rejected for *A. ervi*; there was no significant increase in the number of alfalfa aphids parasitized in the second patch when the first patch contained alfalfa aphid, suggesting that patches of low quality hosts did not increase in value to females when high quality hosts were not encountered, at least within the time frame of this experiment. The decline in rate of parasitization by *A. ervi* females in a second patch of high value hosts is not predicted by models of optimal foraging (Stephens & Krebs 1986), which suggest that high value patches should always be exploited to the same extent (determined by the marginal rate of gain in a patch of that type). However, the result is consistent with a state-variable interpretation in which attack rate declines concurrently with egg load.

For *M. paulensis*, on the other hand, both the first and second hypotheses appeared partially supported (Table 2.8). Fewer alfalfa aphids were parasitized following experience with pea aphids as opposed to alfalfa aphids as hypothesized, although there was no difference in the number of eggs laid per alfalfa aphid parasitized. Thus experience with high quality hosts caused females to lower their reproductive investment in a subsequent patch of low quality hosts. More alfalfa aphids were parasitized in the second patch than in the first in treatment 3, although there

Treatment	Number of hosts parasitized in second patch *vs* first	Number of eggs laid per aphid in second patch *vs* first
1. Ap -> Ap	<*	=
2. Ap -> Mc	<	=*
3. Mc -> Mc	=*	<*
4. Mc -> Ap	>	>

Table 2.7. Effects of host sequence on the oviposition behaviour of *A. ervi* females. Asterisks indicate results contrary to those hypothesized. The hypothesis that fewer alfalfa aphids would be parasitized in second patches when the first patch contained pea aphid as opposed to alfalfa aphid was not supported (there was no significant difference), and neither was the hypothesis that fewer eggs would be laid per alfalfa aphid (no significant difference).

Treatment	Number of hosts parasitized in second patch *vs* first	Number of eggs laid per aphid in second patch *vs* first
1. Ap -> Ap	=	=
2. Ap -> Mc	<	= *
3. Mc -> Mc	>	=
4. Mc -> Ap	>	= *

Table 2.8. Effects of host sequence on the oviposition behaviour of *M. paulensis* females. Asterisks indicate results contrary to those hypothesized. The hypothesis that fewer alfalfa aphids would be parasitized in second patches when the first patch contained pea aphid as opposed to alfalfa aphid was supported, but the hypothesis that fewer eggs would be laid per alfalfa aphid was not (no significant difference).

was no significant difference in the number of eggs laid per alfalfa aphid parasitized. This observation is not consistent with conventional models of optimal foraging which assume the threshold for acceptance of a less profitable host is affected only by encounters with hosts of higher profitability (Jaenike 1978), although it may be consistent with models assuming a fixed host preference hierarchy with floating acceptance thresholds (Courtney *et al.*1989). It is possible that the threshold for acceptance of lower quality hosts decreases over time solely as a result of a lack of experience with high quality hosts.

A. ervi females responded to an increase in patch quality by increasing their rate of attack and oviposition, whereas *M. paulensis* females responded to a decrease in patch quality by reducing their rate of attack and oviposition. Females of both species parasitized more aphids in total when pea aphid was provided in both patches compared to any other treatment (Tables 2.1 & 2.3), and fewer aphids in total when alfalfa aphid was present in both patches compared to any other treatment.

M. paulensis females that encountered pea aphids in both patches parasitized similar numbers in each, as hypothesized, but *A. ervi* females parasitized fewer in the second patch, suggesting that egg load may have been reduced in *A. ervi* females that encountered pea aphids in the first patch. Reduced egg load as a result of foraging in a high quality patch would also explain why the number of eggs laid per aphid parasitized tended to be lower in second patches relative to first patches in treatments in which pea aphid occurred in the first patch.

However, *A. ervi* females superparasitized significantly more aphids in the second patch than in the first when pea aphids followed alfalfa aphids, (Table 2.2), supporting the idea that females increased their reproductive investment per aphid in response to an increase in host quality. Furthermore, in the first patch females laid significantly more eggs per pea aphid

parasitized than per alfalfa aphid parasitized, and superparasitized fewer aphids overall when alfalfa aphid occurred in both patches compared to when pea aphid occurred in one patch only (Table 2.1). These results suggest a tendency in *A. ervi* to adjust the rate of superparasitism according to relative host quality. Thus self superparasitism by *A. ervi* appears to be sensitive to both egg load and host quality in a manner that is independent of experience.

There were no significant differences in rates of superparasitism among first patches, second patches, or any treatments for *M. paulensis*. It should be noted that self superparasitism by *M. paulensis* often occurs during a single bout of host handling (see Chapter Three), whereas in the case of *A. ervi*, self superparasitism most likely results from repeated attacks. Nevertheless, fewer eggs per aphid were laid by *M. paulensis* females in the second patch than in the first when alfalfa aphid followed pea aphid, but there was no significant difference when pea aphid followed alfalfa aphid. Apparently, females decreased their reproductive investment per aphid in response to hosts that were relatively low in quality, but did not increase it in response to hosts that were relatively high in quality. Superparasitism declined in the second patch when pea aphid occurred in both patches, but there was no decline when alfalfa aphid occurred in both patches. This result may reflect a decline in egg load as a result of a high rate of oviposition into preferred hosts in the first patch, although *M. paulensis* provided with pea aphids in both patches parasitized similar numbers in each. Under these conditions, declining egg load in *M. paulensis* females apparently has an effect on rates of superparasitism before it affects the rate of attack on hosts. It can be argued that rates of parasitism and superparasitism are both influenced by egg load and are therefore not independent measurements, however the latter observation indicates that there may be justification for considering them separately.

M. paulensis has a relatively long host handling time (*ca* 45 sec, but see Chapter Three for details), compared to *A. ervi* which oviposits in less than a second, and this may partially explain the different results for these two species. *M. paulensis* females are not only slower than *A. ervi* females, but also subdue aphids by means of a paralytic venom (Calvert & van den Bosch 1972b) whereas *A. ervi* females do not. Thus *M. paulensis* makes a larger investment of both time and energy in each host parasitized, regardless of the number of eggs laid. *M. paulensis* females may be more reluctant to handle alfalfa aphids following experience with pea aphids that struggle less and have less noxious cornicle secretions (Chow 1989). The greater number of alfalfa aphids parasitized by *M. paulensis* females in the second patch suggests that they lowered their acceptance threshold for low quality hosts in the absence of high quality hosts, an effect not observed in *A. ervi* females. Females of different parasitoid species apparently vary in their response to a particular foraging experience, just as they vary in response to sensory cues in host evaluation.

Patterns of host acceptance arise not only from responses to individual hosts, but from strategies for progeny allocation within and among host patches. Decisions on how much to invest in each patch (brood size, patch residence time) and in each host (superparasitism) are somewhat interdependent because both decisions influence, and are influenced by, egg load. Therefore, decisions to accept, reject, or superparasitize individual hosts cannot be fully understood without considering patch investment strategies, just as the reverse is true. Even high quality hosts decline in value after a series of ovipositions because host value declines with egg load. If larger females have more eggs they may place a higher value on hosts and host patches than smaller females. However, these insects are pro-ovigenic and can mature more eggs in time, with the result that depreciation in host value may often be temporary.

However, the apparent decline in host value begins long before eggs are exhausted and may be adaptive in other contexts. The greater the number of hosts already parasitized, the smaller the fraction of a female's total reproductive effort remains to be allocated in the future. For iteroparous females, the balance between present versus future reproductive effort will change over time because an increasingly smaller fraction of total reproductive effort remains to be allocated (Bell 1980). It is therefore reasonable to expect that female oviposition tactics may change with age in a manner which is independent of egg load. At a more proximal level, if there is an optimal brood size (number of offspring placed in one patch) which is finite and independent of host quality, a patch will always be abandoned at some point regardless of the number of acceptable hosts remaining. For example, high rates of hyperparasitism could select for smaller brood size in primary parasitoids if offspring survival is improved by such risk-spreading behaviour (Ayal & Green 1993, Mackauer & Völkl 1993).

A. ervi females parasitized significantly more pea aphids in a 20 min period following exposure to pea aphids previously attacked by conspecific females, but solitary rearing versus rearing in groups had no effect on the number of hosts parasitized. A period of 24 hours is evidently adequate time for chemical changes in the host to occur which are detectable to females wasps. The value of a patch of unparasitized aphids to *A. ervi* females, as estimated by the number of hosts parasitized, appears to increase following encounters with aphids previously attacked by conspecifics, much as it does following encounters with a less-preferred host species.

A. ervi females reared in groups and exposed to previously parasitized pea aphids superparasitized more pea aphids than did females reared in groups and exposed only to unparasitized pea aphids, but the difference in rates of superparasitism between solitary and grouped females exposed only to unparasitized pea

aphids was not significant. Visser *et al.* (1992b) showed that females of *Leptopilina heterotoma* Thompson anticipate competition and superparasitize more hosts when confined with conspecific females prior to the experiment. However, these results indicate that encounters with previously parasitized hosts have a larger effect on rates of self superparasitism by *A. ervi* than do encounters with conspecific females. In the case of *M. paulensis*, the number of pea aphids parasitized, did not vary as a result of rearing in groups or exposure to parasitized aphids. However, there was apparently some interaction between these two experiences that resulted in elevated rates of self-superparasitism relative to solitary females that encountered only unparasitized aphids.

As I have demonstrated in Chapter One, each parasitoid species has a characteristic response profile to a particular array of sensory cues associated with the host. A female parasitoid probably uses innate responses to estimate host quality as a function of positive and negative host attributes. Some host stimuli may be noxious or deterrent (*e.g.* cornicle secretions, certain coloration), but others may be prerequisites for attack and acceptance (*e.g.* shape, movement *etc.*). In phytophagous insects, host preference is often inducable and more a function of experience than of innate response profiles (Dethier 1980, Jermy, 1987, Papaj & Rauscher 1987, Papaj & Prokopy 1989). However, Chow & Mackauer (1992) found that prior host experience had no effect on the subsequent host preference of *A. ervi*, *A. pisivorus* or *P. pequodorum*, and also concluded that females did not switch to exploiting a less preferred host when it became more abundant (Chow & Mackauer 1991). Similarily, my results do not indicate that female parasitoids learn to prefer the familiar, but rather that they compare the relative quality of the hosts and host patches they encounter and may adjust their oviposition tactics accordingly. There are few examples of quantitative variation in reproductive allocation as a function of experience in insects (Prokopy *et al.* 1986; 1989, Drost & Cardé 1990). My results

suggest that the value of a host (or a host patch) to a female aphidiid, as estimated by the number of eggs laid (or aphids parasitized), may vary according to whether the aphid (or patch) is judged inferior or superior in quality relative to aphids (or patches) previously encountered.

Much of the work on learning in parasitoids has focused on associative learning of odour cues associated with the host (Lewis & Tumlinson 1988, Papaj & Vet 1990, Turlings *et al.* 1990, Vet & Groenewold 1990, Lewis *et al.* 1991, Turlings *et al.* 1993). My results do not fall into the categories of associative or non-associative learning, nor of operant conditioning (*sensu* Smith, 1993), but under the broad definition of learning as a change in behaviour that occurs as a result of experience. Although there is some controversy over whether changes in behaviour following foraging experiences can be considered learning (see Rosenheim 1993), such behavioural modifications might have adaptive value if host quality and availability were relatively predictable within parasitoid generations, but relatively unpredictable between generations (Stephens 1993). This is because unpredictable host availability across generations would favour learning ability, and the value of the information would depend on how representative it was of actual host quality and availability at that time.

The complexity of the insect learning process is perhaps best understood for the foraging behaviour of honey bees (Gould 1984; 1991; 1993). Apparently, the rate of learning can vary with the sensory modality of the cue; odors are quickly learned, colours take longer, and shape recognition the longest. Bees can quickly learn which complexes of cues are consistently associated with the highest rewards, in terms of volume and concentration of nectar, and which are unrewarding. However, parasitoid females that have been well fed (as were all those used in these experiments) forage for reproductive opportunities for which they are 'rewarded' with ovipositions, rather than food. While hosts may be continuously variable in quality, they are discretely acceptable

or unacceptable. In contrast to a bee, which may continuously adjust its allocation of time and effort while collecting nectar from flowers that vary in quality, a parasitoid female is limited to making one of two discrete decisions: lay an egg or not, and if yes, to lay one or more. A qualitative response to host quality is also possible in terms of offspring sex allocation and other work has shown that female offspring may be allocated more often to (relatively) larger or higher quality hosts (Charnov 1982, van den Assem *et al.* 1984, Werren 1984, Cloutier *et al.* 1990). However, these results indicate that female parasitoids make quantitative adjustments of local brood size and rate of superparasitism in response to recently obtained information on the availability of hosts and their relative quality.

Chapter III

Intrinsic and Extrinsic Factors Influencing Reproductive Allocation by *Monoctonus paulensis*

INTRODUCTION

In the previous chapter I demonstrated that a female parasitoid may adjust her foraging tactics in response to various experiences. In this chapter I examine factors, both extrinsic and intrinsic, which influence the allocation of eggs by *M. paulensis* females to individual hosts, *i.e.* superparasitism, and to patches of hosts, *i.e.* patch exploitation. Self superparasitism by*M. paulensis* has been previously reported in the field (Calvert & van den Bosch 1972a) and my preliminary observations revealed that mated females self-superparasitized many of the pea aphids they attacked during a single bout of host handling. This finding is unusual in that self superparasitism is most frequently observed when unparasitized hosts are scarce (Singh & Sinha 1982b, Waage 1986). It is also at variance with current theories of adaptive superparasitism by solitary parasitoids (Waage 1986, Visser *et al.* 1992a) which predict that a female should place only a single egg into each unparasitized host she encounters, unless host density is very low.

The laying of more than one egg per host by a solitary parasitoid (superparasitism) results in larval competition as only one offspring can survive and complete development. Self superparasitism (oviposition into a host containing a female's own progeny) can be distinguished from conspecific superparasitism (oviposition into a host containing the offspring of a conspecific female). Conspecific superparasitism can be viewed as a form of interference competition among females attacking the same hosts (Bakker *et al.* 1985, van Alphen 1988, Visser *et al.* 1992a); females competing within a patch must lay additional eggs in hosts they have already parasitized in order to improve the survival of their offspring. Females usually prefer to oviposit in unparasitized hosts, but should accept those parasitized by other females if the second larva has some chance of winning the competition for the host (Bakker *et al.* 1985, Visser *et al.* 1992a, Visser *et al.* 1992c). Because self superparasitism results in

competition among siblings, the fitness payoffs are thought to be lower than those from conspecific superparasitism (Hubbard *et al.* 1987, Mackauer 1990, van Alphen & Visser 1990, van Dijken *et al.* 1992). Nevertheless, self superparasitism by solitary parasitoids has been observed in the field, as recently reported by van Dijken *et al.* (1993) for *Epidinocarsus lopezi* (DeSantis).

A female that lays two eggs in a host makes a greater reproductive investment than does a female laying only one. In Chapter Two I observed that superparasitism by females of *A. ervi* declined after they encountered a patch of their preferred host, *A. pisum*, presumably as a consequence of declining egg load (= the number of mature eggs available for oviposition). Theoretically, self superparasitism should cease at the point where the fitness payoff is greater from maximizing the utility of each egg, rather than that of each host (Iwasa *et al.* 1984). I hypothesized that the first few aphids attacked by a female might have a higher probability of superparasitism than those attacked subsequently. I also hypothesized that host handling time might be correlated with the number of eggs laid in an aphid; time and eggs are both 'currencies' of fitness and it might take a female longer to lay two eggs than one. Recent work on parasitoid foraging behaviour has underlined the importance of physiological 'state' variables (Mangel & Clark 1986), particularly egg load (Iwasa *et al.* 1984, Collins & Dixon 1986). I hypothesized that the tendency of *M. paulensis* females to superparasitize would decline with egg load, *i.e.* eggs would become increasingly valuable relative to hosts after a series of ovipositions. However, an effect of experience with hosts may alter behaviour in a manner which is difficult to distinguish from effects of changes in egg load (Rosenheim & Rosen 1991). I therefore designed an experiment to resolve any confounding effects of prior host experience from true effects of egg load.

For a female with abundant eggs, self superparasitism may constitute adaptive behaviour whenever the survival of her

offspring increases as a positive function of the number of eggs laid. This may be true in at least two circumstances: (1) when hosts are at risk of attack by other parasitoids (Cloutier 1984, Visser *et al.* 1990, Visser *et al.* 1992b, Visser 1993), or (2) when sibling larvae overwhelm host defences that vanquish solitary larvae (Streams 1971, Puttler 1974, van Alphen & Visser 1990). There is evidence that the fitness gained by an aphidiid wasp increases as a function of the number of eggs laid into a multiply-parasitized host (Mackauer *et al.* 1992). Self superparasitism may therefore increase a female's reproductive success when hosts are in short supply relative to eggs. However, there may be a cost of rejection in terms of time and energy for species like *M. paulensis*, which have an extensive host handling time, particularily if discrimination of parasitized hosts is not possible prior to attack. Indiscriminate oviposition should occur whenever the costs of rejecting parasitized hosts outweigh the benefits of discrimination (Speirs *et al.* 1991).

It has been suggested that host density is a factor influencing levels of superparasitism (Cloutier 1984, Laurence 1988), but I suspected that self superparasitism by *M. paulensis* was not a response to low host density. From a female's perspective, host availability can be estimated from her encounter rate with unparasitized hosts. I hypothesized that the number of aphids parasitized in a short period would decline with host encounter rate, whereas the number of eggs laid per aphid would remain constant, on the assumption that rate of parasitism would be more sensitive to encounter rate than rate of superparasitism. To test this, I devised an experiment in which females foraged in petri dishes of three different sizes. A spatial manipulation of density was deemed to be a more realistic way of generating different rates of host encounter than a temporal manipulation of exposure time, or varying the number of hosts in an arena of constant size. Small numbers of pea aphids tend to distribute themselves more or less randomly over the entire surface of a petri dish. Provided that the activity levels of aphids are

relatively constant size across arenas of various sizes, host encounter rate would be a linear function of the number of aphids per unit area.

In gregarious parasitoids, the number of progeny allocated to a particular host constitutes a discrete clutch. Consequently, such species have been the subject of many experiments designed to test theories of clutch size (Werren 1980, Charnov & Skinner 1984, Parker & Courtney 1984) and sex ratio (Putters & van den Assem 1985, King 1987). In solitary parasitoids, female reproductive investment can be measured at the level of the individual host (number of eggs laid per host), and at the level of the host patch (patch time, number of hosts parasitized). Whether one considers a patch to be an infested leaf, plant, or cluster of plants, the offspring allocated to a patch can be considered a brood, which is analagous to the clutch produced by a gregarious parasitoid in a single host. Brood size is a key life history trait (Stearns 1976) and female parasitoids may have evolved particular strategies for distributing broods among host patches. Adaptive adjustment of brood size can occur in response to particular experiences or physiological states (Hemerik *et al.* 1993). I hypothesized that virgin females would employ more conservative oviposition tactics than mated females because of their inability to produce daughters.

A number of parasitoid females may oviposit as virgins and produce exclusively sons (Godfray & Hardy 1993). In some gregarious parasitoids, females produce smaller clutches than mated females (*e.g. Apantales glomeratus* L., Tagawa 1987), while in solitary parasitoids the ovipositional activity of virgins may be lower than that of mated females (Donaldson & Walter 1984, Li *et al.* 1993). Differences in oviposition behaviour between virgin and mated females would be expected if the optimum size of an all-male brood was different from that of a mixed brood, or if all-male broods required some particular distribution among host patches in order to maximize maternal fitness. I hypothesized

that virgin females would allocate fewer eggs per host, and fewer offspring per patch, than their mated counterparts because male offspring produced without female siblings would contribute less to maternal fitness on average than would offspring in mixed broods. I therefore examined the effects of mating status on progeny allocation by *M. paulensis* females at the level of both individual hosts, and host patches.

Older females have a lower life expectancy and fewer remaining reproductive opportunities relative to young females. As a result, the value of individual hosts, or host patches, may increase with female age. When unparasitized hosts were not available to *E. californicus*, older females were more likely to accept pea aphids previously parasitized by a conspecific (Völkl & Mackauer 1990). Discrimination among age classes of aphids was more pronounced in young females of *L. cardui* (Weisser 1994). Furthermore, ovipositions by older females were longer in duration and they remained longer in host patches than younger females. I tested the hypothesis that hosts and host patches might become more valuable to *M. paulensis* females as they aged, predicting that 6-day-old virgins would superparasitize more aphids than their 2-day-old counterparts, remain longer in petri dish patches, and parasitize more hosts.

Parasitoids may sample their environment and modify their tactics for allocating progeny to hosts and host patches according to information available to them (Haccou *et al.* 1991, Roitberg *et al.* 1992, Visser *et al.* 1992b). Do encounters with conspecifics, or hosts previously parasitized by conspecifics, cause females to anticipate competition and adjust their oviposition tactics accordingly? In the previous chapter I observed that these two experiences together increased the rate of superparasitism by mated *M. paulensis* females. In preliminary experiments I observed that virgin females were less prone to self-superparasitize than were mated females. I hypothesized that the experience of encountering other females would cause virgins to

increase their rate of superparasitism. I further hypothesized that exposure to aphids previously attacked by conspecifics would generate 'pessimistic' expectations of host availability (*sensu* Roitberg 1990) and cause virgin females to remain longer in patches, and parasitize more aphids, than virgins that never encountered conspecifically-parasitized aphids.

MATERIALS AND METHODS

Insect Colonies

Both aphid and parasitoid colonies were reared as described in Chapter I. All pea aphids used in experiments were late second instars (72 ± 6 h of age at 20°). All parasitoid females used in experiments emerged from mummies placed singly into gelatin capsules. Adults were transferred to a wax paper cup with a bean stem and honey within 16 h of emergence. Females referred to as 'mated' were caged individually with 2 males each, whereas those referred to as 'virgin' were caged alone. All parasitoid females used in experiments were 24-48 h of age unless otherwise specified.

Aphids were provided to individual females in plastic petri dishes 6 cm x 1 cm unless otherwise specified. In all experiments, aphids from each replicate were reared separately on a bean shoot and a subsample (5/6, 10/12, or 20/25), dissected after 4 days of rearing. In this manner a constant number of aphids could be dissected from each replicate and occasional dead aphids, ignored. Replicates in which no aphids were parasitized were excluded from the analysis.

Serial Ovipositions

I observed 40 mated females individually as they attacked pea aphids in a petri dish. Each female was permitted to attack 4 aphids on her second day of life and another 4 on her third day of life. Aphids were removed following attack and placed in individual clip cages fastened to a bean plant, the handling time, and order of attack, recorded.

Host Density

I tested the effect of host density on rate of superparasitism by providing females with a constant number of pea aphids (25 per petri dish) in arenas that varied in size; large (14.0 cm x 2.2

cm), medium (8.7 cm x 1.2 cm), and small (5.4 cm x 1.2 cm). The interior cylindrical surface areas were determined by the equation: $2\pi r(h + r)$ as 404.6 cm^2, 151.7 cm^2, and 66.2 cm^2 respectively. Two-day-old, mated *M. paulensis* females (n = 60) were each confined in a dish for 75 min, a period that was judged adequate to obtain significant parasitism in all treatments without being sufficiently long to result in high rates of re-attack in the high density treatment. Twenty aphids were dissected from each replicate after rearing for 4 days.

Egg Load

I dissected a total of 48 virgin females to count the mature eggs in their ovaries, 16 at < 1 h post-eclosion, 16 at 24 ± 4 h old, and 16 at 48 ± 4 h old. The ovaries were dissected out, placed in a drop of saline on a glass slide, and ruptured with a coverslip to release the eggs, which were then counted under low magnification in a compound microscope. Twenty females were caged individually with two males and 60 hosts each for 12-18 h while 22 control females were confined with two males each and no hosts. The following day, each female was permitted to attack 6 aphids, a number deemed sufficient to estimate levels of superparasitism without creating significant variation in egg load among females. The parasitoids were then immediately dissected in order to count the number of eggs in their ovaries. Five of the 6 aphids in each replicate were dissected to count the numbers of eggs and larvae they contained.

I repeated this experiment using a slightly different design in order to resolve any effect of prior experience with hosts from an effect of egg load. The experiment was designed so as to reveal whether or not females can mature additional eggs, and regain their tendency to superparasitize, following ovipositional activity. Seventeen females were confined individually with two males each for 2 days without hosts, while another 17 were each confined with 2 males and 60 pea aphids for the first day, but with none for the second day. A third group of 17 were each

confined with 2 males and 60 pea aphids for the first day and received an additional 60 pea aphids on the second day. On the third day, each was permitted to attack 6 aphids, 5 of which were dissected. Each female was dissected immediately following oviposition, the ovaries removed and the eggs counted.

Mating Status

Individual females, virgin (n = 24) and mated (n = 23), were permitted to attack a total of 12 pea aphids in a petri dish. Each attacked aphid was immediately removed to a bean plant and replaced with another. Ten aphids from each replicate were dissected after 4 days of rearing to count the number of larvae they contained. To see if superparasitism was affected by the age of females, this experiment was replicated using virgin females (n = 11) that were 6 days old and the results compared with those for 2-day-old virgins.

I compared the patch residence times of virgin and mated females and the number of aphids they attacked in an open patch. Two-day-old virgin (n = 20) and mated (n = 20) females were each provided with a petri dish containing 10 aphids; attacked aphids were not replaced. Ten aphids was selected as a patch size that would render the experiment manageable. Aphids were placed into the dish < 20 min prior to the start of the experiment so that accumulation of honeydew would be minimal. Once a female attacked an aphid the lid of the dish was removed to permit her departure. The number of aphids attacked by each female was recorded and patch residence time calculated from a females' first attack to the time of her departure. To see whether the value of host patches would increase with female age, this experiment was repeated with 20 virgin and 20 mated females that were 6 days old but had no experience with hosts.

Conspecific Encounter

Upon emergence, females were caged overnight either singly (n = 24) or in groups of 5 (n = 22) and had no contact with males. The following day, females were placed individually into a petri dish containing 15 pea aphids and permitted to attack 12. Each attacked aphid was removed to a bean plant and replaced with another. Ten aphids were dissected from each replicate after rearing for 4 days.

Exposure to Parasitized Hosts

Within 16 hours of emergence, virgin females were divided into 2 groups of 20. Those of one group were then individually introduced into a petri dish containing 20 unparasitized pea aphids, while those of the second group received 20 pea aphids that had been attacked by conspecific females 24 h previously. Given that > 90% of attacks on pea aphids result in successful parasitization, I refer to this treatment as "exposure to previously parasitized aphids". Females were left to forage undisturbed for 20 min and were then removed to individual containers. The following day, each female was introduced into a petri dish containing 20 unparasitized pea aphids. Once a female made her first attack on an aphid, the lid of the dish was removed to permit her to leave at will. The number of aphids attacked by each female was recorded and patch residence time calculated from a females' first attack to her departure from the dish. Attacked aphids were removed and replaced immediately and all aphids were dissected from each replicate after rearing.

Using the SYSTAT© statistical package (Wilkinson 1989), I compared differences between treatment means with a one-way ANOVA followed by a Fisher's LSD test in cases where 3 groups were compared, and by linear regression in the host density experiment.

RESULTS

Serial Ovipositions

Mated *M. paulensis* females superparasitized the majority of pea aphids they attacked in the laboratory during a single bout of host handling on 2 consecutive days (Table 3.0). The mean handling time was 41.7 ± 4.5 sec/host, with a weak correlation between handling time and the number of eggs laid ($Y = 10.624 + 0.382X$, $r^2 = 0.152$, $P < 0.001$). Oviposition sequence did not affect the mean number of eggs laid per parasitized aphid on the first day of testing (mean ± SE = 1.78 ± 0.08, $F = 1.938$, df = 3, 103, $P = 0.128$), or on the second day (mean ± SEM = 1.57 ± 0.06, $F = 0.576$, df = 3, 87, $P = 0.632$). The mean number of eggs laid per parasitized aphid differed significantly between day 1 and day 2 ($F = 7.908$, df = 1, 204, $P = 0.005$).

Host Density

The number of aphids parasitized declined with an increase in the surface area of the arena (Fig 3.0). The relationship between the number of aphids parasitized was adequately described by a linear regression equation ($r = 0.39$; regression coefficient = -2.353 (SE = 0.78); F-ratio= 9.134; df = 1, 51; $P = 0.004$). However, the numbers of eggs laid per aphid parasitized did not vary among treatments (mean = 1.498 ± 0.09; $r = 0.013$; regression coefficient = -0.004 (SE = 0.04); F-ratio = 0.008; df = 1, 49; $P = 0.929$).

Egg Load

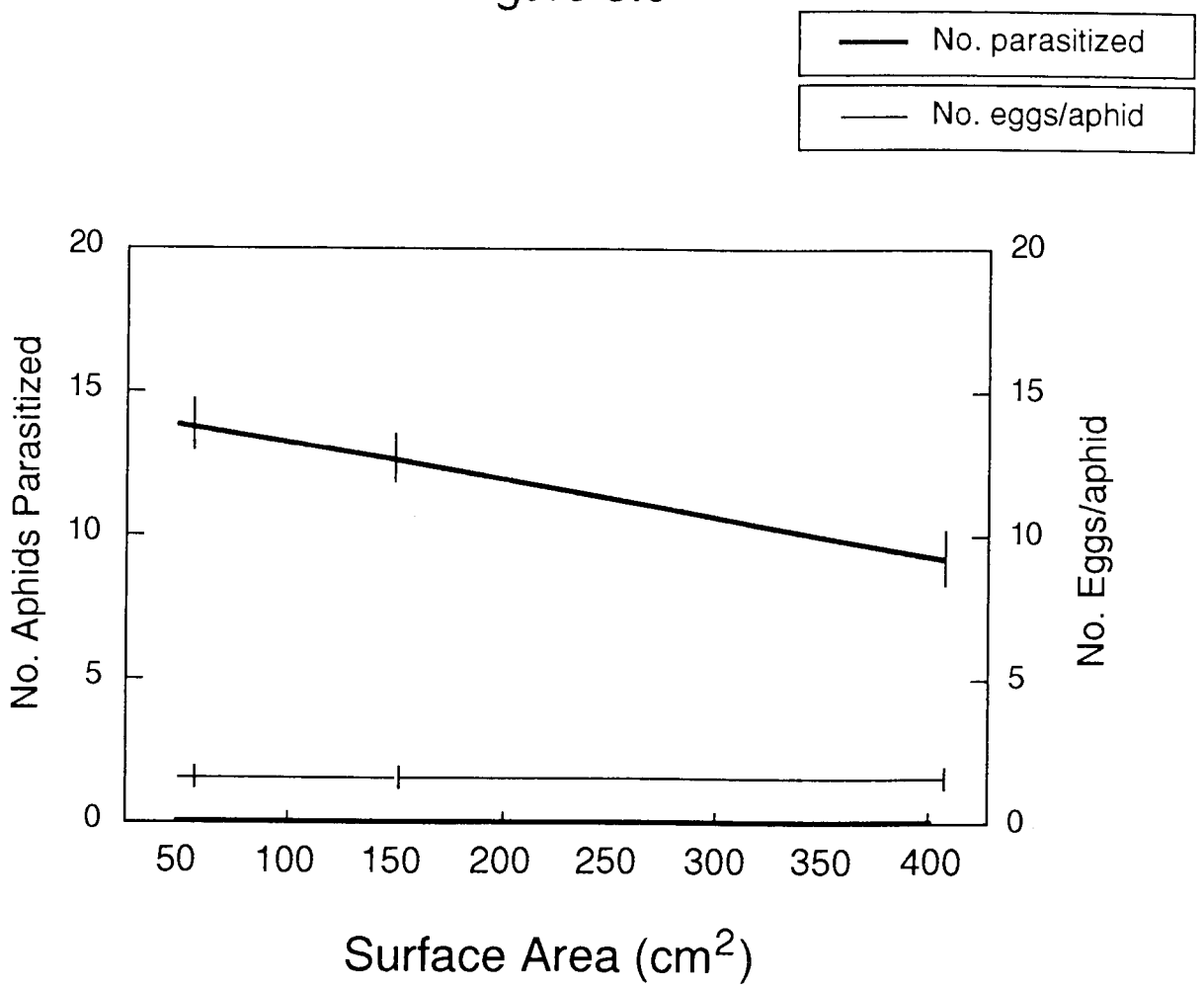
Virgin females (n = 48) contained a mean of 64 ± 3.9 mature eggs in their ovaries at emergence, which increased to 150 ± 4.5 at both 24 h (± 6 h) and 48 h (± 6 h). When mated females (n = 20) were caged overnight with 60 aphids each on their first day of life they laid a mean (± SE) of 1.02 ± 0.01 eggs per aphid the following day and contained a mean of 62.6 ± 3.8 eggs in their ovaries. Females that were caged without hosts for their first day of life

	Day 1				Day 2			
Aphid Attacked	1st	2nd	3rd	4th	1st	2nd	3rd	4th
n	24	29	26	27	22	24	24	20
No. eggs	2.1 ± 0.26	1.8 ± 0.15	1.6 ± 0.13	1.7 ± 0.10	1.5 ± 0.11	1.7 ± 0.13	1.5 ± 0.12	1.6 ± 0.11

Table 3.0. Mean number of eggs laid per aphid parasitized (± SE) in four sequential by mated *M. paulensis* females on 2 successive days. Females were each permitted to attack 4 pea aphids on the 2nd and 3rd day following their emergence. Each aphid was handled by a female only once.

Figure 3.0. Influence of host density on the number of aphids parasitized (± SE) and the number of eggs laid per aphid parasitized (± SE) by mated females of *M. paulensis*. The regression equations were: $y = 7.068 - 2.353\ (\pm\ 0.78)x$, $P = 0.004$ (number of aphids parasitized) and $y = 1.498 - 0.004\ (\pm\ 0.04)x$, $P = 0.929$ (number of eggs/aphid parasitized).

Figure 3.0


No. parasitized
No. eggs/aphid
No. Aphids Parasitized
No. Eggs/aphid
0
5
10
15
20
50
100
150
200
250
300
350
400
Surface Area (cm2)

(n = 22) laid a mean of 1.66 ± 0.07 eggs per aphid the following day and contained a mean of 100.0 ± 4.0 eggs in their ovaries. These differences were significant (No. eggs laid: F = 74.892; df = 1, 40; P < 0.001; No. eggs in ovaries: F = 45.160; df = 1, 40; P < 0.001).

Results of the second egg load experiment are shown in Table 3.1. There were significant differences among treatments in the number of eggs laid per aphid parasitized (F = 11.716; df = 2, 49; P < 0.001) and numbers of eggs remaining in the ovaries of females (F = 45.622; df = 2, 49; P < 0.001). Mated females that were caged without aphids contained significantly more eggs in their ovaries on the third day than those which had received aphids continuously over 2 days (Fisher's LSD, P < 0.001). However, females caged with aphids on their first day of life, but none on the second, contained more eggs in their ovaries on the third day than those with continuous access to aphids (Fisher's LSD, P < 0.001) or no aphids (Fisher's LSD, P = 0.004). Females with continuous access to aphids laid fewer eggs per aphid than those that received either no aphids (Fisher's LSD, P < 0.001), or aphids only on the first day (Fisher's LSD, P < 0.001). The difference between the latter 2 groups was not significant (Fisher's LSD, P = 0.699).

Mating Status

The effects of mating status on superparasitism are shown in Table 3.2. There were differences among treatments in the number of aphids parasitized (F = 3.308; df = 2, 55; P = 0.044), but the only significant difference among means was between 2-day-old mated females and 6-day-old virgins (Fisher's LSD, P < 0.036). There were also differences in the numbers of eggs laid per aphid parasitized (F = 11.098; df = 2, 55; P < 0.001). Two-day-old mated females laid more eggs per aphid than did either 2-day-old or 6-day-old virgins (Fisher's LSD, P < 0.001 and P = 0.008 respectively, Table 3.2). There were no significant differences between 2

Variable	Treatment		
	No Hosts	Hosts on day 1 only	Hosts on days 1 and 2
n	17	18	17
Eggs / aphid	1.37 ± 0.06[b]	1.31 ± 0.07[b]	1.04 ± 0.02[a]
Eggs present in ovaries	130.2 ± 6.6[b]	167.3 ± 9.4[c]	63.0 ± 6.9[a]

Table 3.1. Mean numbers of eggs laid per aphid parasitized and numbers of eggs remaining in the ovaries of *M. paulensis* females (± SE) receiving one of three treatments. Each mated female received; (1) no aphids for the first two days of life, (2) 60 pea aphids on the first day but none on the second day, (3) access to 60 pea aphids on both days. Means within rows bearing the same letter were not significantly different ($P > 0.05$) in a one-way ANOVA followed by Fisher's LSD.

Variable	2-day-old mated	2-day-old virgin	6-day-old virgin
n	23	24	11
No. aphids parasitized	9.52 ± 0.17^{b}	9.33 ± 0.14^{ab}	8.64 ± 0.47^{a}
No. eggs / aphid	1.50 ± 0.04^{b}	1.27 ± 0.04^{a}	1.30 ± 0.05^{a}

Table 3.2. Mean number of pea aphids parasitized and number of eggs laid per aphid parasitized (± SE) by 2-day-old mated *M. paulensis* females, and 2- and 6-day-old virgins. Means within rows bearing the same letter were not significantly different ($P > 0.05$) in a one-way ANOVA followed by Fisher's LSD.

and 6-day-old virgins in the number of eggs laid per aphid parasitized (Fisher's LSD, P = 0.890).

Two-day-old mated *M. paulensis* females remained in host patches longer than their virgin counterparts (F = 37.373; df = 1, 38; P < 0.001), and attacked a larger proportion of available hosts (F = 12.034; df = 1, 38; P < 0.001; Fig 3.1). There were no differences in patch residence times (F = 1.651; df = 1,38; P = 0.206) or numbers of hosts attacked (F = 0.143; df = 1, 38; P = 0.707) for 6-day-old virgin and mated females (Fig 3.2). No differences between 2- and 6-day-old mated females were observed in either patch time (F = 2.120; df = 1, 38; P = 0.154) or the number of aphids attacked (F = 1.462; df = 1, 38; P = 0.234).

Exposure to Conspecific Females

Virgin females caged overnight in groups of 5 laid significantly more eggs per aphid parasitized on their second day of life than did virgin females caged alone (Table 3.3). Grouped females appeared to be more successful in parasitizing aphids than solitary females, but a t-test of significance revealed that the null hypothesis could not be rejected (t = 1.875, df = 1, 21), *i.e.* the difference observed in ANOVA was not significant.

Exposure to Parasitized Hosts

Virgin females that encountered conspecificly-parasitized aphids on their first day of life remained longer in host patches than did females exposed to unparasitized aphids (Table 3.4), attacked more aphids, and laid a larger number of eggs. The difference in number of eggs laid per aphid parasitized was not significant.

Figure 3.1. Mean patch residence times and numbers of aphids attacked (+ SE) by 2-day-old virgin and mated *M. paulensis* females. Each female was released into an open petri dish containing 10 aphids. Patch times were calculated from time of first attack to a female's departure from the dish. Differences in patch times and numbers of hosts attacked were both significant to $P < 0.001$ in a one-way ANOVA.

Figure 3.1

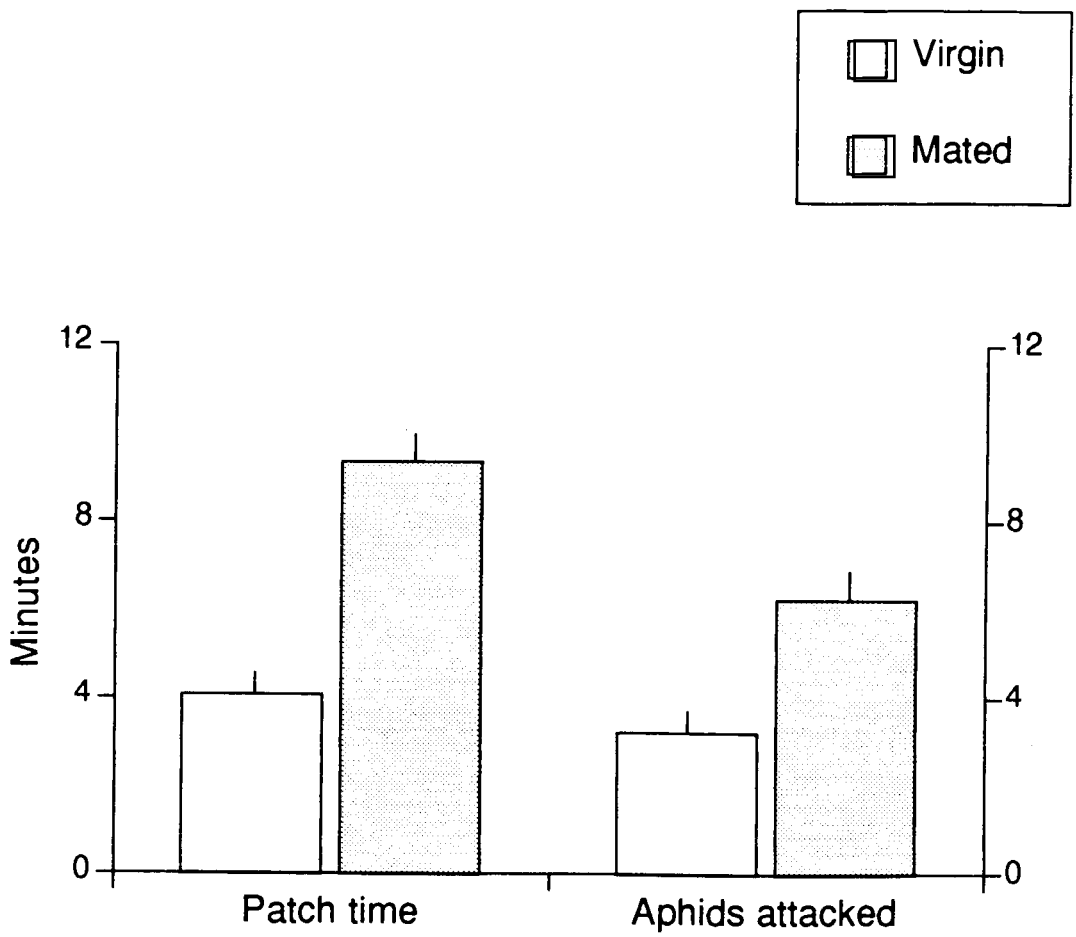
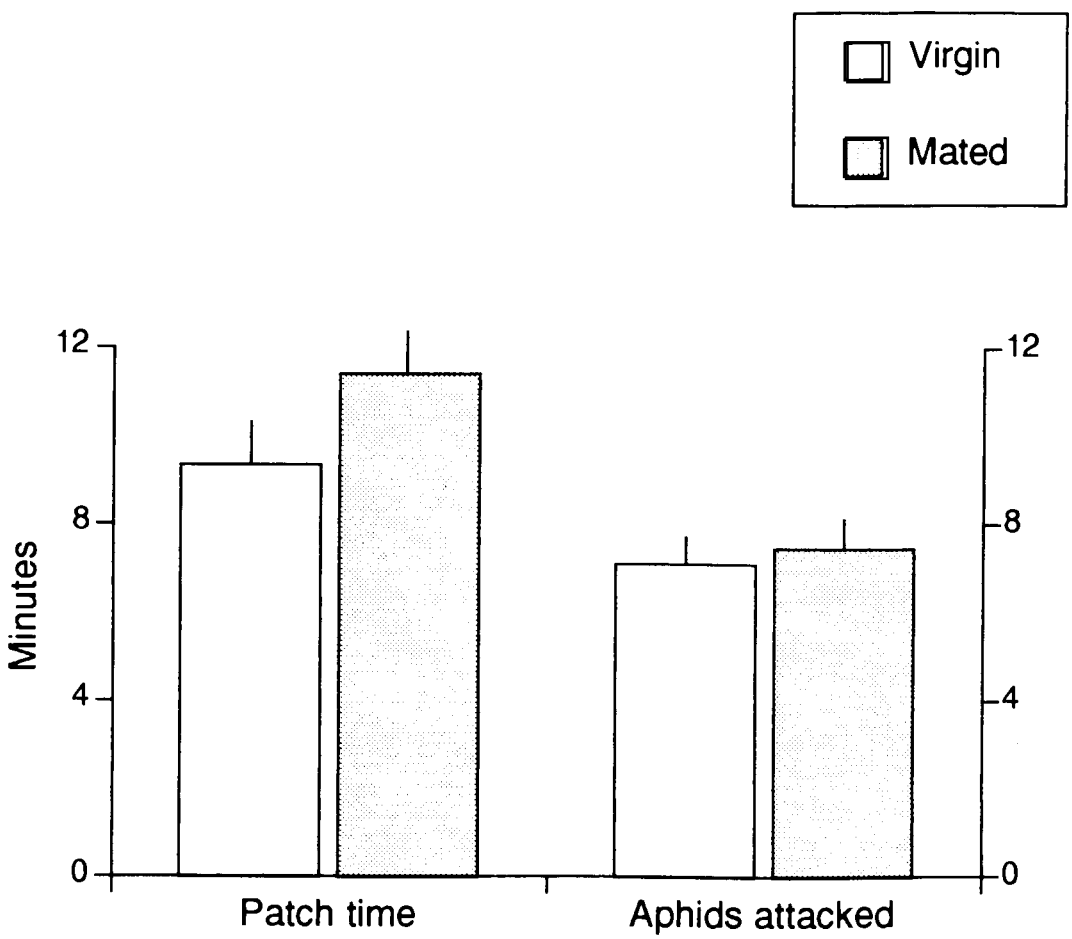


Figure 3.2. Mean patch residence times and numbers of aphids attacked (+ SE) by 6-day-old virgin and mated *M. paulensis* females. Each female was released into an open petri dish containing 10 aphids. Patch times were calculated from time of first attack to a female's departure from the dish. Differences in patch times and numbers of hosts attacked were not significant ($P > 0.05$) in a one-way ANOVA.

Figure 3.2



Variable	Solitary	Grouped	ANOVA
n	24	22	
No. parasitized	6.58 ± 0.41	7.73 ± 0.40	$F = 3.96$, P = 0.052*
No. eggs / aphid	1.15 ± 0.03	1.30 ± 0.05	$F = 6.45$, P = 0.015

Table 3.3. Mean numbers of pea aphids parasitized and eggs laid per aphid parasitized (± SE) by 2-day-old virgin *M. paulensis* females caged either alone or in groups of five overnight. * *t*-test of significance revealed that there was no difference in number of aphids parasitized.

Variable	Unpar'd Host Experience	Parasitized Host Experience	ANOVA
n	20	18	
Patch time (min)	6.1 ± 0.74	11.8 ± 1.26	F=16.03, P<0.001
No. attacked	5.0 ± 1.03	10.1 ± 1.64	F=07.09, P=0.012
No. eggs laid	4.3 ± 0.77	11.2 ± 1.83	F=13.03, P=0.001
No. eggs / aphid	1.36 ± 0.10	1.41 ± 0.07	F=00.16, P=0.689

Table 3.4. Mean patch residence times, number of aphids attacked, number of eggs laid per female, and number of eggs laid per aphid parasitized (± SE) by virgin *M. paulensis* females receiving one of two conditioning treatments. Females were exposed for 20 min to either 15 unparasitized aphids, or 15 aphids attacked by a conspecific female 24 hrs earlier. The following day females were released into an open plastic petri dish containing 15 aphids. Patch residence times were calculated from the first attack to a female's departure from the dish.

DISCUSSION

Mated *M. paulensis* females frequently laid more than one egg in a pea aphid during a single attack (Table 3.0). This is at variance with the prediction that solitary parasitoids should never self-superparasitize when they search a patch alone (Visser *et al.* 1992a). Although self superparasitism by *M. paulensis* could function to secure hosts against attack by other females, this behaviour was observed even when females had not encountered conspecifics.

In a study designed to resolve the influences of egg load and prior host experience on the clutch size of a gregarious parasitoid, *Aphytis lingnanensis* Compere, Rosenheim & Rosen (1991) showed that clutch size was reduced when egg load declined, or following recent host encounters. My results are consistent with these findings and reveal that *M. paulensis* females only self-superparasitize when they possess an abundant egg supply, *i.e.* when they have been deprived of hosts for the previous 24 h. Additional eggs are matured within this period, a process apparently stimulated by exposure to hosts; females that received hosts on their first day of life had more eggs in their ovaries on the third day than did females that received no hosts (Table 3.1). The fact that females of the former group did not superparasitize more than those of the latter suggests that there is a threshold effect of egg load on superparasitism. Some critical number of eggs must be present before a female will superparasitize, but further increases in egg load apparently do not result in more superparasitism. Females ovipositing on the first day, but not the second, superparasitized as many aphids on the third day as did females that had not previously oviposited, indicating that superparasitism is dependent on egg load and does not result from a lack of experience with hosts.

Two-day old virgin *M. paulensis* females superparasitized fewer pea aphids than did mated females of the same age (Table 3.2). The lower rate of superparasitism among virgin females

may reflect a smaller reproductive investment in each host relative to mated females. This would be expected if sons represent a high-risk investment when produced without female siblings. Males must mate to leave offspring, but unmated females can achieve some fitness through the production of sons. Aside from the possibility that sperm competition may occur within females if they mate more than once, the reproductive success of males will be largely determined by the number of matings they achieve, whereas that of females will be determined by the number of hosts they succeed in parasitizing (Hamilton 1967). Thus the fitness acquired by a female through her sons is a function of the number which succeed in mating, whereas that acquired through daughters is a function of the number which succeed in finding hosts. Mate competition is expected among male parasitoids because females of most species are thought to mate only once, whereas males can mate with many females (Stary 1970). Wilkes (1965) observed that males of *Dahlbominus fuscipennis* (Zett.) can inseminate at least 25 females. Daughters represent a safe investment relative to sons, not only because they need not mate to reproduce, but because the reproductive success of males is unpredictable (Thornhill & Alcock 1983) and varies with sex ratio and local mate competition (Hamilton 1967). Hence the mean fitness of unmated females is probably lower than that of mated females.

In an independent study I determined that mated *M. paulensis* females produce female-biased broods when foraging under the conditions of these experiments (Mean sex ratio = 83.4 %, n = 174 broods, containing 3005 offspring). Self superparasitism may therefore reflect a propensity to invest more per host when daughters are produced but, since only surviving adults could be sexed, I was unable to confirm that mated females superparasitize with fertilized eggs. Alternatively, virgin females may conserve eggs for the purpose of producing daughters later on if a mate can be found, or if their lifespan can be extended by so doing. The latter effect might be important in species capable

of resorbing eggs, but Aphidiids cannot (Stary 1970). However, the lower rate of superparasitism by virgin females seems to be independent of age; 6-day-old virgins did not superparasitize any more hosts than did 2-day-old ones, despite presumably lower expectations of finding a mate. Thus virgin females behave as though they were egg-limited and maximize the utility of eggs, whereas mated females behave as though they were time-limited and maximize the utility of each host (Iwasa *et al.* 1984). In this context, the higher rate of self superparasitism by mated females may reflect an increase in the value of hosts after mating that is independent of their quality. Thus two of my first three hypotheses were supported; superparasitism was positively correlated with egg load (up to some threshold) and with mated status, although not with female age.

Virgin *M. paulensis* females also invested less in each host patch than mated ones. Given that the sons of virgins will not encounter sisters within the patch, a strategy of scattering them widely over many patches may improve the chances that at least some of them will encounter the female progeny of other wasps. Whereas mated females should maximize their fitness by producing daughters and exploiting patches thoroughly, virgin females may minimize the possibility of zero fitness (no sons mating) by distributing male offspring widely. Unless males disperse following eclosion, those in unisexual broods will experience local mate competition for unrelated females, the intensity of which will increase with the size of the all-male brood. Under these circumstances, the optimum size of an all-male brood will be smaller than that of a mixed brood. In contrast, a mated female producing mostly daughters behaves in a pessimistic manner (*sensu* Roitberg 1990) and seeks to exploit each patch thoroughly before risking emigration to search for another. My hypothesis that virgin females would employ oviposition tactics distinct from those of mated females was therefore supported, with differences evident at both the level of the individual host and the host patch.

Young virgin females may also leave patches earlier than mated females in order to seek a mate and produce daughters. Although the value of hosts to virgin females, as estimated by the rate of superparasitism, did not increase with age, the value of host patches apparently did; 6-day-old virgin females of *M. paulensis* exploited host patches more intensively than did 2-day-old virgins, and just as intensively as mated females of the same age (Fig 3.2). In contrast, there was no significant difference between 2-day-old and 6-day-old mated females in patch residence time or number of aphids attacked, suggesting that the behaviour of virgin females was more sensitive to age than was that of mated females.

It has been suggested that low host density is a factor influencing levels of superparasitism (Cloutier 1984, Laurence 1988), but I found no difference in rates of superparasitism by *M. paulensis* across three host densities, as measured by the number of hosts per unit area (Fig 3.0). The linear decline in numbers of hosts parasitized with increasing surface area of the arena indicates that rates of host encounter did, in fact, vary across treatments. The absence of an effect of host density on superparasitism supported my fourth hypothesis, that self superparasitism by *M. paulensis* is not a response to low host density, *i.e.* that host value is independent of short-term differences in encounter rate. Nevertheless, it is possible that the host densities, and the responses of females, generated under such conditions are not comparable to natural situations in which aphids are probably settled in clusters feeding on a plant, as opposed to wandering around in a petri dish.

Virgin females that encountered conspecific females on their first day of life superparasitized more pea aphids than did solitary females (Table 3.3). This is similar to the effect observed for mated females in Chapter Two, although in that experiment rates of superparasitism were elevated by a combination of conspecific encounter and parasitized host exposure. The result is also similar

to that of Visser *et al.* (1992b) who showed that experience in groups prior to foraging increased rates of superparasitism by mated females of *Leptopilina heterotoma*. In this context, self superparasitism represents an 'insurance' strategy for securing hosts in anticipation of attacks by other females. My study is somewhat different from that of Visser *et al.* (1992b) in that I employed unmated females, and suggests that superparasitism in this context is not contingent on mated status.

Exposure of virgin females to aphids previously attacked by conspecifics caused them to remain longer in a subsequent patch of unparasitized aphids, and parasitize more hosts, compared with an exposure to unparasitized aphids (Table 3.4). This result suggests again that female parasitoids may assess patch quality relative to previously encountered patches and adjust their reproductive allocation accordingly. Contact with previously parasitized hosts apparently serves as evidence of competition from conspecifics and causes virgin females to assess unexploited patches as higher in value compared to females that have encountered only unparasitized hosts. Female foraging experiences may therefore influence the subsequent allocation of progeny to patches, as well as to individual hosts, a finding which underlines the importance of examining oviposition tactics at both the level of the host, and the host patch.

Whereas the roles of egg load and experience have received much theoretical attention (see references above), I am aware of no model of parasitoid foraging behaviour that considers mating status as a state variable. I suspect that mating status may have an important influence on oviposition behaviour in any parasitoid with haplodiploid sex determination, particularily in species attacking hosts that are highly aggregated. A scarcity of hosts, a high cost of dispersal, and conspecific competition are all factors that should select for increased patch residence times independent of mating status. However, when hyperparasitism is a significant source of mortality which varies among patches,

females should reduce their patch residence times and invest less in individual hosts, again regardless of their mating status (Ayal & Green 1993). In the following chapter I test whether or not distinct virgin oviposition strategies are the general rule among aphidiids, and whether or not virgin and mated females behave the same in some species.

Chapter IV

Variation in Foraging Strategy of Aphidiid Wasps with Mating Status

INTRODUCTION

Many recent studies of insect foraging behaviour have departed from classical mechanistic approaches and focussed instead on developing models that include rules for decision-making by individuals (Mangel & Clark 1986). Models have been developed that are 'behaviour-rich' and take into account physiological state and individual experience (Mangel 1989, Roitberg 1990). Many parasitoids, including aphidiids, forage for hosts which are usually distributed in clumps or patches. Patch residence time, or the amount of time invested by a female in exploiting a particular clump of hosts, is therefore a central issue in much of the current theory on parasitoid foraging. Observations of aphid parasitoids in the field suggest that many females leave infested plants long before suitable hosts are all parasitized (Mackauer & Völkl 1993). An understanding of patch leaving decisions will be essential for predicting, and possibly manipulating, the behaviour of parasitoids in various biological control programmes.

If hosts are encountered in patches, how does a female parasitoid decide when to leave one patch and search for another? From a maternal perspective, the decision is how large a reproductive investment to make in a current patch before accepting the risks of emigrating to seek another. If the decision to leave were influenced only by encounter rates with unparasitized hosts, we would expect females to exploit patches until they either either ran out of eggs, or exhausted the patch. However, the optimum patch residence time will also be influenced by (1) the probability of finding other patches, (2) the risk of mortality while seeking them, (3) the survival of offspring within patches, and (4) the sex of progeny that can be produced. At best, we can only expect foragers to possess imperfect estimates of host availability based on their recent experience, but among haplodiploid insects with control of fertilization, offspring sex can be determined with some certainty. In this chapter I will

test the hypothesis that the foraging strategy of a female aphidiid changes after she mates and becomes able to produce female offspring.

Individuals face uncertainty in foraging and may rely on recent experiences to assess host availability and determine their strategies. Furthermore, many decisions made by a female parasitoid within a host patch may be contingent on previous decisions. For example, upon encountering a host, a female makes a decision to accept (oviposit) or reject it. If it is accepted, a decision is made regarding the number of eggs to lay and, in cases of maternal control of fertilization, their sex. Following an oviposition (or rejection), a further decision is made whether to remain within the patch and continue searching, or to leave and seek another patch. Unless the female is disturbed by a predator, the decision to leave may be influenced by recent experiences within the patch (Haccou *et al.* 1991, Visser *et al.* 1992b) or by her physiological state (Rosenheim & Rosen 1991). Two of the state variables which have received much attention are age (Roitberg *et al.* 1992), and egg load (Iwasa *et al.* 1984, Rosenheim & Rosen 1991, Weisser 1994). An older female may benefit from remaining longer in a current patch because her chances of finding another patch are lower. A reduced egg supply may decrease a female's propensity to search and increase her tendency to emigrate (Collins & Dixon 1986), presumably because there are few benefits to remaining when mature eggs are not available. However, emigration may also entail a cost in terms of allocation of energy to flight.

Mating status is a potentially important state variable which has been largely ignored in theories of parasitoid foraging behaviour. In the previous chapter I observed that, under these specific laboratory conditions, mated females of *M. paulensis* remained longer in host patches than did their virgin counterparts, attacked more hosts, and laid more eggs in each host they parasitize. In this chapter, I test the hypothesis that these

differences due to mating status are a general rule for most aphidiid species. I suggest that models of foraging behaviour applied to haplodiploid parasitoids should take into account mating status as a discrete, or categorical, state variable. I hypothesized that the optimal size of all-male broods would be smaller than that of mixed broods for most parasitoid species because the incremental fitness gain of laying additional male eggs within an all-male brood declines more rapidly than the incremental gain of laying additional female eggs within a mixed brood.

For these experiments I selected five species of aphidiid, all from different genera, to gain a broad perspective on the generality of mating status effects within the family. Furthermore, I examined foraging behaviour in a more natural setting than in the previous chapters, *i.e.* by provisioning wasps with aphids feeding on a plant shoot.

MATERIALS AND METHODS

Insect Colonies

A colony of *Lysiphlebus testaceipes* Cresson was established from individuals parasitizing *Aphis hederae* Kalt. on *Hedera helix* in West Vancouver, B.C. and reared on black bean aphid, *Aphis fabae* Scop. Individuals of *A. smithi*, *E. californicus*, *M. paulensis* and *P. pequodorum* were obtained from our stock colonies and reared on pea aphid as described in Chapter One. Pea aphids were used in experiments when they were 3 days of age at 20° C (late second instar nymphs), whereas black bean aphids were used at 4 - 5 days of age when they were 3rd or 4th instar nymphs. This was done to standardize host size as the black bean aphid is smaller than the pea aphid. Furthermore, earlier instars of black bean aphid are difficult to manipulate without inflicting mortality. All parasitoids emerged alone in gelatin capsules and were transferred to their own wax paper cup with a bean stem and diluted honey within 16 hours of eclosion. Females referred to as 'mated' were caged overnight with 2 males each, while virgins were caged alone. All females were used in experiments when they were 32-48 h of age without prior exposure to aphids. Following every experiment, mated females were each placed into a petri dish containing 20-30 aphids for 40 min. These aphids were then reared through to mummification and emergence so that mating could be verified. Data for mated females that failed to produce daughters was then excluded from the analysis.

Direct Observations of Behaviour

The first series of experiments was designed to determine if differences in patch residence time or attack rates were evident between virgin and mated females when they foraged on a bean shoot. Female parasitoids (12 virgin, 12 mated) of each species, except *L. testaceipes*, were released into individual vented plastic mini-cages (16 cm diameter x 5 cm deep) containing a single bean shoot at the 6 leaf stage on which 40 unparasitized pea aphids had

settled several hours earlier. Female *L. testaceipes* (12 virgin, 12 mated) were each provided with a 6-leaf bean shoot on which 20 black bean aphids had settled. This species is far more persistent within a patch and fewer hosts were provided in order to render the experiment managable. Each female was observed continuously as she searched and attacked aphids; no dissections were performed as rates of parasitism were determined in a separate experiment. An attack was defined as a strike with the ovipositor that made contact with an aphid. Following the first attack, the lid of the cage was removed to permit the female to leave if she wished. Patch residence time was calculated from the time of the first attack to the time the female left the minicage, either on the wing or by walking over the lip of the cage. The data were analysed by one-way ANOVA.

Confinement Experiments

The second series of experiments was designed to provide independent confirmation of the first, and to control for any influence of the observer on attack rates. Furthermore, if virgin and mated parasitoid females differ in oviposition rate, differences in the number of aphids parasitized should be evident even when females are confined within a patch. Thirty females of each species (15 virgin, 15 mated) were each placed into a vented plastic mini-cage containing a single bean shoot at the 6 leaf stage on which 26 aphids had settled several hours earlier. *L. testaceipes* females were provided with black bean aphids, those of all other species, pea aphids. The number of aphids was selected based on known rates of parasitism by these species so that even the most active females would not exhaust available hosts within the 2 h trial period, which itself had to be long enough to give slower or more reticent females a chance to encounter aphids and begin foraging. The aphids from each replicate were reared separately for 4 days, whereupon 20 aphids were dissected from each to count the numbers of parasitoid larvae they contained. Replicates in which no aphids were

parasitized were excluded from analysis. The incidence of superparasitism was estimated as the number of eggs laid (= total number of live and dead larva) per host parasitized. The data were analyzed with a one-way ANOVA.

Virgin Receptivity Following Oviposition

It has been suggested that females ovipositing as virgins may subsequently refuse to mate (Subba Rao & Sharma 1962, Stary 1970). If this were true, it would have an important bearing on the interpretation of results, since virgins leaving host patches would not be motivated by a search for mates. In order to test whether virgins remain sexually receptive, unmated females of all five species were each confined with 40 aphids in a cup for a period of 1 h on their first day of life. In the case of *L. testaceipes*, freshly emerged virgins appeared reticent to attack aphids and were therefore confined with hosts overnight. Following their initial exposure to aphids, females were transferred to a wax paper cup with a bean stem and diluted honey and provided access to males of similar age for 14-16 hours (overnight). The following day, females were each introduced to a plastic petri dish containing 30-40 aphids for 1 h. The aphids from each replicate (a single female's brood) were reared through to emergence of adult wasps so that the proportion of females successfully fertilized could be determined.

RESULTS

Mated females of *A. smithi* (Fig 4.0) *M. paulensis* (Fig 4.3) and *P. pequodorum* (Fig 4.4) remained significantly longer in patches than did their virgin counterparts (*A. smithi*, $F = 6.734$, $P = 0.017$; *M. paulensis*, $F = 9.610$, $P = 0.005$; *P. pequodorum*, $F = 20.223$, $P = 0.000$). However, the differences were not significant for *E. californicus* ($F = 1.656$, $P = 0.212$, Fig 4.1) and *L. testaceipes* ($F = 1.090$, $P = 0.310$, Fig 4.2). Mated females of all species except *L. testaceipes* attacked a larger number of aphids than their virgin counterparts (*A. smithi*: $F = 8.416$, $P = 0.008$; *E. californicus*: $F = 7.209$, $P = 0.014$; *L. testaceipes*: $F = 0.001$, $P = 0.992$; *M. paulensis*: $F = 28.740$, $P < 0.001$; *P. pequodorum*: $F = 8.922$, $P = 0.007$). Mated females of *P. pequodorum* also attacked more aphids per minute in the patch than did virgins ($F = 9.345$, $P = 0.006$).

With the exception of *L. testaceipes*, mated females of all species parasitized a larger number of aphids in the 2-hour time interval than did their virgin counterparts (Table 4.0). The experiment had to be repeated with *P. pequodorum* and the results pooled ($n = 52$) since a total of 18/28 (= 64%) virgins and 6/24 (= 24%) mated females did not parasitize any aphids. This can be attributed to the fact that females of this species take a long time to loose their initial flight tendency and begin foraging. Superparasitism was significantly higher by mated females than virgins for *A. smithi* ($F = 18.576$, $P = 0.000$) and *M. paulensis* ($F = 12.795$, $P = 0.001$). Mated females of *E. californicus* also laid more eggs per aphid than their virgin counterparts. Although the difference was not quite significant in ANOVA ($F = 3.791$, $P = 0.063$), a power test yielded a t value of 2.372 ($df = 1, 25$; $P < 0.05$). No difference in rate of superparasitism between virgin and mated females was observed for either *L. testaceipes* ($F = 0.328$, $P = 0.572$) or *P. pequodorum* ($F = 0.031$, $P = 0.861$).

Figure 4.0. Mean patch residence times and number of pea aphids attacked (+ SE) by virgin and mated females of *A. smithi*. ANOVA significance levels, $P < 0.05$ (patch time) and $P < 0.01$ (attacks).

Figure 4.0

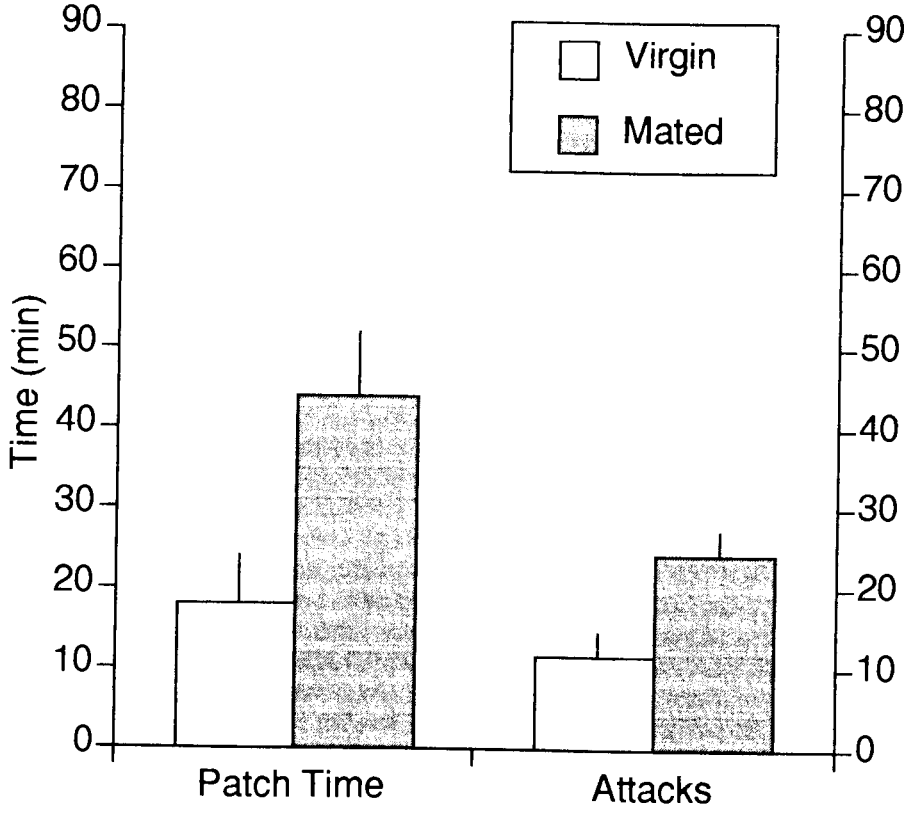

Virgin
Mated
Time (min)
90
80
70
60
50
40
30
20
10
0
Patch Time
Attacks

Figure 4.1. Mean patch residence times and number of pea aphids attacked (+ SE) by virgin and mated females of *E. californicus*. ANOVA significance levels, $P > 0.05$ (patch time) and $P < 0.05$ (attacks).

Figure 4.1

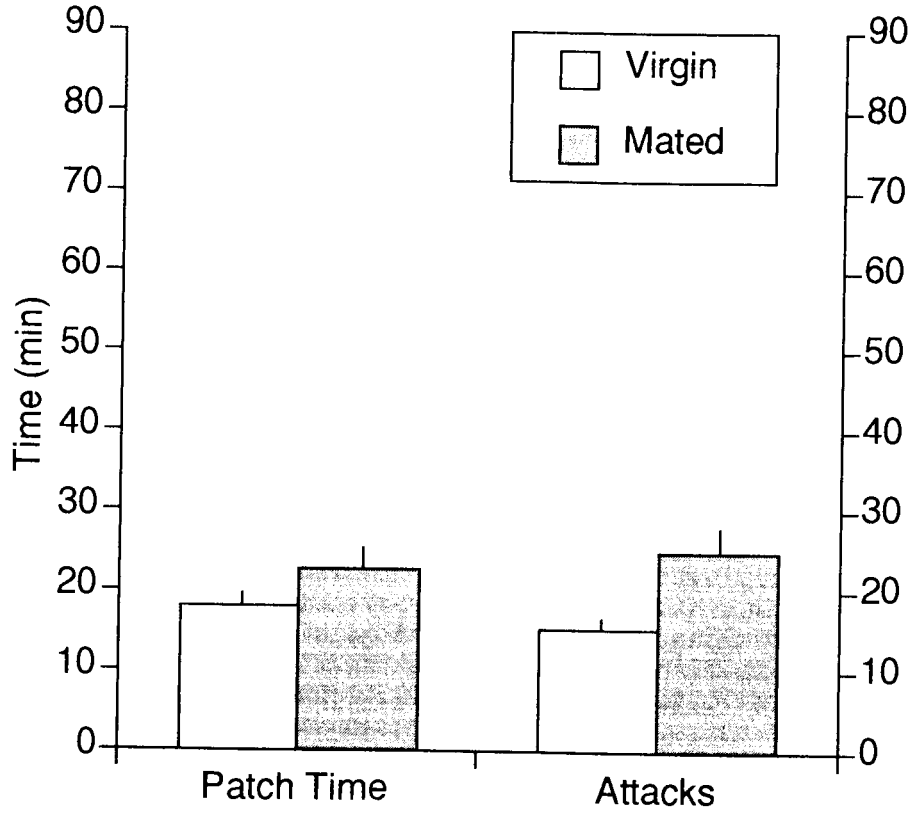

Virgin
Mated
Time (min)
90
80
70
60
50
40
30
20
10
0
Patch Time
Attacks

Figure 4.2. Mean patch residence times and number of black bean aphids attacked (+ SE) made by virgin and mated females of *L. testaceipes*. ANOVA significance level, $P > 0.05$ in both cases.

Figure 4.2

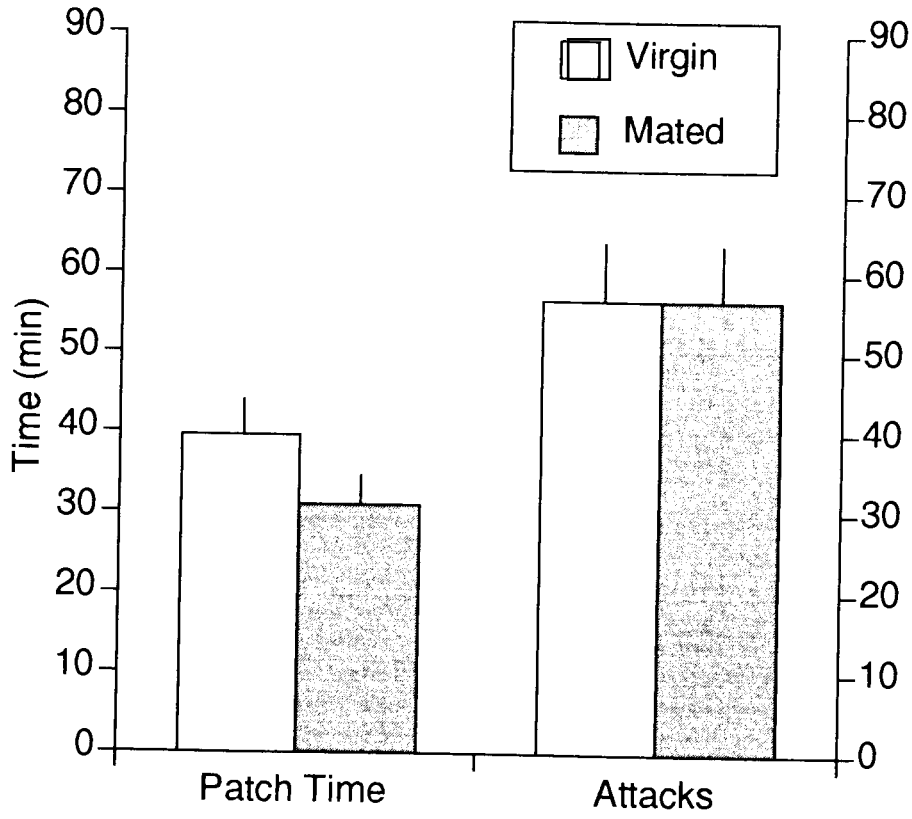

Virgin
Mated
Time (min)
90
80
70
60
50
40
30
20
10
0
Patch Time
Attacks

Figure 4.3. Mean patch residence times and number of pea aphids attacked (+ SE) by virgin and mated females of *M. paulensis*. ANOVA significance levels, $P < 0.01$ (patch time) and $P < 0.001$ (attacks).

Figure 4.3

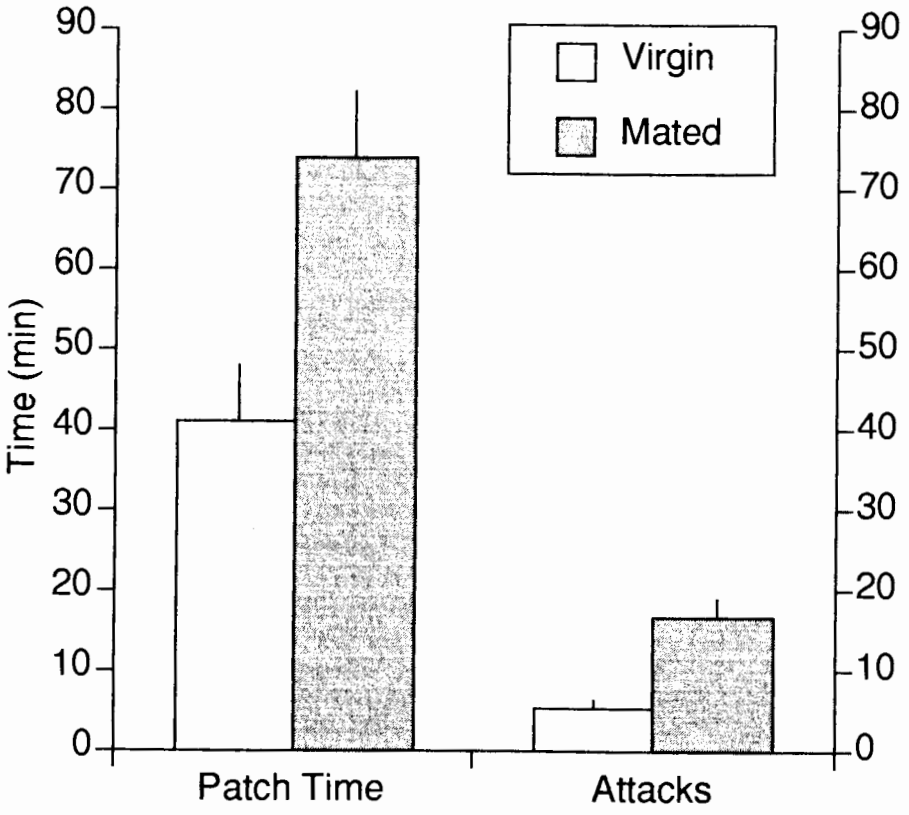
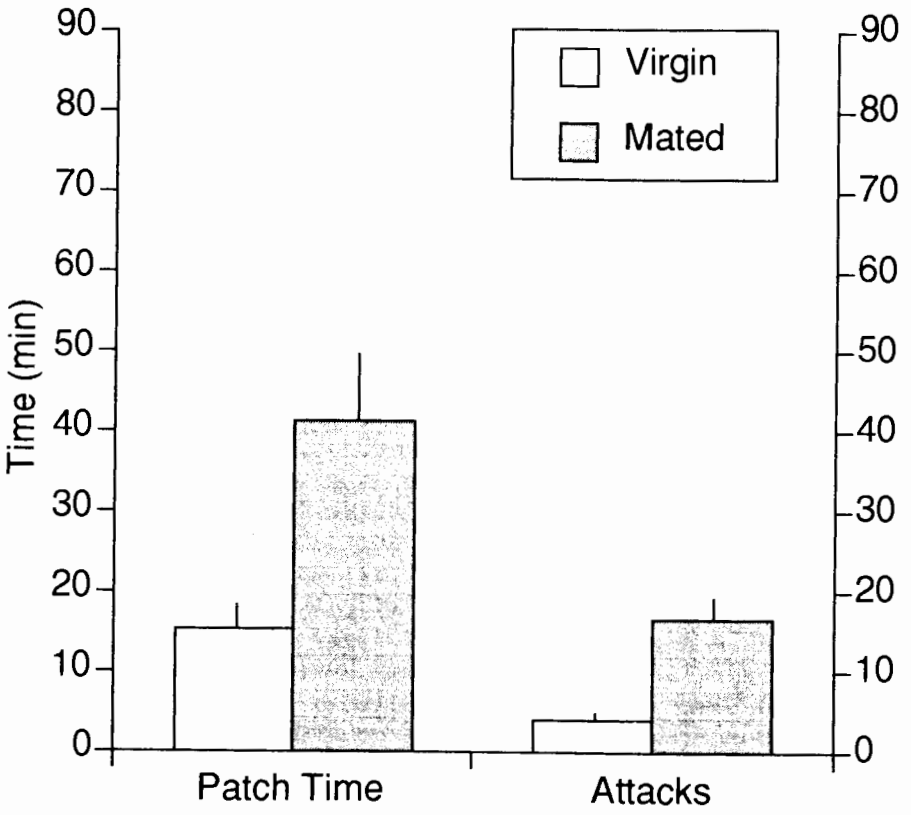

Virgin
Mated
Time (min)
90
80
70
60
50
40
30
20
10
0
Patch Time
Attacks

Figure 4.4. Mean patch residence times and number of pea aphids attacked (+ SE) by virgin and mated females of *P. pequodorum*. ANOVA significance levels, $P < 0.001$ (patch time) and $P < 0.001$ (attacks).

Figure 4.4


Virgin
Mated
Time (min)
90
80
70
60
50
40
30
20
10
0
Patch Time
Attacks

	Virgin			Mated			
Species	No. parasitized	Eggs/A	n	No. parasitized	Eggs/A	n	*F*
A. smithi	12.4 ± 1.06	1.23	18	16.3 ± 0.50	1.48	13	11.59**
E. californicus	9.8 ± 0.88	1.21	12	14.7 ± 0.88	1.61	14	15.49***
L. testaceipes	14.9 ± 0.70	1.85	15	16.1 ± 0.79	1.96	14	1.32^{ns}
M. paulensis	10.4 ± 0.74	1.46	16	17.0 ± 0.66	1.83	15	44.44***
P. pequodorum	2.6 ± 0.71	1.03	28	8.8 ± 1.30	1.04	24	18.75***

Table 4.0. Mean number of aphids parasitized (± SE) and mean number of eggs laid per aphid parasitized (Eggs/A) by virgin and mated females of five aphidiid species. Females of *L. testaceipes* were provided with third and fourth instar nymphs of black bean aphid, all other species received second instar pea aphids. A sample of 20 aphids was dissected from each replicate after 4 days of rearing. The data for 'No. parasitized' were analyzed by one-way ANOVA, ^{ns} = P > 0.05, ** = P < 0.01, *** = P < 0.001).

A majority of females of all species mated successfully following oviposition as virgins: *A. smithi*: 30/33 (91%); *E. californicus*: 15/16 (94%); *L. testaceipes*: 32/34 (94%); *M. paulensis*: 23/30 (77%); *P. pequodorum*: 20/32 (63%).

DISCUSSION

Mated females of *A. smithi*, *M. paulensis* and *P. pequodorum* all remained in host patches significantly longer than their virgin counterparts, and attacked more aphids within the patch. Although virgin females of *E. californicus* remained in host patches as long as their mated counterparts, they attacked significantly fewer aphids. *L. testaceipes* was exceptional in that there was no difference between virgin and mated females in either patch residence time or numbers of aphids attacked. Results of the confinement experiments confirmed an identical pattern of differences in terms of numbers of aphids parasitized in a 2h period of undisturbed foraging. One consequence of these differences in behaviour would be the production of all-male broods by virgin females that are smaller than the mixed broods produced by mated females. Similarly, Tagawa (1987) observed that larger clutches are produced by mated females of the gregarious parasitoid *Apanteles glomeratus* than by virgin females, and Walter and Clarke (1992) noted that unisexual male broods of the polyembryonic encyrtid, *Copidosoma sp.* are smaller than either bisexual or unisexual female broods, although in the latter species the mechanism of sex determination is different.

The potential influence of mating status on the foraging behaviour of female parasitoids has received little attention in recent entomological literature. Previous studies have noted that males may interfere with searching females and reduce their oviposition rates (Kumar *et al.* 1988), while others have shown that females search more widely for hosts in the presence of males (Kfir *et al.* 1975). McColloch and Yuasa (1915) were perhaps the first to observe a difference in fecundity between virgin and mated females of a solitary species. Subsequent work has suggested that mated females may often be more fecund than virgin females (Avidov *et al.* 1967), although some studies have found no difference (Rechav 1978, Yu *et al.* 1984). Li *et al.* (1993) found that mated females of *Trichogramma minutum* Riley laid

more eggs than virgin females on their first day of life, but that virgins increased their rate of oviposition on subsequent days so that there was no difference in overall fecundity between the two. However, in most experiments designed to measure fecundity, females are caged with an excess of hosts for extended periods, circumstances which may often obscure intrinsic differences between virgin and mated females with respect to attack rates or patch-leaving tendencies.

Browne (1922) studying the gregarious parasitoid *Melittobia acasta* Walker was perhaps the first to observe a higher oviposition rate among mated than virgin females. In a study of sex ratio in *Spalangia endius* Walker, a solitary parasitoid of house fly pupae, Donaldson and Walter (1984) discovered a higher rate of oviposition in mated compared to virgin females which they attributed to a higher level of activity. Similarily, Antolin (1989) observed that mated *Muscidifurax raptor* Girault and Saunders, another solitary parasitoid of fly pupae, remained longer in arenas containing hosts, and attacked 50% more hosts, than did unmated females. However, the possible adaptive significance of such behaviour was not explored in any of these studies. Tagawa (1987) suggested that "it may be of advantage to virgin mothers to have a larger number of patches from which male offspring can disperse", since "they must disperse to search for other non-related females". The author demonstrated that virgin and mated females of *A. glomeratus* had similar numbers of available eggs and that virgins increased their clutch sizes following mating.

In all species except *L. testaceipes*, mated females seemed to allocate more time to seeking hosts once on a plant, while virgins spent more time grooming or resting. Although virgin *E. californicus* spent as long in host patches as their mated counterparts, they did not attack as many aphids. Virgin females of *P. pequodorum* were also less active than their mated counterparts; less than half of the virgins confined with hosts oviposited, whereas 3/4 of mated females parasitized some

aphids. Mated *P. pequodorum* females made more attacks per unit time in the patch than did virgins, indicating they were more active in searching for hosts. Virgin females of all species, except *L. testaceipes,* displayed an initial tendency to fly from a plant when released onto it, and took longer than their mated counterparts to begin searching for aphids. This may be analogous to the findings of Loke & Ashley (1984) who observed that mated females of *Cotesia marginiventris* (Cresson) responded more intensely to host kairomones than did unmated females. Possibly, mating triggers neurological changes within a female parasitoid that primes her for host seeking behaviour.

A significant proportion of females in many parasitoid populations may oviposit as virgins, although estimates vary greatly among species (Godfray & Hardy 1993). Results of the mating experiment indicate that females of all species retained sexual receptivity following oviposition as virgins, despite previous suggestions to the contrary (Vevai 1942, Subba Rao & Sharma 1962, Stary 1970). This would suggest that, under some conditions, ovipositing virgins may continue to seek mates and produce mixed clutches later in life.

Whereas virgin females produce only sons, mated females of all five species tend to produce a preponderance of daughters within each brood (spanandry). The sex ratios of broods produced by inseminated females of *A. smithi* and *P. pequodorum* in the laboratory are slightly female-biased, usually around 60%, an estimate consistent with field data for these two species (Mackaeur 1976). Successfully mated females of *E. californicus* frequently produce broods in the laboratory that are > 90% female, while brood sex ratios of *L. testaceipes* and *M. paulensis* both average around 85% female. It is often assumed that a female gains fitness with a female-biased brood by economizing on the production of males when daughters are predominantly sib-mated (Hamilton 1967, Waage 1982). This is because a female

can best maximize the number of her grandprogeny by maximizing the number of daughters seeking hosts.

It has been noted that "Mothers of uniparental sons share a greater genetic identity with grandchildren than mothers of biparental sons" (Bull 1979) - the average genetic identity being greater by a factor of two. Furthermore, females should favour sibmating among their daughters since the resulting grandaughters will carry three times as many maternal genes as those of outcrossed daughters. Sons may therefore be produced for either of two maternal purposes: to mate with their sisters, or to seek outcrossed matings with the daughters of other females. In this context it is reasonable to expect all-male broods to require a distinct distribution across host patches relative to mixed broods. Sons deposited without sisters may be distributed sparsely among patches if this improves their chances of encountering the female offspring of other wasps, or if mate competition among them can be reduced by so doing. The (presumably) lower mating success of males in unisexual broods should favour smaller broods distributed over a larger number of patches. This may be similar to the effect noted by Werren (1980) for the gregarious parasitoid, *Nasonia vitripennis*, in which the proportion of male offspring increased with decreasing brood size. However, if hosts are sufficiently rare, females should opt for exploiting each patch thoroughly regardless of mating status. This may be the case for *Dendrocerus carpenteri*, a hyperparasitoid of aphidiids. Mummified aphids containing primary parasitoids in suitable stages of development are much rarer than unparasitized aphids and *D. carpenteri* females, regardless of their mating status, exploit clumps of mummies completely (A Chow, unpublished). However, this is not a likely explanation for the behaviour of virgin *L. testaceipes* as there is no reason to expect their natural hosts to be any more scarce than those of the other aphidiid species in this study.

Mated females of *A. smithi* and *M. paulensis* laid significantly more eggs per aphid parasitized than did their virgin counterparts, indicating they made a greater reproductive investment in each host, as well as in each patch. It is possible that increasing the number of eggs in a host increases the probability of one offspring surviving; two larvae may sometimes overwhelm host resistence mechanisms that kill a large proportion of solitary larvae (Streams 1971) or increase the probability of securing a host in the event of competition from the larvae of other females (van Alphen & Visser 1990). The fact that self-superparasitism was higher among mated females of *A. smithi*, *E. californicus* and *M. paulensis* than among their virgin counterparts suggests that mating increased the value of individual hosts to females of these species. Nevertheless, the highest rate of superparasitism was observed in *L. testaceipes*, the species in which there was no difference between virgin and mated females.

The similarity in oviposition behaviour between virgin and mated females of *L. testaceipes* is difficult to explain, given that there are no salient differences in the life history or biology of this species compared to the others. Why should virgin females remain so long in patches that lack males? What fitness payoff can there be for filling up a patch with exclusively male offspring? One possibility is that males mate predominantly outside their natal patch, as observed in *Spalangia cameroni* (Myint & Walter 1990) and in *Pachycrepoideus vindemiae* (Nadel & Luck 1992). In these cases there would be little advantage to virgins reducing the size of their all-male broods. Males of most aphid parasitoids are thought to locate and identify virgin females by their scent (Stary 1970), but they may also orient to the same odours that attract females, *i.e.* honeydew (Read *et al.* 1970) and plant odours (Powell & Zhang 1983). It is therefore possible that the host complex serves as a rendezvous site for males and females of some species. An adaptive strategy for distributing all-male broods might not evolve in females if virgin ovipositions were

rare events as a result of highly efficient mate-finding by males, or ovipositional restraint in newly-emerged virgins.

Yet another possible explanation for the absence of distinctive virgin oviposition behaviour is ecological in nature. The strain of *L. testaceipes* used for this experiment was collected from an ant-tended aphid, *A. hederae*, and may be adapted to exploiting hosts which are relatively free of hyperparasitism. Females of *Lysiphlebus cardui* and *L. hirticornis* are known to exploit host patches very thoroughly, often remaining overnight in the patch and exhausting their egg supply (Mackauer & Völkl 1993). A similar behaviour has been observed in the solitary parasitoid *Coccophagus atratus* Compere (Donaldson & Walter 1991). *Lysiphlebus cardui* is particularily effective in parasitizing aphids tended by ants. Many ant species are not aggressive toward this parasitoid as they are toward other primary parasitoids and hyperparasitoids, possibly due to some form of chemical camouflage (Völkl & Mackauer 1993). When offspring survival rates are high, fitness payoffs may be greatest for females which invest heavily in each host patch and postpone emigration until most available hosts had been parasitized. However, mortality from hyperparasitism is often high in many aphidiid species (Mackauer & Völkl 1993). Whenever there is significant variation among patches in rates of hyperparasitism, smaller brood sizes should be favoured which spread reproductive investment over a larger number of patches (Ayal & Green 1993).

CONCLUSIONS

The discovery of a 'patch' of hosts by a female parasitoid, whether this is an infested leaf, plant, or cluster of plants, begins with a threshold event, that of host recognition. This event can be determined empirically as an observable change in female behaviour. Searching ceases and the female begins to assess the sensory profile of the host in a species-specific manner. Host recognition triggers a sequence of behavioral events, the outcome of which is contingent on a series of decisions. One decision a female can make is to probe the host with her ovipositor in order to investigate it further, a behaviour I have referred to as 'attack'. An attack therefore indicates a female's readiness to oviposit and handle the host, but does not necessarily correlate with acceptance. If the female decides to accept the host, further decisions may be made as to whether or not to fertilize the egg, and whether to lay more than one egg. Subsequently, decisions are made as to whether to resume searching, engage in some other activity, or emigrate from the patch.

The Decision to Search

Female parasitoids that are not motivated to oviposit may bump into hosts and either retreat or walk over them without apparent recognition. Searching behaviour is sometimes triggered by contact with honeydew and is often reinforced by an oviposition. However, newly-eclosed virgin females may encounter and taste honeydew but choose to rest or groom for an extended period without initiating search behaviour. In contrast, a searching female antennates the substratum repeatedly and makes quick forays on foot, usually with a high frequency of turning. It may be difficult to ascertain when the decision to search for hosts is originally made, as females may arrive at a host patch in various behavioral states. Long range orientation to patches is guided by the odor profile of the host complex and may occur for purposes of mate location or feeding, apart from oviposition.

Perhaps the most important physiological states influencing search behaviour are egg load and mating status. Mated females of many species may be more inclined to search for hosts than virgin females because the relative value of hosts is greater when daughters can be produced. On the other hand, host value is positively correlated with egg load. Consequently, a female with few eggs has low propensity to seek hosts. An oviposition may result in an intensified search locally, whereas a series of host rejections may reduce the searching tendency and increase the probability of emigration. Search behaviour may also diminish following encounters with hosts that are low in quality, distasteful, or costly to handle. Furthermore, a search may be temporarily discontinued to avoid a predator, or to groom away residues on the antennae and ovipositor that interfere with host recognition and evaluation.

The Decision to Attack

A majority of the aphids recognized as potential hosts are usually attacked, but the probability of attack hinges on detection and evaluation of a series of visual cues in *Aphidius* spp, *M. paulensis*, and *P. pequodorum*, although not in *E. californicus*. Although the role of host odor at this stage is ambiguous, antennation of the aphid cuticle seems to confirm host identity in many species. Pre-strike responses to the host are stereotyped for each species, but even at this early stage of evaluation a female's response may be influenced by her physiological state or recent experiences. Egg load appears to be the critical state variable influencing attack propensity. Furthermore, positive (reinforcing) and negative (deterrent) host cues may be learned through experience that influence the probability of attack on recognizably distinct host phenotypes. If unsuitable or distasteful hosts are frequently encountered, females may learn to recognize distinctive visual characteristics that facilitate their avoidance without the risks of host handling and the attendant losses of time and energy if they are ultimately rejected. Furthermore,

recognition of 'signal' cues associated with acceptable hosts may improve foraging efficiency by reducing the time spent in pre-strike evaluation.

The Decision to Accept

When a female attacks a host she decides whether to accept or reject it for oviposition. In some species this decision is made in less than a second, while in others the ovipositor may be inserted repeatedly, or a single insertion may last up to a minute or more. A female generates some estimate of the quality of the host by sampling its internal chemistry and assessing the information so obtained according to criteria that are largely heritable and species-specific. However, the probability of acceptance may be influenced by recent experiences with other hosts, and whether the present host is judged to be higher, lower or equivalent in quality.

How Many Eggs to Lay

The laying of more than one egg in a single host by a solitary parasitoid represents an increased reproductive investment in that host. The decision to self-superparasitize will reflect a female's assessments of both host quality in an absolute sense, and host value in a relative sense. Rates of self superparasitism may vary among host species and across different physiological states. Both a large egg load and mated status tend to increase the relative value of hosts to female parasitoids and are consequently associated with higher rates of superparasitism. Virgin females are less prone to superparasitize than are mated females because hosts are of lower value to females when daughters cannot be produced. Eggs are relatively expendable to a female with a large egg load and fitness may be gained through self superparasitism whenever offspring survival is greater in superparasitized hosts This may be the case when sibling larvae overwhelm host defenses that would vanquish solitary larvae, or when there is risk of subsequent oviposition by

other female wasps. Contact with potential competitors (*i.e.* conspecific females) or encounters with previously parasitized hosts are experiences which may cause females to increase their rates of superparasitism.

The Decision to Leave the Patch

As long as a female has some probability of encountering another patch, she should spend only a finite amount of time in any patch, regardless of its quality. When a female elects to leave a patch she may do so on foot or on the wing. One mode of travel is often characteristic of a species, but the choice may also depend on a female's available energy and physiological state. The decision as to mode of travel will influence the probability of encountering an adjacent patch versus landing in a whole new habitat. Some parasitoids are more prone than others to startle responses that result in flight, *e.g. Aphidius* spp. and *P. pequodorum*, although emigration following a series of undisturbed ovipositions is more likely to occur on foot. Other species, such as *E. californicus*, *L. testaceipes* and *M. paulensis*, appear reluctant to fly under most circumstances. If a female elects to remain in a patch, she may engage in other activities such as resting, feeding or grooming before renewing her search for hosts.

A female's experiences in other patches, along with state variables, such as egg load and mating status, will influence her assessment of patch value and consequently, her patch residence time. When a female's egg load is high, an oviposition will usually result in a renewed search locally, whereas emigration becomes more probable as her egg supply becomes depleted. A female's reproductive investment in a patch may vary depending on whether host quality is judged to be higher or lower than in previous patches. A threat of conspecific competition, such as encounters with other females or the hosts they have parasitized, may increase the value placed on unexploited patches and cause a female to remain longer in them than she would otherwise.

Unmated females tend to leave patches earlier than mated females because the optimal size of an all-male brood is usually smaller than that of a mixed brood. The fitness of a mated female will be primarily determined by the number of her daughters that succeed in finding new host patches, whereas for a virgin it will be determined by the number of her sons that succeed in mating the daughters of other females. Males without sisters are best distributed among many patches to minimize mate competition among them. A mated female can economize on the production of sons by producing a spanandrous brood as long as sufficient sons are produced to mate her daughters, and the risk of outcrossing among daughters is low. However, the value of hosts within a patch declines with successive ovipositions because a female's optimal brood size is usually finite. Furthermore, there are risks associated with placing too many offspring in one patch when mortality from hyperparasitism is high, but varies among patches.

Future Research Directions

In its final stages, the host evaluation process is inevitably guided by responses to contact-chemosensory cues. These responses are probably the most ancestral of all those guiding the process, and yet we know practically nothing about them. Is host acceptance a discrete response to the presence of chemicals that are either pre-requisites for host acceptance or rejection? Do various elements of host physiology interact in a quantitative or additive manner to determine host quality on a sliding scale? The answers to these questions will first require the isolation of chemical factors involved, a difficult task given the small size of the host. The hemolymph of a large number of aphids could be fractionated, but the activity of fractions would require testing in some sort of host model. The construction of such a model would be difficult, not only because of the small size required, but because of the range of sensory cues that may be necessary to elicit attack and oviposition (*e.g.* movement).

Other questions arise from the results described in Chapter Two. Are there additive effects of experiences such as conspecific encounters and encounters with previously parasitized hosts on parameters such as rate of attack or superparasitism? A repeat of the second experiment using a fully balanced design might resolve such an interaction. Levels of self-superparasitism by *A. ervi* females varied among host types when patches contained only one kind of host, but no such effect was evident in the Chapter One experiments in which both hosts were present in the same patch. This result suggests that choice tests may in some cases obscure behavioural responses that are evident when females encounter only one kind of host at a time. Given the current emphasis on choice tests for resolving host preferences, more detailed experiments are required which contrast female responses in choice and no-choice situations.

The results described in Chapter Three suggest a possible interaction between age and mating status which could be tested more carefully by repeated observations of the same females at different ages. It is also possible that effects of age on the foraging behaviour of mated females might be evident in patches of larger size than those employed in this experiment. Given that exposure to conspecific females increases superparasitism among virgin *M. paulensis* females, it would be interesting to test whether or not patch residence time is affected by this experience. It is also conceivable that the impact of various experiences varies with female age, an hypothesis that would be relatively easy, if time-consuming, to test. Furthermore, it is possible that the self-superparasitism behaviour of *M. paulensis* is specific response to the species of host, *i.e.* pea aphid and alfalfa aphid. Preliminary work suggests that such behaviour may be absent when *M. paulensis* attacks other species, such as the cereal aphid, *Sitobion avenae* (F).

The consequenses of mating status effects on female foraging behaviour need to be investigated in field studies. What

proportion of females can be expected to oviposit as virgins in the wild? How efficient is mate-finding at low population densities? Is it justifiable to consider mating status as a categorical state variable, or are some females in fact 'more mated' than others? The effects of mating could conceivably vary with the quality of the male or the quantity of sperm transferred. What is the fequency of second matings among females, and can females that exhaust their sperm supply re-mate?. It would also be useful to have precise estimates of the frequency of sib-mating and levels of local mate competition in field populations in order to more precisely extrapolate the consequences of various offspring allocation strategies to real world situations.

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