FORAGING FOR HOSTS: HOST DISCRIMINATION AND PATCH EXIT DECISIONS BY THE LEAFMINER PARASITOID *OPIUS DIMIDIATUS*

by

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Title of Thesis/Project/Extended Essay

Foraging for Hosts: Host Discrimination and Patch Exit Decisions by the Leafminer Parasitoid, *Opius dimidiatus*

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ABSTRACT

Optimal foraging theory is often used to investigate the allocation of patch time by insect parasitoids. Using a perspective based on this theory, this study investigated the "decision rules" used by *Opius dimidiatus* in determining how long to remain searching a patch of its host, the chrysanthemum leafminer.

In preparation for patch time experiments, elements of the parasitoid's biology and foraging behaviour were examined, including life history and development, fecundity, host instar preference and host discrimination.

The detection of, and response to, already-parasitized hosts is an important factor in a female parasitoid's decision to remain in a patch or emigrate. Experiments showed that *O. dimidiatus* females can discriminate between unparasitized and previously-parasitized hosts, either before or after probing them. This discrimination is time-dependent; pre-probing discrimination lasts for 2 1/2 hours, post-probing discimination lasts 3 1/2 hours. The reasons why superparasitism occurs and why it might be adaptive are discussed.

A number of models have been designed to describe patch time allocation. One of them, the "Giving-Up Time" (GUT) model, predicts that a parasitoid will leave a patch when the time since the last oviposition exceeds some value, its "giving-up time". Flexible GUT models assume that a parasitoid can vary her GUT as she searches a patch, in response to information about its profitability.

Laboratory experiments were conducted in which *O. dimidiatus* females were timed as they searched patches of hosts, in order to assess how information gained about patch size, presence of host stimuli, host density, ovipositions and encounters with parasitized hosts influence their searching time.

The results suggest that when *O. dimidiatus* females arrive in a patch, they use a variable GUT to set their foraging time. Initially, their GUT is a function of the density of host mines. An oviposition in a healthy host results in a resetting of the GUT to a value that increases with each successive oviposition. However, an encounter with a parasitized host causes the setting of a new, lower GUT that decreases with each parasitized host encounter.

It appears that, rather than using simple, fixed foraging rules, *O. dimidiatus* females employ more complex, dynamic strategies that allow them to update information as they forage, and adjust their searching effort accordingly.

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CHAPTER I

INTRODUCTION

Contemporary studies of animal behaviour rely to a great extent on optimal foraging theory as a useful tool in sorting out the complexities involved in foraging behaviour (for review see Krebs et al. 1983, Pyke 1984). One of the key questions investigated is how animals use resources which vary in quality and/or distribution. If we assume, for example, that natural selection is favouring those individuals who most effectively exploit the available resources, then we would expect that, when faced with resources that are patchily distributed, they would apportion their foraging time in an optimal manner among the patches. The allocation of foraging time can be predicted using such models as the Marginal Value Theorem (Charnov 1976), which predicts that animals searching a "set" of patches for their prey should employ a fixed, or "threshold", capture rate based on the average rate of capture over all patches in that set. According to the model, when a predator arrives in a patch its initial rate of prey capture will depend on the density of prey within that patch, but the rate will decline as the patch becomes depleted. When it reaches the threshold rate for that habitat, the predator should move to another patch in the habitat. Patches containing relatively few prey should be avoided if the capture rate would be lower than the threshold rate. This strategy would, if used, result in a more even exploitation of patches within a habitat.

Though such models may predict the outcome of an animal's foraging strategy, they do not often provide insight into how the strategy is accomplished. For example, the Marginal Value Theorem assumes that predators know the abundance and distribution of their prey in order to set a critical capture rate for the habitat. Unless they engage in extensive pre-sampling of the patches, animals cannot be expected to act on the basis of this kind of knowledge.

Instead, they are assumed to be using simple behavioural rules, "rules of thumb" (sensu Cowie and Krebs 1979), which, if followed, should allow them to come close to optimal strategies. One analogy to illustrate this situation would be the decision a person must make about when to remain in a job or leave to find another. An optimal strategy would be to leave when one is no longer likely to be successful in that job or achieve one's goals, but without a vision of the future, this likelihood is difficult to assess. One uses certain "rules of thumb" to direct behaviour: "If you get a 5% raise, stay in job longer, otherwise leave", "If you are passed over for promotion twice, leave job". In other words, the rules of thumb have evolved because they direct an individual towards a general solution to the problem.

Many studies, for example, are beginning to focus on the rules that animals employ in deciding how long to remain foraging in a patch (Waage 1978,1979; Iwasa et al. 1981, Morrison and Lewis 1981, Roitberg and Prokopy 1984, van Alphen and Galis 1983, Ydenberg 1984, Krebs and McCleery 1984, Green 1984). It is becoming particularly important to understand the behaviour of insect parasitoids that forage for hosts in which to lay their eggs, since their foraging behaviour is so closely linked with their reproductive capacity, and therefore their success as biological control agents of pests.

This study is an attempt to understand the rules of thumb used by one such parasitoid, *Opius dimidiatus* (Ashmead) (Hymenoptera:Braconidae) as it searches on leaves for its host, the chrysanthemum leaf miner, *Liriomyza trifolii* Burgess (Diptera:Agromyziidae).

This system was chosen for the study of patch time allocation for a number of reasons: first, the host, the chrysanthemum leafminer, is sessile and trapped within a mine, and so its numbers and distribution could be easily controlled and manipulated.

Secondly, both host and parasitoid have short generation times, and are relatively easy to rear, so that large numbers can be produced quickly for experimental purposes. Finally, there was the enjoyment of being able to work with a parasitoid which might someday contribute to the control of a serious agricultural pest. The drawbacks to this system became apparent when only sketchy information could be found on the genus *Opius*, and none on the species *O. dimidiatus*. It became necessary, therefore, before the experiments on patch time could be attempted, to conduct extensive biological and ecological studies of *O. dimidiatus* in order, not only to maintain a healthy colony of experimental subjects, but also to gain the information essential to the proper interpretation of later behavioural studies. Chapter II outlines the biological and ecological data gathered in the early part of my investigations.

During these studies, experiments to determine the ability of *O. dimidiatus* to discriminate between parasitized and unparasitized hosts revealed a more complex pattern of response than anticipated, necessitating a more detailed study of this subject. Chapter III, therefore, outlines the findings concerning host discrimination by *O. dimidiatus*.

Finally, Chapter IV deals with contemporary theories of patch time allocation, and with the experiments conducted with *O. dimidatus* to understand the foraging rules used by this species and compare them with existing models.

CHAPTER II

BIOLOGY AND ECOLOGY OF *Opius dimidiatus*

Opius dimidiatus (Ashmead) is a solitary braconid of the family Opiinae, but a species about which little has been reported. Most references to this species mention only that it has been found as part of the parasitoid complex found on *Liriomyza* spp., in particular, *Liriomyza sativae* Blanchard, the vegetable leafminer (McClanahan 1974, Minkenburg and van Lenteren 1986). Part of the reason for the scarcity of information about this species may be the frequent confusion of *O. dimidiatus* Gahan with *O. bruneipes* and *O, dissitus* Cresson (Wharton 1984). Nevertheless, individuals have been reported in low numbers in collections in North America from Florida (Petitt 1984) and California (Parrella 1987), to Ontario (McClanahan 1974).

O. dimidiatus is an endoparasitoid which lays its eggs in leafminer larvae found in the leaves of a large number of greenhouse and vegetable crops, and which completes its development within the puparium of its host. In order to conduct studies using *O. dimidiatus*, a colony of the parasitoid has been maintained continuously at Simon Fraser University, Burnaby, B.C., since the spring of 1985. To provide hosts, *L. trifolii* , the chrysanthemum leafminer, has also been in continuous rearing.

A. Rearing of hosts and parasitoids:

(i) Hosts:

The host insect, *L. trifolii*, is an agromyziid fly which infests leaves of a wide range of vegetable and horticultural crops both in greenhouses and in the field. Among the commercially produced crops most seriously affected by the leafminer are celery (Trumble 1985), tomatoes (Parrella 1987), bell peppers (Chandler and Gilstrap 1987), watermelon (Lynch and Johnson 1987), and in particular, chrysanthemums (Jones et al.

1986, Parrella 1987). An adult fly punctures leaves with its ovipositor, feeding from some puncture holes, and laying eggs in others, just below the epidermis of the leaf. After 5 days, the egg hatches and the larva begins to feed within the leaf tearing at tissues with a rasping mouthhook, and leaving a serpentine mine containing frass. The early thread-like mines are almost invisible, but they increase in length and width as the larvae grow. These mines severely disfigure plants, causing reduction in crop value and yield (Parrella 1987). A leafminer reared on broad bean (*Vicia faba* L. c.v. "Broad Windsor") passes through three larval instars within approximately 5 days, after which it bites its way out of the mine, drops to the ground and burrows into the soil to pupate for 10 days. Development of *L. trifolii* is influenced greatly by temperature, humidity, type of host plant, and plant quality (Minkenburg and van Lenteren 1986, Parrella 1987); therefore, the life history parameters presented above are approximations.

To begin a colony of *L. trifolii*, infested leaves were obtained from a chrysanthemum greenhouse in Langley, B.C. The leaves were placed on mesh screens above moist paper towels, covered with moist towels and sealed within aluminum pans covered with plastic wrap and aluminum foil to keep the humidity high. When larvae dropped to the towels and pupated, they were collected with a fine paintbrush, and placed in capped vials with moist filter paper until emergence of adults. Once the colony had been established, it was augmented by adult flies and larvae collected from this greenhouse monthly during the summers of 1984-1987, in an attempt to maintain genetic variation.

Adult flies were kept in a large wood and screen cage (110cm x 45cm x 45cm) and provided with water; yellow plastic garden labels streaked with honey were hung from the cage ceiling to provide a carbohydrate source. *L. trifolii* was reared on broad bean leaves; three-week-old plants were left in the fly cage for 24 hours to receive oviposition, after which they were removed from the cage and kept under fluorescent lights (16 hours: 8 hours) for approximately 8-10 days until larvae were close to pupation. Leaves were then removed and held as described above until puparia could be collected. *L. trifolii* adults live approximately one week under these conditions; life expectancy may be higher in the humid conditions provided by a greenhouse.

(ii) Parasitoids:

In late spring of 1985, 50 adults of *O. dimidiatus* were received from D. Gillespie (Agriculture Canada Research Station, Agassiz, B.C.) and used to start a colony which has been reared continuously since that time. Adults have been kept in two types of cages: the main colony was kept in the same type of wood/screen cage, and under the same conditions, as described for the leafminer adults. For experimental purposes, parasitoids were transferred to plexiglass and screen cages (30cm x 30cm x 30cm). In addition to honey and water, parasitoid adults were provided with hosts so that they could host feed to gain the protein needed for oogenesis (Jervis and Kidd 1986). Twice weekly, 8-10 plants containing nearly-mature host larvae were placed in the large cage for up to 5 hours for parasitization. (At this parasitoid-host density, longer exposure would have resulted in larvae being killed by repeated parasitoid probes.) The leaves were removed from the plants and host puparia collected. To obtain unmated females for experiments, puparia were placed in individual gelatin capsules until parasitoid emergence. The male:female sex ratio in this colony remained approximately 1:1 with a slight male bias.

B. Biology and Ecology of *O. dimidiatus*:

Though *O. dimidiatus* is being considered as a biocontrol agent against *L. trifolii*, its biology and behaviour are as yet unstudied. Such information should be important considerations in the selection of natural enemies, not only to facilitate rearing of a species, but to exploit its strengths. Wellington (1977) argued that in control programs pay more attention should be paid to the attributes that promote survival of the pests, and to the strengths and weaknesses of the natural enemies. In this way, we can control pests rather than just attacking their numbers, "putting the 'insect' back into 'insect ecology'".

An understanding of a parasitoid's searching behaviour, for example, may reveal why it is more effective on some leaf surfaces than others. By studying the complexities of cues involved in oviposition we may get a clue as to why a control program may not have been successful. When conducting behavioural studies such as these, one must understand the organism in order to interpret the results in the most realistic way possible, and to understand how the animal gathers and uses information as it forages. Since no information on this species was available, it was necessary to investigate elements of the insect's biology and behaviour before attempting behavioural experiments.

(i) Life History and Development:

O. dimidiatus lays a single egg per oviposition into the haemocoel of a *L. trifolii* larva. The egg begins to absorb fluid and increases in size within the first half hour, and at 17 hours the first instar can be distinguished inside the egg. Approximately 24 hours after oviposition, the egg hatches into a mandibulate first instar. This instar can be found in host larvae or puparia (depending on the age of host larvae at parasitization). When a cohort of 4-day-old host larvae were parasitized on the same day, then sampled daily and dissected, the first instar larvae of *O. dimidiatus* were found in greatest numbers one day after oviposition.

The duration of the first instar is variable and subject to the developmental stage of the host at the time of oviposition. *O. dimidiatus* apparently delays its moult into the second instar until after its host has pupated; regardless of the age of the host larva when parasitized, the second parasitoid instar is found only in the host puparium. This observation agrees with the findings for other *Opius* species (Pemberton and Willard 1918, Lathrop and Newton 1933) and for *Chelonus* sp. on *Pyrausta nubilalis* (Bradley and Arbuthnot, 1938). Minimum duration of the first instar of *O. dimidiatus* is approximately 2 days, after which it moults into the hymenopteriform second instar.

Though determination of the total number of larval instars was not necessary for this study, researchers have found that other *Opius* species pass through 4 larval instars (Pemberton and Willard 1918, Lawrence et al. 1976).

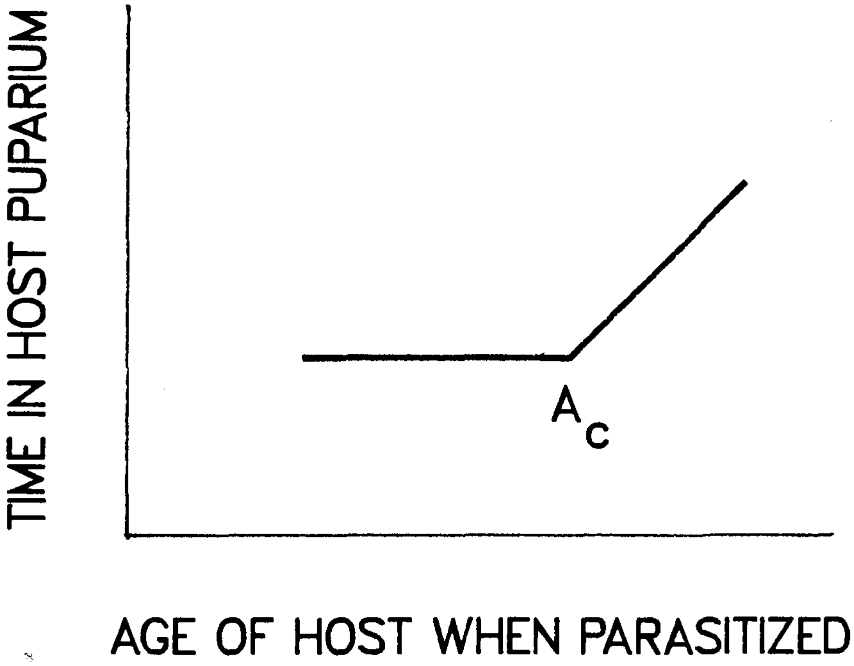
Under crowded conditions such as those found in colony rearing, superparasitism sometimes occurs. As in other solitary species, only one adult of *O. dimidiatus* emerges from a host. I believe that the destruction of supernumeraries takes place before the moult into second instar. Though several eggs and/or first instars are frequently found in a host larva upon dissection, more than one second instar/puparium is never found, suggesting that the mandibulate first instar larval form may be removing the competition, possibly by biting and killing them. In fact, I have observed fighting between two first instars of this species, and between first instars of a related species with a similar mandibulate stage (pers. obs.).

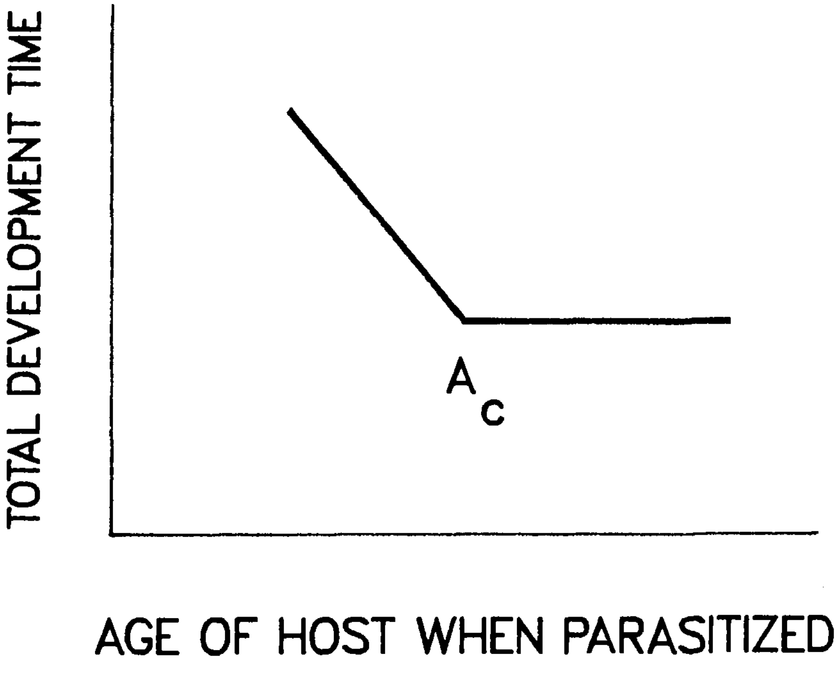
The total development time of *O. dimidiatus*, from egg to adult is difficult to determine, in view of the possibility of a delayed moult. An immature *O. dimidiatus* usually begins development of the egg and first instar within the host larva, and completes its development in the host puparium. Whether or not the development of the egg and first instar continues after host pupation depends on the age of the host larva at parasitization. At some critical host age "A_C", the host age at parasitism will be such that the parasitoid can just complete the first instar before the onset of host pupation, and can moult into a second instar immediately after the host pupates. If host larvae are parasitized prior to A_C, parasitoid larvae must spend a longer period inside the host larval form, and delay moult until host pupation. For parasitoid eggs laid after host age A_C , there will not be enough time for full development of the egg and first instar, so completion of these stages may continue into the pupal host.

The possible effects of host age at parasitization on both the duration of time spent by the parasitoid in the host puparium, and the total time taken to complete its development are demonstrated in Figures 2.1 and 2.2. In Figure 2.1, it is shown that the

Fig. 2.1: Predicted time spent in host puparium as a function of host age at parasitization. When hosts are parasitized at age A_C, parasitoids can complete first instar development without a delay in moult to second instar.

Fig. 2.2: Predicted total development time as a function of host age at parasitization. A_C is described in previous figure.


TIME IN HOST PUPARIUM
A_c
AGE OF HOST WHEN PARASITIZED


TOTAL DEVELOPMENT TIME
A_c
AGE OF HOST WHEN PARASITIZED

time spent in a host puparium should be constant and a minimum as long as the host at parasitization is young enough that egg and first instar can be completed within the larva, leaving only the second and succeeding parasitoid instars and pupa to develop in the puparium. If the first instar is complete prior to host pupation, a moult into the second instar will be delayed, but the duration of the development which then takes place inside the host puparium will not be affected. If parasitization occurs after A_C, however, the egg and first instar development will be incomplete at host pupation, and the period spent in the puparium should be increased by the time necessary to complete these stages.

Total development time of *O. dimidiatus* (Fig. 2.2) will be the sum of the periods spent in host larva and puparium. The duration of total development will be greatest in youngest hosts (because of delay in moult within the host larva) and decrease as the host age when parasitized approaches A_C. At host age A_C, total development time of the parasitoid will be minimum, since the parasitoid will just be ready to moult into the second instar at the point of host pupation. Parasitoid eggs laid in hosts older than this, closer to metamorphosis, will still take the minimum total development time: the extended time in the puparium is balanced by the shorter period within the host larva.

This theory is based on a number of assumptions: first, that the rate of development of any stage of the parasitoid will be the same whether in the host larva or puparium; second, that host larval development is not increased or decreased by parasitism; and thirdly, that a delayed moult does not affect the normal development of the second and succeeding parasitoid instars.

To determine the total development period of immature *O. dimidiatus*, the following procedure was followed:

Method and Materials

Over a three day period, several hundred host larvae were offered to parasitoids in the large cage for 4 hours at a time. Hosts were allowed to pupate and puparia were kept in individual gelatin capsules at 20.1°C until adult parasitoid emergence. A daily record was kept of dates of parasitization, host pupation, adult parasitoid emergence, and the sex of adults. From these puparia, 226 adult parasitoids emerged (118 male, 108 female). For each adult, both the total development time from parasitization, and the time within the host puparium were calculated. T-tests were performed to compare male and female development, with respect to both the time spent in the host puparium and the total development time (from egg to adult). When each developmental period was considered separately, adults were grouped according to the age of their hosts at parasitization. Group means within each period were compared using Scheffés Multiple Range test. In addition, an analysis of covariance compared the slopes of the regressions for males vs females.

Results

Overall development times for males and females are shown in Table 2.1. Mean duration of development from egg to adult for males (18.2 days) and females (20.1 days) were significantly different from each other (t-test, $p<0.001$), as were the mean development times measured from host pupation (male: 15.8 days; female :17.6 days) (t-test; $p<0.001$).

Hosts remained in larval form for up to four days after parasitization. Therefore, as pointed out above, parasitoid adults were separated into four groups corresponding to the age of the host when parasitized, relative to its pupation. Group 1 individuals, for example, developed in hosts that were 4 days from pupation when parasitized; these parasitoids, therefore, spent the longest period within the host larvae. Mean total development times between groups and development times within the host puparia between groups were compared using a Scheffé's Multiple Range test. (Males and females within each group were not compared further.)

Table 2.1: Development of *Opius dimidiatus* in its host, *Liriomyza trifolii*

	DEVELOPMENT TIME WITHIN HOST PUPARIUM (days)			TOTAL DEVELOPMENT TIME (days)		
	X	S.E.	n	X	S.E.	n
MALE	15.839	0.153	118	18.153	0.173	118
FEMALE	17.569	0.218	108	20.139	0.179	108
	t-test, $p < 0.001$			t-test, $p < 0.001$		

As seen in Figures 2.3 and 2.4, the expected patterns of development predicted in Figures 2.1 and 2.2 did not emerge for either males or females. For males, the time spent in the host puparium (Fig. 2.3) was least when the youngest hosts were used and the greatest time was spent in the host larva. As predicted, the time spent in the host puparium increased significantly when the hosts were less than 3 days from pupation at parasitization. In these hosts, the development of the egg and first instar may have continued into the host puparium, increasing the duration within that stage. However, the length of time in the puparium did not continue to increase as expected, when even older hosts were used. Regardless of whether the host remained as a larva for 2 days or only 1 day, development of the parasitoid seemed to be such that time in the puparium was unchanged. The slope of the regression line for male development in the puparium (+0.324) further suggests that males spend the same amount of time in the host puparium regardless of the age of the host at parasitism.

Females showed a pattern opposite to that predicted, increasing the time in the puparium as older hosts were used, rather than displaying a constant time after a delayed moult in the larva. It appears that every less day spent in the host larva is compensated for by an extra day in the puparium. (The slope of the regression line is 0.748, indicating an increase in time spent in the puparium as the time in the larva is shortened.) Less than 3 days prior to pupation, their time in the host puparium was constant regardless of the age of the host larva when parasitized. Even when hosts used were only 1 day from pupation, the duration of development in the puparium was not lengthened, as I had predicted would be necessary to allow completion of the first instar.

Figure 2.4 shows the total development time of males and females. Males developed for the longest total time when the longest period was spent in the host larva (4 days), necessitating a delay in moult until the host metamorphosis and an extended total development time. Their total development time was least when older hosts were used. When male eggs were laid only 1 day before their hosts pupated, the shorter development

Fig. 2.3: Time spent developing in host puparium as a function of host age at parasitization. (Means ± S.E.) Scheffés Multiple Range test compared development times among host age groups. (Comparison of groups was not made between sexes.)

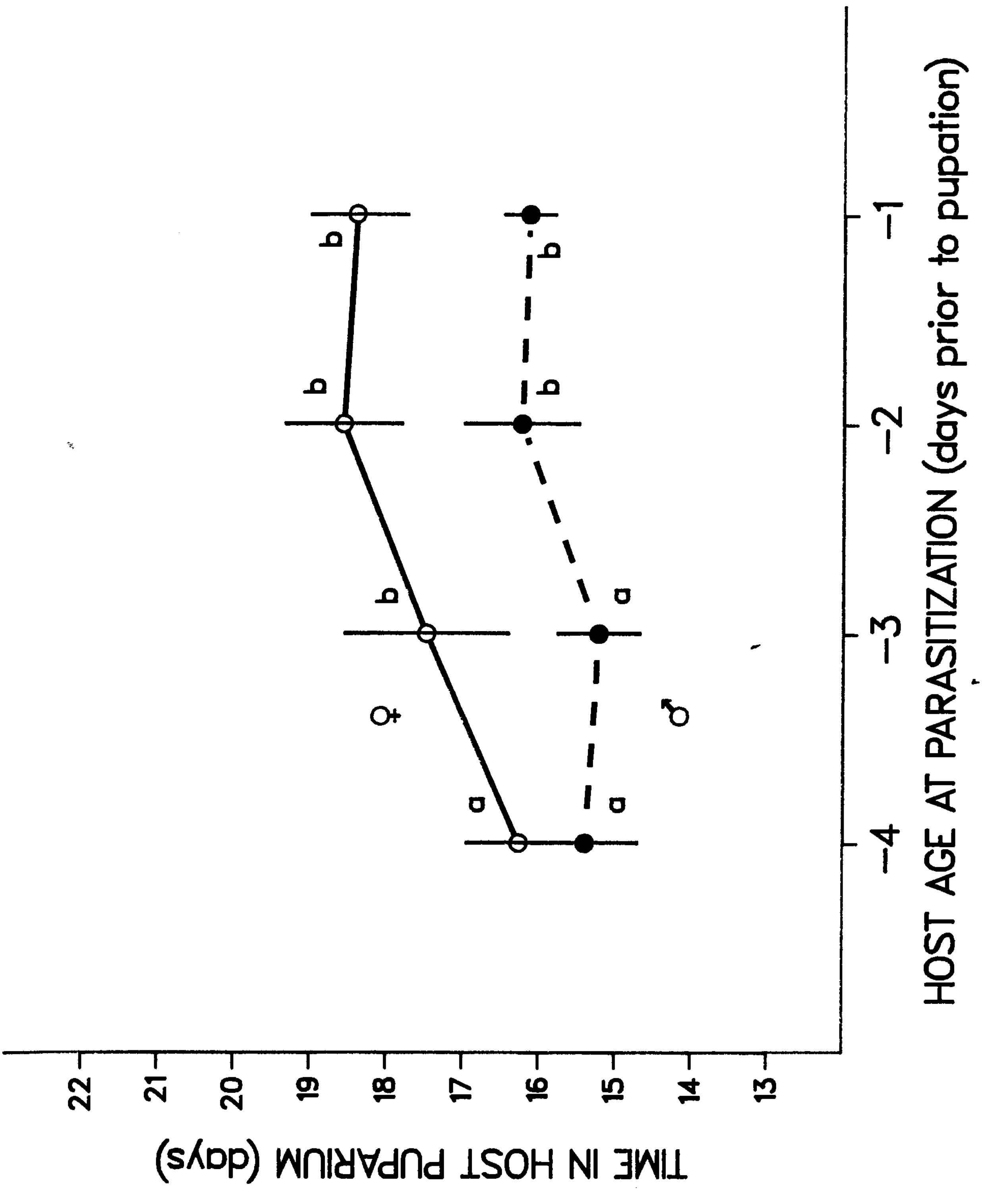
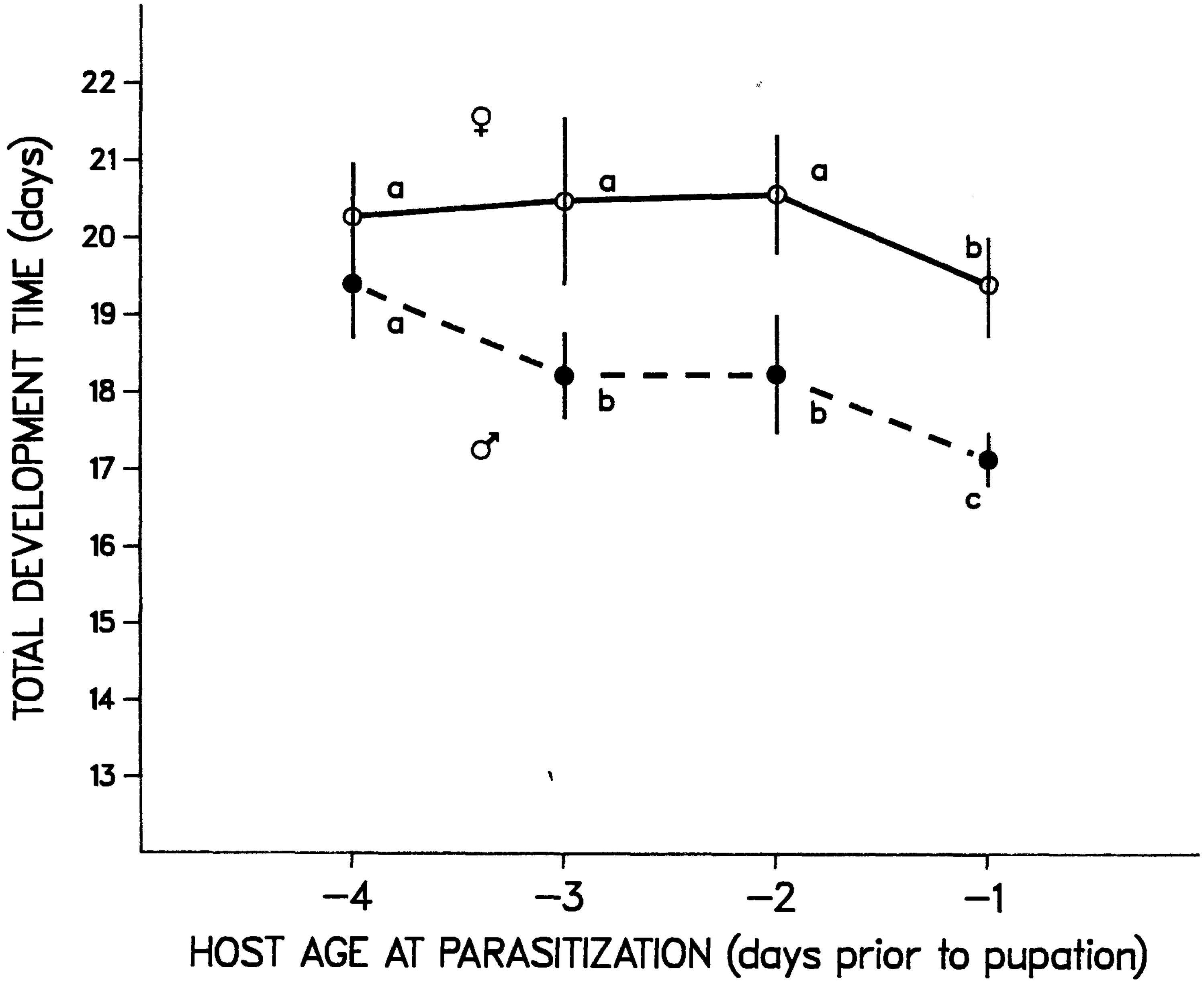

TIME IN HOST PUPARIUM (days)
22
21
20
19
18
17
16
15
14
13
HOST AGE AT PARASITIZATION (days prior to pupation)
−4
−3
−2
−1
♀
♂
a
a
b
a
b
b
b
b

Fig. 2.4: Total development time as a function of host age at parasitization. (Means ± S.E.) Scheffé's Multiple Range test compared total development times among host age groups (Comparison of groups was not made between sexes.)


TOTAL DEVELOPMENT TIME (days)
22
21
20
19
18
17
16
15
14
13
♀
♂
a
a
a
b
a
b
b
c
−4
−3
−2
−1
HOST AGE AT PARASITIZATION (days prior to pupation)

time reflected the lack of increase in the duration of development in the puparium for this group (Fig. 2.3). This decrease in total development time with decreasing time in host larva is reflected in the negative slope of the regression line (-0.676).

Females continued to show a divergence from the predicted pattern when their total development is examined (Fig. 2.4). When hosts are parasitized more than 1 day prior to pupation, and time in the host larva exceeds 1 day, the total development time is a constant, and maximum. (The regression of the data shows only a slight decrease in total time regardless of the time spent in the larval form (slope=-0.252)) This situation would occur if, even given 4 days within the larva, a female parasitoid did not have a delayed moult, and did not complete the first instar in those 4 days. Equally puzzling is the fact that, as in the results for males, the shortest development time resulted when eggs were laid in hosts 1 day prior to their metamorphosis.

Discussion

The results for male development appear to suggest that development of egg and first instar are completed within 3 days in the host larva (compatible with the observations of 24-hour egg and 48-hour first instar development); parasitoids in host larvae longer than 3 days must delay moult, extending their total development time. However, when eggs are laid in hosts 1 day prior to host pupation, total development time is least. Two possibilities come to mind: either the parasitoid larvae are speeded up in their development, or there is an upper threshold to the duration of time it is possible to spend within the host puparium.

Female development patterns seem also to indicate that there is a reduction in total development when the host is one day from pupation. As conjectured for males, female parasitoid development may be speeded up in pre-pupal hosts, or the maximum time spent in a host puparium may be limited. For females, the results appear to suggest that, regardless of how young the host is when parasitized, there may be no point at which the egg and first instar are completed before host pupation, so a delayed moult may not be necessary.

When the regressions of male and female developments are compared they show a significant difference both for the time they each spend in the host puparium (t-test, $p<0.01$), and the total development times (t-test, $p<0.01$). Male development in the host puparium does not increase noticeably as the time in the larva decreases. In comparison, female development time increases in the puparium with decreased time in the larva. When a comparison is made of the regressions of total development times of males and females, this pattern translates into a decrease in total time for male parasitoids as time in larva decreases, while females show a continued development time. These results could be explained if females, by remaining longer, could attain a larger size and increase their fecundity. For males, however, larger size may not be an advantage, so their best strategy may be to spend a minimum amount of time developing.

Although the results presented here cannot be satisfactorily explained by the original predictions, they do suggest a number of factors which may influence the duration of the parasitoid immature stages. One of these, the host age at parasitization, must still be considered to play an important role, especially in view of the consequences of a delayed moult. Beckage (1985) describes several species of hymenopteran parasitoids whose first larval moult occurs in synchrony with the beginning stages of host metamorphosis and whose first instar, therefore, varies in duration. The possibility exists, therefore, that there may be no maximum duration of first instar for these species. In fact, they may need host ecdysone to moult and continue their own development. Lawrence (1982) demonstrated that when parasitized third-instar larvae of *Anastrepha suspensa* (Loew) were ligatured, the parasitoids moulted to second instars only when they were isolated on the same side of the larvae as the ecdysteroid-secreting ring glands.

Other factors are suggested by looking at the validity of the assumptions underlying the predictions. It was assumed that the rate of development of any one stage of the immature parasitoid would be the same regardless of the age of the host. However, the physiological environments may be different inside hosts of different ages. The food found by a parasitoid larva within a pupated host may be more fluid and perhaps more accessible than in a larva, and so may affect the growth rate of a first instar. This might explain the apparent "speeding-up" of parasitoid development in the oldest larvae. Conversely, a parasitoid might delay its feeding in small larval hosts until the host has reached a size where it is better able to support parasitoid development (Mackauer 1986). A parasitoid larva might respond to the higher levels of ecdysone present within a pre-pupal host. It is unlikely, however, that *O. dimidiatus* passes through an obligatory moult in response to host ecdysone levels, since dissections of host puparia often produce eggs and living first instar larvae.

The second assumption to be questioned is that host development is not affected by parasitization. One species of parasitoid, *Microplitis croceipes* (Cresson) delays the metamorphosis of its host, *Heliothis virescens* (F.), maintaining the larval form to satisfy its own nutritional requirements. It also seems able to initiate metamorphosis in the host so that its own pupation and emergence may take place in a protected environment (Beckage 1985). However, not immediately obvious is how control of a host's development would vary the development time of the parasitoid.

Likewise, it is difficult to predict the effects of a delayed moult on succeeding instars. It may be possible that an extension of the first instar to achieve synchrony with the host metamorphosis might cause physiological or metabolic changes in the immature parasitoid that would then influence its later development.

A final explanation for the lack of predicted results must derive, not from the theory, but from the method of collecting data. Pupae were checked for emergence at the same time each day; therefore, the emergence times were accurate only to within 24 hours. Likewise, it was impossible to determine more accurately when, within the four-hour period described, each host had been parasitized. Future work in this area would benefit by a more frequent (perhaps hourly) collection of emergence data, and by exact timing of the parasitism of each larva used.

In conclusion, factors affecting the developmental period of *O. dimidiatus* appear to be quite complex. An endoparasitoid develops in, and is exposed to, a constantly changing world, where host metabolic processes and nutrition and varying host hormone levels may affect its own development. In particular, the possible suppression of the first larval moult until host pupation is a factor that must be considered in any calculations.

(ii) FECUNDITY:

In order to insure that females were never egg-limited, I undertook a fecundity study so that experimental females would be approaching the age of maximum egg laying.

Methods and Materials

Twelve newly-emerged, virgin females were kept separately in plexiglass cages and given at least 100 host larvae/day (early 3rd instar) until their death. Plants containing larvae were replaced at 24-hour intervals, and puparia were collected and dissected to count the number of eggs deposited. The Kolmogorov-Smirnov test was performed using the cumulative fecundity data of each female to compare the fecundity distributions of the females.

To determine whether this species carries a finite number of eggs from birth (pro-ovigenic) or produces them continuously (synovigenic) (Flanders 1950), the ovaries of 22 newly-emerged females, inexperienced in oviposition, were dissected and the numbers of fully- and partially-formed eggs counted.

Results

All 12 females survived for 12 days, 6 surviving for 16 days after emergence under these conditions. A Kolmogorov-Smirnov test confirmed that the egg distributions were not different for different females ($p>0.05$). Figure 2.5 shows the mean age-specific fecundity. There is a lower oviposition rate immediately after emergence, increasing to a peak in the egg-laying period between 6-8 days, after which it decreases steadily. The mean lifetime fecundity was 494.3 eggs /female (S.E.=45.5, n=12). Dissection of ovaries revealed a mean number of eggs of 57.4 (S.E.=2.66, n=22), of which 22.3 (S.E.=1.63, n=22) were fully formed and discrete.

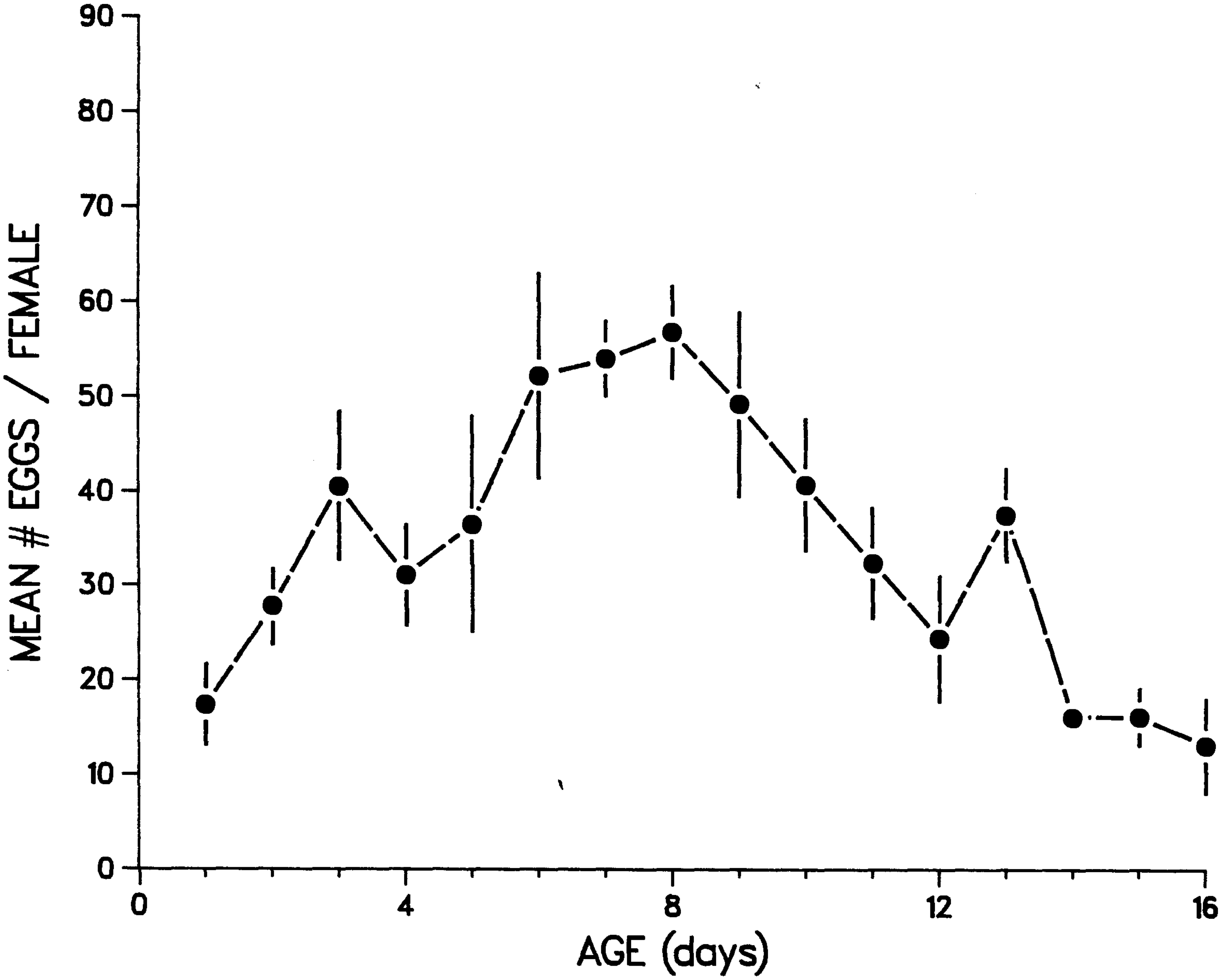
Discussion

O. dimidiatus females produce eggs continuously throughout their life and can begin ovipositing within the first day after emergence. Fecundity rises to a peak at 8 days, and begins to decline with increasing age. Since providing a supply of 8-day-old females in adequate numbers for future experiments would be very difficult, it was decided to use 4-7 day old females which would be approaching their period of maximum fecundity, and would be convenient to produce using the existing rearing routine.

(iii) Search and Oviposition Behaviours:

Patch time experiments require that detailed observations be recorded of both the host-searching behaviour of *O. dimidiatus* and the behaviours associated with oviposition. The following describes the series of behaviours common to *O. dimidiatus* females.

Fig. 2.5: Age-specific fecundity of *Opius dimidiatus*.


MEAN # EGGS / FEMALE
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
AGE (days)

a) *Host Location*:

These parasitic females appear to be strongly attracted to the plants, flying directly to leaves as soon as plants are placed in their cage. The attraction of females to host medium regardless of the presence of a host has been noticed for several species of Opiinae (Glas and Vet 1983, Greany et al. 1977). Once on a leaf, a female pauses several seconds to groom, and then begins to search, tapping her antennae from one side to another, until she finds a mine. She appears to follow the mine by using this side-to-side antennal tapping to ascertain when she has moved away from the edges of the mine, or has lost contact with the odours of the frass in it. A trail made from frass dissolved in water will elicit the tracking response. Every few steps, she hesitates, pulls her abdomen beneath her and taps the leaf with the tip of her ovipositor. This tapping has been speculated to be for the purpose of depositing a trail-marking pheromone (Petitt 1984), or a means of probing the leaf briefly to locate a host (Minkenburg and van Lenteren 1986). My observations do not support either theory. No liquid has been seen being deposited on the leaf, nor does the ovipositor do more than briefly touch the mine. Trails she has followed are neither accepted nor rejected noticeably upon re-encounter with them, and the ovipositor is never "unsheathed" during this behaviour. It is my belief that tapping on the surface of the leaf, and the vibrations this produces, may indicate to her when she is above a hollow mine or on more solid leaf tissue, or that it enables her to better detect frass deposits. Sensillae on the tip of the ovipositor may contribute to such functions.

The odour of frass inside the mine is probably an important component of the stimuli followed by the searching female, in that frass may be correlated with larval presence. As she begins to encounter fresher frass closer to the larva's position, her attention to the trail becomes more directed. Once in the vicinity of the trail's end, a female concentrates her tapping and probing in a small area, and is not easily distracted. Even when the host larva has been removed previously from the leaf, she may circle and probe the hole left in the leaf for several minutes.

Another cue that appears to be important to the parasitoid in finding the precise location of the host is the vibration produced by the mouthhook of the feeding larva. Because the larva is embedded in the leaf and not visible to the parasitoid female, these vibrations produced by feeding may serve to pinpoint the larva's position once she has found its general location using trail stimuli. Even when searching randomly, and not following a mine, a female can be led to a host by means of this vibrotaxis. This phenomenon has been reported for other members of the Opiinae, *Biosteres (=Opius) longicaudatus* Ashmead (Lawrence 1981), and *Diachasma alloeum* (Muesebeck) (Glas and Vet 1983), both endoparasites of Tephritidae. Both Lawrence, and Glas and Vet further speculate that the vibrations produced by feeding larvae may also stimulate oviposition behaviour.

Furthermore, in observations made during experiments with *O. dimidiatus*, it was observed that many "non-moving" larvae (those whose mouthhooks were not moving) went undetected, even by females passing right over them. There are at least two reasons why larvae become immobile: they may still be temporarily paralysed from a recent oviposition, or they be reacting to the approach of tapping antennae by temporarily ceasing all movement or "freezing". This "freezing" response lasts for about ten seconds, after which the larva resumes feeding, but it is often long enough to distract the female, and prevent her from locating the host.

To test the hypothesis that detection of a host is independent of movement of larval mouthhooks, parasitoids were observed encountering both parasitized and "healthy" (i.e. unparasitized) hosts; as the larvae were approached, the movement or non-movement of their mouthhooks was noted, as was parasitoid detection of them (recognizable by behavioural changes and probing). Hosts were recorded as "healthy" (moving or non-moving), parasitized (and paralysed), or parasitized (moving or non-moving). A G-test compared the detection of moving hosts, both "healthy" and parasitized, with non-moving hosts.

Of a total of 182 encounters, 75% of all moving hosts were probed as compared with 20% of all non-moving hosts, significantly different (G=48.8, p<.001), a fact indicating that detection is a function of the movement of mouthhooks. There was no difference in the non-detection of "healthy" or parasitized hosts that were immobile (G=.10, p>.50), and both "healthy" and parasitized hosts showed an equal tendency (22% of encounters) to "freeze" when approached by a female parasitoid (G=.06, p>.50).

It is clear that a parasitoid's ability to find leafminer larvae depends to a great extent on host-feeding vibration, and that in future experiments, the non-detection of immobile hosts by a searching female should not be mistaken for host rejection.

Once having located the host, the *O. dimidiatus* female begins to probe the immediate area with her ovipositor, turning 180 degrees and taking 2-3 steps forward after each miss until she makes contact with the larva. If she is unsuccessful after several probes, she stops for several seconds with her antennae quivering (possibly to relocate the host-related cues) and then attempts to reprobe the leaf. The female seldom has difficulty probing smaller larvae once she has located them. However, as hosts increase in age, they increase in size and activity, and are able to move more freely within the mine, often using directional change to elude a probe, and so require more handling to probe them.

b) *Oviposition Behaviours*:

Once the parasitoid has located the larva with her ovipositor, she straddles the leaf above it; after pulling her ovipositor under her towards the anterior part of her body, she inserts the ovipositor with a single thrust. It is at this point that a female appears to judge the suitability of a host for oviposition. If she does not find the host suitable for oviposition, she withdraws her ovipositor without paralysing it. If the host is suitable, she apparently injects a venom which paralyses the host for up to 3 minutes. Injecting the venom requires several seconds (or longer if the host is large and wriggling) until the larva becomes elongated and motionless. At this time the *O. dimidiatus* female also ceases movement, her antennae extended horizontally and just slightly quivering, until the egg has been laid. Immediately after, the antennae wave, her abdomen dips as the pair of third valvulae which form the sheath (Richards and Davies 1977) assist in withdrawal of the ovipositor, and the ovipositor is retracted. The mean length of an oviposition is 59.46 seconds (S.E.=4.31, n=50), and a rejection of a parasitized host after a probe takes a mean time of 39.06 seconds (S.E.=3.12, n=50), significantly less than an oviposition (t-test,p < .05).

Once the oviposition is completed, the female steps away from the larva immediately, often stopping to groom her antennae and ovipositor. A second oviposition can take place in as little as 30 seconds; there does not seem to be the need for a long period of time to elapse, to bring down eggs for example, (Rogers 1972), and one female was observed to lay 12 eggs consecutively in 6 minutes. Despite a report by Lema and Poe (1979), I have never observed this species ovipositing in larvae emerging from the leaf; in fact, when faced with a larva out of the leaf, females reacted strongly in avoiding it.

(iv) Choice of Instar:

In patch time experiments, females will be presented with hosts for oviposition, so it is important to determine which instar(s) will or will not be acceptable to the parasitoid as potential hosts. As outlined by van Alphen and Drijver (1982), egg distribution by parasitoid females over different age classes of larvae is a function of the following parameters:

a) total density of host larvae

b) relative density of different age classes

c) encounter rate with the different age classes (which

is a function of the numbers of each age class, the size of host at each age, and the amount of stimulus it produces.

d) the properties of the host larva at each age that influences its chances of "avoiding" parasitism (e.g.activity, position in the substrate, etc.)

e) preference by the parasitoid for a particular age or class of host.

To test instar choice, they suggest, (a) and (b) must be kept constant, leaving the last 3 factors to cause an egg distribution that will result in the best survival for the parasitoid. It is assumed that "best survival" implies the greatest number of eggs laid for the number of hosts available.

In this experiment, the total density and relative densities of three instars of hosts were kept constant, and female parasitoids were given the opportunity to choose to oviposit or not at each encounter. I expected to find, first, that the encounter rates of the three instars would vary, with the small, first instars being encountered least frequently and the third instars the most frequently. The mines made by first instar larvae are very small so that the stimuli they produce are relatively small. The larger the larva, the greater the vibration and movement, and the more likely it is to be encountered (Vinson 1976).

It was expected, too, that females would choose not to oviposit in first instars. First instars of this species (and many small second instars) do not usually survive a probe and oviposition (pers. obs.), so a female would not benefit from depositing an egg there. Even if they did survive, the early host instars might remain longer in the leaf, subject to possible superparasitism or multiparasitism, or premature death due to environmental conditions.

The oldest larvae (late third instar), on the other hand, are sometimes difficult to parasitize due to extreme activity when probed, and try to escape. (All three instars can show "freezing" behaviour when approached.) Nevertheless, an egg deposited in a third instar, and thus vulnerable for a shorter time, would seem to have the best chance of survival. Therefore, I expected that a female would persevere and differentially deposit her eggs in the larger, third instar larvae.

Methods and Materials

Leaves were prepared to contain three different instars of host larvae in the following way: 8 days, 6 days, and 5 days before the trials, the same plants were given to flies for oviposition for 4 hours. After 8 days, (allowing 4 days for all eggs to hatch), there were three classes of larvae: third instars (4-day-old larvae), second instars (2-day-old larvae), and first instars (1-day-old larvae). From these plants, leaves were chosen that contained three larvae of each instar, for a density of 9 larvae/leaf.

Experienced females, 4-7 days old, were given the chance to encounter and probe any host. The acceptance (oviposition) or rejection (no oviposition) of each larva was scored. A χ^2 test tested the prediction that the choice of oviposition in, or rejection of, a host was independent of host stage (instar).

Results

There was no instar effect on the proportion of hosts accepted for oviposition by *O. dimidiatus* in this experiment (Table 2.2), contrary to prediction. The largest instars were not parasitized more frequently when encountered than the other hosts though they were encountered significantly more often (χ^2=34.1, p<.05). Moreover, the smallest hosts, though encountered at the lowest rate, were accepted as frequently as the larger hosts.

Table 2.2: Effect of *Liriomyza trifolii* instar on acceptance for oviposition by *Opius dimidiatus*.

INSTAR	1	2	3
# ENCOUNTERED	9	48	67
# ACCEPTED	7	25	42
% ACCEPTED	79	52	63

$X^2 = 0.516$
$p > 0.05$

Discussion

It was not surprising to find a significant difference in the encounter rate of the three classes of hosts. As suggested previously, all larval instars do not have the same chance of being encountered. Females use various cues to find hosts, olfactory cues associated with the mine, and the movement of the larva and its mouthhook among them. The probability of encountering a host usually depends first on the probability of encountering a mine and following it. Larger mines (belonging to older larvae) are easier to detect, cover a greater leaf area, contain more frass, and often cut across the mines of young larvae, which are thread-like and difficult for a parasitoid to detect. The large amounts of frass in larger mines may also mask the weaker odours being produced from the small mines. In addition, larger hosts may produce more intense vibration, and so be more often tracked by parasitoids. For these reasons, larger hosts have a far greater probability of being encountered.

However, the predictions that parasitoid females would preferentially oviposit in third instar hosts and reject the first instars was not supported. Taken at face value, the results obtained would seem to suggest that the choice of instar by *O. dimidiatus* has not evolved to favour production of the greatest number of surviving offspring. Classical foraging theory predicts that when high quality prey (i.e. hosts) are available, low quality prey should not be used (Krebs and McCleery 1984). If the parasitoid were aware of the presence of better quality hosts, she would be expected to have rejected low quality, first instar hosts when she found them. However, the foraging model also predicts that when the rate of encounter with highest quality hosts falls below a low threshold, she should include lower quality hosts. It is possible that, at the density of hosts presented in this experiment, the average rate of encounter with any stage was so low that it was a better strategy to include lower quality hosts and lay an egg in any larva rather than waste an opportunity to oviposit. Also, when recognition time is added to the model, the addition of poorer quality hosts depends partly upon their density.

Of course, if all instars proved to be of the same quality and promised the same chance of offspring survival, the inclusion of first instars could also be explained. This is difficult to accept, given the high probability of mortality of first instars from probing. (First instars and small second instars seldom survive a probe, from my observations both in the laboratory and in the field, and are usually found dead and discoloured.)

As well, *O. dimidiatus* is not a specialist on *L. trifolii*. It parasitizes a number of leafminer species, so that it may have evolved on a broad size range of hosts.

A further possibility exists why there appeared to be no instar choice, having to do with the method of recording "acceptance" and "rejection". If a larger host was probed but not parasitized, it was assumed "rejected", whereas in many cases, the choice to oviposit may have been overridden by the difficulty in paralysing and controlling the host. Whether this situation should be considered an acceptance (because of supposed "intent") or a rejection (because the extreme amounts of venom necessary to subdue such larvae may cause them to be selected against), is difficult to determine. Regarding them as "acceptances" would increase the percent acceptance of third instars.

Unclear, too, is the basis on which the parasitoid determines the relative "acceptability" of each instar. The time when the choice is determined is critical. I have assumed that the choice was made after probing a host, but some parasitoids "measure" their hosts before probing them in order to test their suitability (Schmidt and Smith 1987). If such pre-probing assessment did occur, then "encounters" that did not lead to probes must also be considered as rejections.

Finally, there has been no evidence of (nor attempt to investigate) encapsulation of parasitoid eggs by this host and its implications to instar choice . In fact, the large percentage of parasitoids emerging from parasitized host puparia (>90%), suggests that encapsulation is not a cause of high mortality of eggs.

The conclusions reached from this experiment indicate that females of *O. dimidiatus* make no choice for oviposition among instars at the density presented. This suggests that any instar used in future experiments has the same chance of being parasitized once it has been encountered. However, since the time spent searching for, and encountering, hosts will be measured, it is important that all hosts used have a reasonable probability of being encountered, and will be equally easy to parasitize. Keeping these requirements in mind, an arbitrary choice was made to use the late second, and early third instar larvae (approx. 3-4 days old) in future experiments.

(v) Host Discrimination:

Future experiments would examine, among other factors, the effect of patch experiences on the time budget of *O. dimidiatus*. It was anticipated that the frequency with which a female encounters parasitized hosts might prove to be one way in which she assesses the changing quality of the patch she is searching. It was therefore necessary to determine if, in fact, an *O. dimidiatus* female could detect a parasitized host, and if so, when this discrimination took place. Because of the complexity of the experiments performed to investigate host discrimination, this topic will be the subject of the following chapter.

CHAPTER III

HOST DISCRIMINATION

A. Past and Present Theory

An important tool in the reproductive and foraging strategy of many parasitoid species is an assessment of the quality of a host patch being searched for oviposition sites. This may be done by using some measurement of host numbers or host quality. Further valuable information would concern which of those hosts might be unsuitable for oviposition, having previously been parasitized.

The laying of a second egg in an already-parasitized host, or "superparasitism", is considered to be disadvantageous for a parasitoid, because the clutch size fitness function increases at a decreasing rate. In solitary species, like *Opius dimidiatus*, only one egg/host can develop and produce an adult; supernumerary eggs and larvae are often destroyed by physical combat of larvae (Fisher 1961, van Strien-van Liempt 1983), by physiological suppression (Fisher 1963) and sometimes by both (Fisher 1961, Chow and Mackauer 1986, Hubbard et al. 1987). In many parasitic hymenoptera species, therefore, mechanisms have evolved by which a searching female can distinguish between unparasitized or "healthy" hosts, and those already-parasitized, a phenomenon defined as "discrimination" by G. Salt in the 1930's (van Lenteren 1981).

Prior to Salt's work, the sole criterion on which the determination of "discrimination" was based was that a female, distributing her eggs non-randomly among available hosts, showed that she possessed the ability to distinguish between parasitized and non-parasitized hosts, and was therefore avoiding superparasitism. However, on the basis of both laboratory research and field collections, Salt provided direct evidence that the process involved in the avoidance of superparasitism was flexible and could lead to superparasitism under some circumstances (Salt 1934). In fact, it is the absence of restraint to oviposit which is more often a cause of superparasitism than the lack of ability to discriminate (van Lenteren 1981).

Since then, many researchers studying the causes of superparasitism have extended the original definition, and define "discrimination" as both the ability to detect differences between unparasitized and parasitized hosts, and the subsequent tendency to refrain from oviposition in parasitized hosts (Force and Messenger 1965, Rogers and Hassell 1974, Bakker et al. 1985), despite the ambiguities resulting from a two-part definition. For example, a female that chooses to override her knowledge of a parasitized host and oviposits in it because of "oviposition pressure" must be considered a "non-discriminator" unless a disclaimer is added. Similarly, a gregarious parasitoid which lays fewer eggs in already-parasitized hosts than in healthy ones, and must therefore be able to distinguish between them, would not be credited with the ability to "discriminate". Other authors, however, avoid including the outcome of discrimination in their usage of the word, and use the simplest sense: to distinguish between parasitized and unparasitized hosts (van Alphen 1980, van Alphen and Nell 1982). For the purposes of this paper, I, too, intend to apply the latter, simpler definition of "discrimination".

Discrimination appears to be a widespread ability among parasitic hymenoptera, reported in about 200 species studied, in 5 families belonging to 4 superfamilies (van Alphen et al. 1987). In most cases, neither the mechanism of discrimination nor the site of its detection have been determined (Fisher 1961, Bakker et al. 1967, Cloutier et al. 1984, Legaspi 1986).

To be able to discriminate, a parasitoid must detect a change in the host as a result of parasitization. The cue may be an external one: an oviposition wound in the host or surroundings, leakage of host fluids, or a mark deposited by the parasitoid before, during, or after oviposition (van Lenteren 1976, Vinson 1976, Klomp et al. 1980, Harrison et al. 1985). A marking substance originating in the Dufour's (or alkaline) gland has been identified as the source of the external pheromone laid down by *Microplitis croceipes* and *Cardiochiles nigriceps* Viereck (Vinson and Guillot 1972) and by *Nemeritis (=Venturia) canescens* (Gravenhorst) (Harrison et al. 1985).

Though an externally-deposited contact chemical is more quickly encountered by a searching female than is an internal mark, and may therefore save her time, it may also be washed off by rain if water-soluble, be dispersed, or evaporate, and so may be a more transitory and less reliable signal. Van Lenteren (1981) suggested that most hosts marked by external cues are non-moving or slow moving hosts (eg. eggs, sessile larvae, pupae) and hosts living in clusters. He further conjectured that, in contrast, strongly-moving hosts should be marked internally only, since by their movement they may transfer external marks to unparasitized hosts, causing them to be misidentified by the parasitoid.

Likewise, internal cues may originate in a number of ways, because of physical changes in the host (van Lenteren 1981) or the haemolymph (Vet et al. 1984) as a result of parasitization, a substance emitted by or associated with the egg, a change in concentration of body fluids (Harrison et al. 1985), or a chemical injected by the parasitoid at the time of oviposition (Harrison et al. 1985). The source of such an internal chemical marker might be the venom injected to temporarily paralyse the host (Wylie 1970), or a pheromone produced by the female that marks the exploited larva. Again, an ingredient found in Dufour's gland extract has been linked to the internal marking of hosts by *N. canescens* (Hubbard et al. 1987). Internal marks are detected during subsequent probes by a female, by the ovipositor; sensillae found on the ovipositor tip could be involved in detection of these marks (van Lenteren 1981, Harrison et al. 1985).

Whether the parasitoid actively lays down a mark for later detection, or passively perceives a host change, it is clear that discrimination has evolved to benefit the "marker", and the possible advantages of discrimination to a searching female are frequently discussed (van Lenteren 1976, Bakker et al. 1985, Roitberg and Mangel 1988). First, by allowing a female the opportunity to avoid superparasitism and probable elimination of her egg, it prevents wastage of eggs. For a species which has a limited egg supply (e.g. pro-ovigenic species), or which lays few eggs/day, each egg becomes an important commodity; the energy involved in egg production is likely to be wasted if it is deposited in a host in which it has a low probability of survival. On the other hand, syn-ovigenic species, such as *O. dimidiatus*, are continually producing eggs, so wastage of eggs and the energy they represent are less likely to be a major constraint. However, since many of these parasitoids are also able to resorb mature eggs when deprived of hosts, using much of the energy they contain for adult maintainance and oogenesis (Jervis and Kidd 1986), there may be some benefit to be gained by avoiding egg wastage even by these species.

Secondly, if oviposition itself takes more time than the handling time involved in assessing the host, time may be saved for further search by discrimination. Time may, in fact, prove to be a more serious constraint than egg limitation. It is unlikely that most parasitoids in the field will exhaust their egg supply during their lifetime, but their foraging time, often limited by risk of predation or by adverse environmental conditions, is more likely to be at a premium.

Discrimination prevents wastage of hosts from multiple stings, often a cause of high host mortality. Finally, discrimination may be an information source, allowing a female to gauge the profitability of a patch, and the extent to which it has already been exploited by herself and other females. She can then make the "decision" whether to superparasitize or migrate from that patch.

As mentioned previously, early researchers assumed that the ability of a parasitoid to discriminate would result in the avoidance of superparasitism, an "all or nothing" situation. When examples of superparasitism were found in the field, they were considered "mistakes" (van Lenteren 1981), and investigations were conducted to discover why such "mistakes" might occur. Van Lenteren (1976) proposed a list of 6 possible causes of superparasitism in *Pseudeucoila bochei* Weld; of these, only two, the increased tendency to oviposit, and inexperience of females were considered to be causes serious enough to result in the degrees of superparasitism found.

"Oviposition pressure" describes the increasing tendency of a female to oviposit when deprived of healthy (unparasitized) hosts for a period of time. According to van Lenteren, if this pressure builds up and she is forced to stay on the patch, it may surpass her inhibition to superparasitize, and she may oviposit in parasitized hosts. Otherwise it may cause her to migrate from the exploited patch. After oviposition, the tendency to oviposit decreases and she will again reject parasitized hosts.

The second causal factor, inexperience of females, led to some controversial conclusions. Proponents of this theory (van Lenteren and Bakker 1975, van Lenteren 1976, Klomp et al. 1980) noted that, when females which had never encountered unparasitized hosts were offered parasitized hosts, a substantial degree of superparasitism occurred. After experiments showed a change in behaviour from acceptance of parasitized hosts by inexperienced females, to significant rejection of parasitized hosts after being given experience with healthy hosts, they concluded that parasitoids must "learn" to discriminate by first experiencing healthy hosts, and that "non-learning" by inexperienced wasps was a main cause of superparasitism. (However, it is unclear how a wasp can determine which characteristics of an unparasitized host are recognizable as "acceptable".)

The phenomenon of inexperienced females superparasitizing was also noted by van Alphen and Nell (1982); they reasoned, however, that many factors other than "non-learning" might cause inexperienced females to not refrain from oviposition. Klomp et al. (1980) concluded not only that females learned discrimination, but that they could, after a period of non-oviposition, "forget" the association they had made between the characteristics of a healthy host and deposition of their egg, and fail to refrain from superparasitism again. They suggest that a "forgetful" female will continue to oviposit in parasitized hosts until she again encounters a healthy host. If this behaviour were taken to its logical extreme, a female might never leave a fully exploited patch. A recent paper by van Alphen et al. (1987) re-examines the earlier work done by van Lenteren and Bakker (1975) with contrasting results, and suggests adaptive reasons why inexperienced females may superparasitize. (These reasons will be discussed later in this chapter)

Nevertheless, it does seem to be true, that as a causal mechanism of superparasitism, a high parasitoid/host ratio (leading to a high encounter rate with parasitized hosts) may result in a buildup of a tendency to oviposit, and a high degree of superparasitism. The resulting distribution of eggs might appear more random, pointing up the danger in using egg distributions from field samples to test the existence of discriminatory ability.

There exists a further possibility, seldom investigated, why discrimination would not be absolute, namely that discrimination is time-dependent. If one considers the character of the mechanisms on which discrimation is based, it becomes logical to investigate the variation in their effectiveness over time. If, for example, a chemical marks the host, the mark may lose its strength and dissipate, or be metabolized by the host and be undetectable after a period of time. In contrast, a substance injected into the hemolymph may be concentrated at the point of entry and take some time to diffuse throughout the host, where it can be detected by a probe. In experiments with *Aphidius nigripes* Ashmead, Cloutier et al. (1984) found no avoidance of superparasitism for the first few hours, but after 2 hours the level of avoidance increased for up to 24 hours, suggesting a gradual biochemical change in the host as a result of parasitism, or a gradual release of an egg factor (Cloutier et al. 1984).

Many species of parasitic hymenoptera have been found to aggregate in areas of high host density (Waage 1983). If hosts do not leave a patch, as in leafminer systems, aggregation would increase the probability of a female parasitoid encountering hosts already exploited by conspecifics. *O. dimidiatus* does not search a patch randomly, but follows the track of the mine, often long and convoluted, to reach the larva. This means that after a female parasitizes a host, even if she resumes searching some distance away on the leaf, her mode of search frequently causes her to re-encounter and follow the mines of larvae she has already parasitized. If *O. dimidiatus* has no means of detecting and rejecting these hosts, the resulting contest competition of sibling larvae within the host would destroy all but one survivor. The egg of *O. dimidiatus* hatches within approximately 24 hours, into a mandibulate first instar that probably kills supernumerary larvae with its large mandibles. Discrimination, then, would confer an advantage on *O. dimidiatus* females by reducing the time spent in handling and ovipositing in hosts that are unlikely to provide them with further offspring. I would expect to find that the ability to discriminate has evolved in this species, provided that discrimination takes less time than oviposition.

The time-saving benefit would be greatest if *O. dimidiatus* could detect parasitized hosts when she comes in contact with them by means of external cues associated with the parasitized hosts or their surroundings. The parasitoid locates her host's position by following frass deposits in the mine with her antennae. If she has evolved the techniques that permit her to locate her host by following olfactory cues, it is not unlikely that olfactory mechanisms might also help her avoid parasitized hosts.

However, *L. trifolii* larvae feed within the leaf, moving forward as they create a mine, at a rate of one body length per hour, sometimes moving backward in the mine to change direction. An external stimulus on a leaf would not be associated with the larva for long, and so may not be as useful as an internal cue. To be sure, internal stimuli would involve more handling of hosts before they are detected, but they would provide a more reliable signal. Given these constraints, the most dependable solution might be a "two-edged sword", one mechanism that acts externally to "warn" a female as she searches, and a "backup" or internal stimulus that can be detected when the first does not prevent probes.

B . Host Discrimination by *O. dimidiatus*

The objectives of these experiments are:

(1) To confirm the presence of discrimination in the *O. dimidiatus-Liriomyza trifolii* system, and

(2) To investigate the level at which discrimination occurs.

Experiment 1: Ability to discriminate

Methods and Materials

a) *Hosts*:

In all the following experiments, broad bean leaves were approximately 20 cm^2, each containing 6-8 chrysanthemum leafminer larvae of late 2nd and early 3rd instar. Leaves were chosen whose mines appeared to be distributed in a relatively homogeneous manner over the leaf area. For presentation to a parasitoid, a leaf was placed on moist filter paper in a petri dish, and replaced for each trial. Prior to the trial, the position of each larva and its mine was mapped on paper in order to identify later the parasitized from healthy larvae during the experiment without contaminating the leaf or larvae with a physical mark that might interfere with the parasitoid's natural searching behaviour.

b) *Parasitoids*:

Parasitoids were 3- to 7-day-old unmated *O. dimidiatus* females. For 24 hours prior to the trial, they were given numerous hosts on which to gain experience and host feed. In addition, as a check of a female's ability to track and parasitize a host, and to avoid buildup of "oviposition pressure", each experimental female was placed on a host leaf and observed ovipositing. She was then transferred to the experimental leaf, using half a gelatin capsule, and allowed to walk onto the leaf. During this first presentation of hosts to prepare the experimental setup, the female was allowed to parasitize up to half the larvae in the leaf before she was removed. These prepared leaves containing both healthy and parasitized hosts in equal numbers were placed on moist filter paper in petri dishes until re-used for the experiment.

After the first ovipositions in hosts were completed, the female was placed in a numbered petri dish containing hosts and held until she could be presented with the same leaf during the trial. Petri dishes containing prepared leaves or *O. dimidiatus* females were numbered to insure that parasitoid and leaf were matched up properly. Each experimental female was able to oviposit and host feed during the holding period, reducing the possibility that physiological pressures might affect her oviposition decisions. Finally, experiments were conducted randomly throughout a foraging day, so that time of day was, likewise, not a factor (Iwasa et al. 1984).

c) *Experimental Protocol*:

Females were placed on leaves giving them a choice of equal numbers of healthy hosts and hosts which they themselves had previously parasitized, and were allowed to find and oviposit in larvae.

Since it had been speculated that discrimination of the hosts might take place either upon encounter with a host, or after testing it with an ovipositor probe, all relevant aspects of the contact with each host were recorded. Using a stereo microscope, the following behaviours were noted:

1. Encounters with hosts: A host was considered to be encountered when a female, engaged in search behaviour, contacted the leaf surface above the larva with her antennae, tarsi or ovipositor.

2. Movement of larval mouthhooks when approached by a female: During previous experiments it was demonstrated that parasitoids were unresponsive to the

presence of hosts whose mouthhooks were not moving (because they had recently been paralysed during an oviposition or because of a "freezing response" that the host larvae often demonstrate when approached by a tapping antenna). Since the vibration of mouthhooks is used by searching females to locate hosts, the absence of this stimulus may make the hosts less "visible". Therefore, only larvae whose mouthhooks were moving when approached , and therefore "visible", could be included in the final analysis.

3. Probes into larva with ovipositor.

4. Paralysis of a host. This always took place before oviposition and seldom when a host was rejected. Non-paralysis of a host, then, was one indication of rejection of a host.
5. Oviposition: Though characterized by distinct behaviours (see chap. II), oviposition was confirmed by dissection after each trial. Slides were made of dissected larvae in water, and a compound microscope used to determine the number of new and previously-laid eggs present. By noting the changing sizes and densities of eggs, it was possible to tell the difference between a newly-laid egg and one that had been laid even half an hour previously, so that a new oviposition could be confirmed.
6. Rejection of a host: A rejection was scored when examination of a host by antennating or ovipositor tapping did not result in a probe, or when probing did not result in an oviposition. Dissections after trials confirmed rejections of hosts.

The proportions of healthy and parasitized hosts encountered in which ovipositions occurred were compared using a G-test.

As suggested previously, a lack of restraint from oviposition by discriminating females could increase the instances of superparasitism and place the results in question.

An attempt was made to remove the factors that might result in this situation by using young, experienced females, by providing opportunities for oviposition just before a trial, and by randomizing the trial times throughout the day.

Therefore, if the rates of oviposition in parasitized hosts and in healthy hosts were not significantly different, it would indicate a lack of discriminatory ability by *O. dimidiatus* females. A significant reduction in the rate of oviposition in parasitized hosts, however, would confirm their ability to discriminate and avoid laying eggs in hosts already parasitized.

If the overall discrimination results do determine that *O. dimidiatus* can discriminate, then a further analysis will be made, using the detailed observations described, to reveal whether discrimination of leafminer larvae takes place before or after probing. First, a comparison will be made between the non-probing of "moving" healthy hosts, and the number of "moving", parasitized hosts that were examined but not probed. Greater rejection of parasitized hosts would indicate that discrimination took place before probing. Likewise, by looking at the hosts that were probed, and comparing the rates of oviposition in healthy and parasitized hosts, it may be possible to determine if there is differential use of these hosts and therefore whether discimination occurred after probing them.

Results

The results of this experiment (Table 3.1) confirm that eggs were being laid significantly less frequently in parasitized hosts than in healthy hosts. Overall acceptance of healthy hosts was 54% compared with 9% acceptance of parasitized hosts. When probing or non-probing of hosts was considered first, it was revealed that there is

Table 3.1: Acceptance of "healthy" and parasitized hosts

	Overall Acceptance		Acceptance Before Probing		Acceptance After Probing	
	%	n	%	n	%	n
Healthy Hosts	54	114	68	114	79	77
Parasitized Hosts	9	68	38	68	23	26
p	< 0.001		<0.001		<0.001	

differential probing of parasitized versus healthy hosts. After they were encountered and examined, 38% of parasitized hosts were probed, significantly lower than the 67% of healthy hosts that were accepted and probed. However, there was an even greater rejection of parasitized hosts for oviposition after probing, based on information gained from probing; only 23% of already-parasitized hosts were accepted for oviposition, as compared with 79% of the healthy hosts.

Discussion

The findings of this experiment lead me to conclude, not only that an *O. dimidiatus* female can discriminate between healthy and parasitized hosts and can avoid superparasitism, but that she does so on two levels. First, she must be reacting to some stimulus, as yet unidentified, either on the leaf above the larva, or on the larva itself, which has indicated a previous oviposition, and is chosing to reject a significant proportion of these hosts. Second, when she does probe parasitized hosts, detection of a further internal cue (also unidentified) causes her to reject a high percentage of them. As a result of two discriminatory mechanisms acting at different levels, females were able to reject 91% of the parasitized hosts with which they came in contact.

Similar results involving a dual method of discrimination have been reported for *N.canescens* which reacts to both an unidentified external marker (Rogers 1972) and an internal mark produced by the Dufour's gland (Harrison et al. 1985). Dissections of *O. dimidiatus* females confirm the presence of a Dufour's gland in this species. However, it has not been determined if the excretions produced by this gland elicit the avoidance reactions demonstrated by *O. dimidiatus*.

Recent studies on avoidance of superparasitism have attempted to tease apart the discrimination process in order to determine the nature and source of the mechanisms acting, when they are manifested, and the duration of their effect (Cloutier et al. 1984, Harrison et al. 1985, Chow and Mackauer 1986, Hubbard et al. 1987). Though determining the source of the stimuli involved is beyond the scope of this study of *O. dimidiatus*, it has now been determined when in the searching process they act. The temporal nature of their effect is a question for further investigation. If there is an ephemeral nature to these mechanisms (if, for example, a volatile chemical marker is involved, or a substance emitted by the developing egg or larva), they may, in fact, have a limited life. I would expect that , whatever the cues a female is detecting, they would produce their greatest effect during a period of time when the knowledge they impart would be most valuable to her, that is, when the probability of reencountering hosts is high. Immediately after oviposition, and while a female is still searching a patch, her chances of reencountering parasitized hosts is high, and decreases as she migrates to different patches. The possibility exists, therefore, that the longevity of the response to stimuli involved in discrimination by *O. dimidiatus* may be limited, reaching its peak shortly after oviposition.

C. Discrimination as a Function of Time:

Further experiments were performed to examine the function of time in both levels of discrimination shown by *O. dimidiatus*.

Experiment 2: Discrimination Before Probing

Methods and Materials

To test the hypothesis that discrimination of a parasitized host before probing , upon encounter with it, is a function of time since the first oviposition in that host, equal numbers of healthy and parasitized larvae were offered to a female as described in the previous experiment; again, the female was the parent of eggs already in the hosts. In these experiments, times were recorded with a stopwatch for each encounter, probe, and initial and second oviposition in a host in order to calculate the time period until re-encounter. Although the hosts were presented for re-encounter at intervals ranging from 30 seconds to greater than 6 hours from the first oviposition, ultimately, the time a female took to locate each host determined the exact period between the first and second encounters. At the conclusion of the experiment, therefore, these time periods were grouped into half-hour intervals and the percentage of probes in each type of host for each interval were compared with a control group of healthy hosts using Scheffé's Frequency Multiple Range test. A polynomial regression was fitted to the arcsin-transformed data.

Results

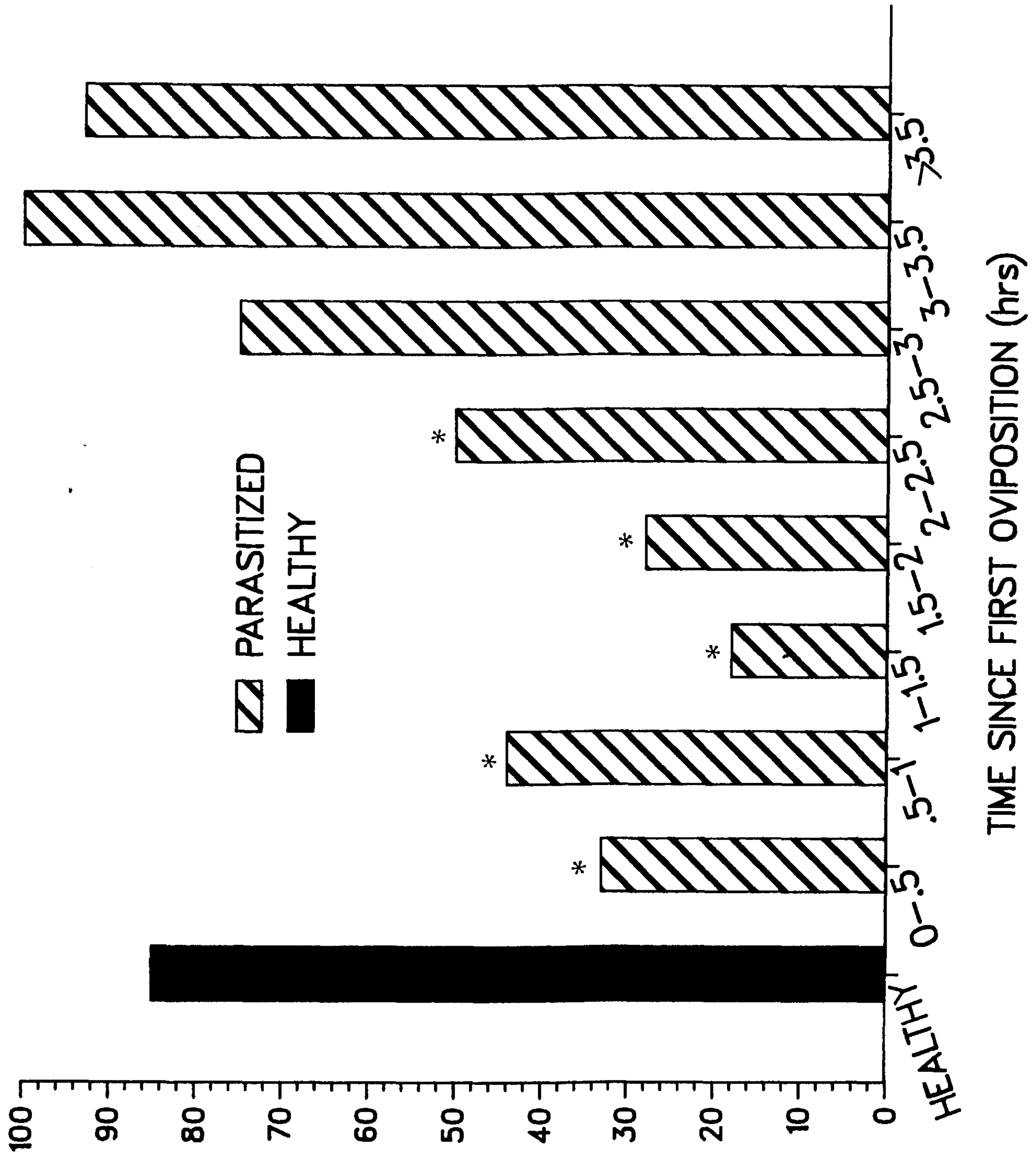
Results of this experiment (figure 3.1) show the differential probing of hosts: the control group, healthy hosts, were probed in 85% of the trials, whereas the once-parasitized hosts showed significantly fewer reprobes for the first 2 1/2 hours after the first egg had been laid. The polynomial regression ($r^2=0.89$) indicates a gradual decline in rejection of hosts up to this point. After 2 1/2 hours, the number of reprobes was no different from probes in healthy hosts.

Experiment 3: Discrimination After Probing

Methods and Materials

In this experiment, which looked at the importance of time in the discrimination of hosts after probing, the same procedure was followed as in the previous experiment, with one adjustment. In order to achieve a suitable sample size of probed hosts, it was necessary to remove mechanisms which might have deterred a female from probing a larva. Since one possible source of deterrent was a mark laid down on the leaf, leaves were washed for 10 seconds with running distilled water following the first ovipositions, and patted dry. They were then held, as before, on moist filter paper until reused. Times

Fig. 3.1: Discrimination of parasitized hosts before probing. (* indicates significant difference from healthy hosts at $p < 0.05$)


PERCENT PROBED
0
10
20
30
40
50
60
70
80
90
100
HEALTHY
0-.5
.5-1
1-1.5
1.5-2
2-2.5
2.5-3
3-3.5
>3.5
TIME SINCE FIRST OVIPOSITION (hrs)
▨ PARASITIZED
■ HEALTHY
*
*
*
*
*

for each probe and oviposition were recorded, and a comparison made of the percentage of already-parasitized hosts for each time interval with the percent parasitism of healthy hosts. As before, a polynomial regression was fitted to the arcsin-transformed data.

Results

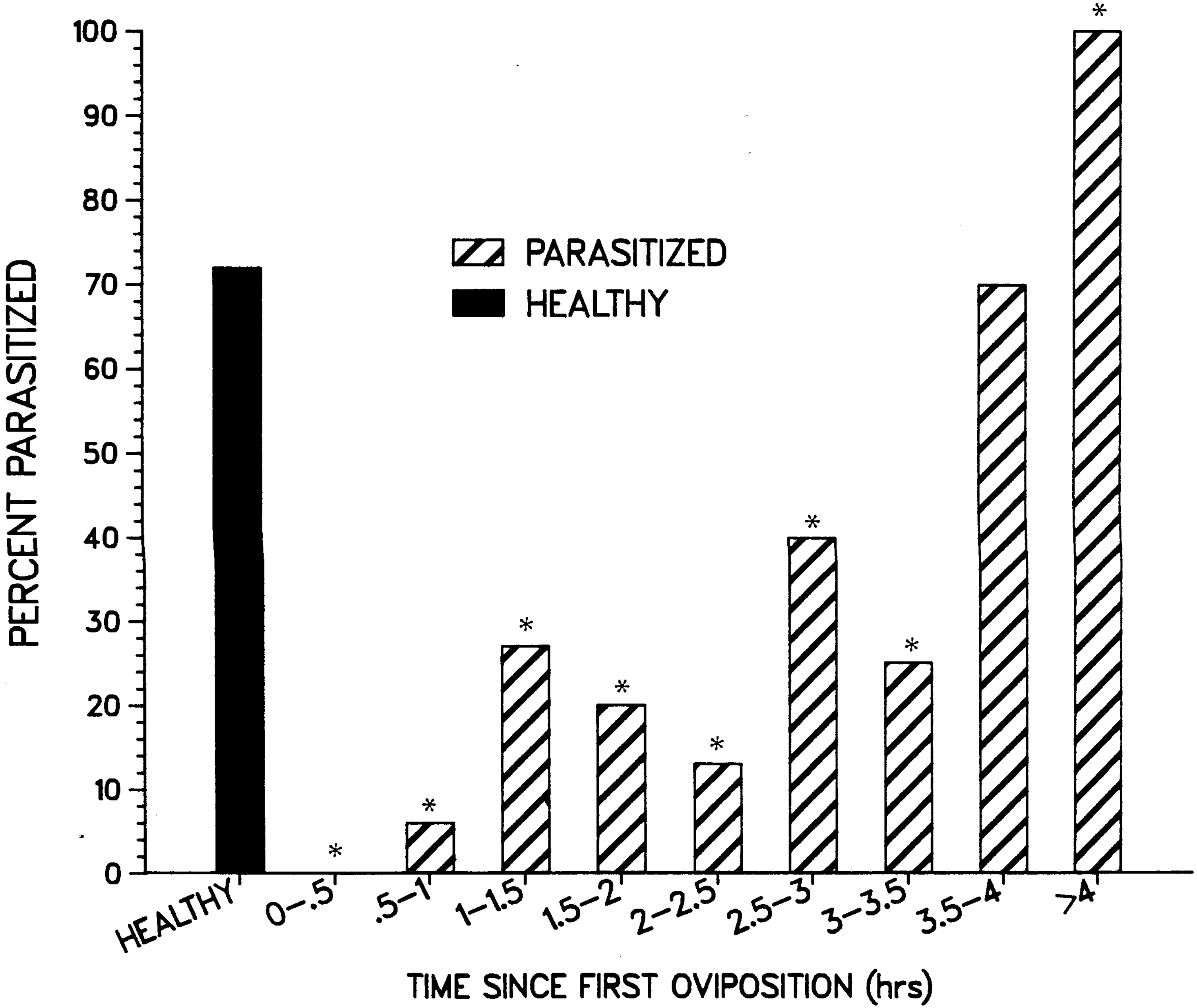
A similar pattern of time-dependent discrimination was revealed in Experiment 3, the investigation of discrimination after a host has been probed (Figure 3.2). Larvae containing eggs less than 1/2 hour old were detected, and superparasitism avoided, in every instance. Even after one hour, only 5% of experimental hosts were superparasitized. In fact, the rate of superparasitism was significantly lower than in the group of healthy hosts in every group up to 3 1/2 hours. The regression line (r^2=0.94) indicates that rejection of hosts decreased up to this point. Between 3 1/2 and 4 hours, there is no difference in the oviposition rate , and after 4 hours, the oviposition rate reached 100%, significantly higher than for healthy hosts.

Discussion

These findings confirm that *O. dimidiatus* females can detect stimuli associated with parasitized hosts on contact with them shortly after oviposition, and that the discrimination reaction elicited by these external cues can last up to 2 1/2 hours. They further suggest that females who probe parasitized hosts can detect them immediately, this response lasting up to 3 1/2 hours post oviposition.

Though the phenomenon of discrimination has been confirmed for many hymenopterous parasitoids, the sources of the stimulus whose detection results in differential avoidance of parasitized hosts are often difficult to identify, with the exception of a few marking pheromones (Vinson 1976, Harrison et al. 1985, Hubbard et al. 1987). Some knowledge can be gained as to the character of the stimuli, however, from the time periods during which the parasitoid avoids superparasitism. Thus, the fact

Fig. 3.2: Discrimination of parasitized hosts after probing. (* indicates significant difference from healthy hosts at $p < 0.05$)


100
90
80
70
60
50
40
30
20
10
0
PERCENT PARASITIZED
PARASITIZED
HEALTHY
*
*
*
*
*
*
*
*
HEALTHY
0–.5
.5–1
1–1.5
1.5–2
2–2.5
2.5–3
3–3.5
3.5–4
>4
TIME SINCE FIRST OVIPOSITION (hrs)

that *O. dimidiatus* responds both before and after probing to some cue or cues, and the fact that these cues are effective only for several hours, allow speculation about the nature of these stimuli.

First, a female of *O. dimidiatus* responds to some change in the leafminer larva upon encounter with it during normal searching behaviour on the leaf. If she is detecting an oviposition wound in the leaf tissue, or leakage of host fluids within the mine or through the oviposition hole, I would expect that the discrimination response would appear to decay over time. Because the larva is continuously moving away from the point of prior oviposition, it becomes less and less likely that the parasitoid will be able to associate the discrimination cue with that particular larva, and she is more apt to probe it.

Similarly, a chemical marker on the leaf surface above the larva would not mark the position of a moving larva for long, and might, in addition, be subject to decay. (The mark on a leaf surface described here to mark a newly parasitized larva should not be confused with the general patch mark described by Price (1970) which identifies a searched patch.) If a marking pheromone were being produced by *O. dimidiatus* for deposition on the leaf, the longevity of the mark should reflect the probability of re-encounter with that host. As discussed previously, the probability of re-encounter is high immediately after oviposition, but decreases as she moves to new patches. On this assumption, and the fact that a day's foraging period lasts only approximately 5-6 hours, a parasitoid need not invest a great deal of energy in a long-lasting, "expensive" mark. Roitberg and Prokopy (1987) point out, however, that since many parasitoids aggregate in patches of high host density, it would be to a female's advantage to allow her mark to last long enough to advertise the presence of her young to conspecifics, since the first egg in a host is not always the "winner". Though a mark may function originally to inform the marker of her own exploitation of a host, it may also serve to protect her progeny by warning conspecific females, until her larva is old enough to ensure its own superiority over supernumeraries. Any of these examples, then, could explain the time-dependent nature of discrimination displayed by *O. dimidiatus* before probing.

Van Lenteren (1981) reports that *O. pallipes* "finds" its host, the tomato leafminer, *Liriomyza bryoniae*, less often after marking both the leaf and the host with pheromone than when hosts have not been preciously detected and marked. Though I have never observed nor detected the deposition of a pheromone by an *O. dimidiatus* female, there is circumstantial evidence that suggests a chemical is involved in marking the leaf, perhaps leaking from the host.

First, as reported in the third experiment, washing leaves after the first ovipositions increased the overall percentage of hosts reprobed, from 50% to 77%. This suggests that a water-soluble mark may be present after parasitization. Secondly, I observed that the oviposition wounds on both the leaf and the parasitized larvae were stained with a rusty-coloured mark. This stain spread out on the upper surface of the mine beneath the leaf's epidermis, and was particularly evident when very small larvae had been killed by repeated probing and paralysis. Attempts to duplicate this stain by probing unparasitized larvae were unsuccessful, but hemolymph drawn from a parasitized larva with a hypodermic needle produced a rusty stain when deposited in an empty mine. By injecting hemolymph from a just-parasitized host into the mine above a healthy host, it was possible to produce a rejection response from females encountering these healthy hosts. Haemolymph from healthy larvae, deposited above healthy hosts, did not elicit rejection of these hosts.

Therefore, it is possible that *O. dimidiatus* females have evolved the ability to detect fluids leaking from hosts they have recently parasitized. Whether their response is to the venom they inject to induce temporary paralysis, or to an internal marker they deposited, is still unclear.

O. dimidiatus also reacts to some cue it detects within the host, rejecting nearly all hosts within the first hour after oviposition, the earliest rejection occurring after less than one minute. This rapid rejection response at first, and the lack of response after 3 1/2 hours suggest the existance of a chemical marker associated either with the act of oviposition or with the egg itself. As hypothesized for external markers, such a chemical need last only as long as it is necessary to protect the host larva from being reparasitized by the same female. Finally, it is interesting, from an evolutionary point of view, that *O. dimidiatus* females fail to avoid laying eggs in hosts containing mandibulate first instars, which have been observed engaged in physical attack of supernumerary larvae.

Until recently, very little work had been done on the time period within which discrimination is manifested. As mentioned, Cloutier et al. (1984) found that the period during which *A. nigripes* could discriminate was between 2-24 hours after oviposition in that host. Harrison et al. (1985) were able to treat hosts topically with Dufour's gland secretion and determined that the percentage avoidance of parasitized hosts by *N. canescens* increased, from 5 minutes after oviposition to 24 hours, and then decreased until 32 hours, when it was no longer effective. Thus, they were able to identify an external marking pheromone from Dufour's gland as at least one mechanism of discrimination by *N. canescens*, lasting for about 32 hours after deposition. They further suggested that *N. canescens* is able to detect an internal mark; this was confirmed by Hubbard et al. (1987).

In the latter's experiments, females rejected hosts on the basis of Dufour's gland secretions, deposited externally, for several days after oviposition. They also reported a different pattern of avoidance between mothers of the first egg and conspecific mothers. Avoidance by mothers was 30% higher than by non-mothers for the first 24 hours after oviposition, after which the rates of avoidance were maintained at the same lower level (Hubbard et al. 1987). *O. dimidiatus* females used in the experiments reported here were all "mothers" of the first egg deposited, so that conspecific superparasitism was not tested.

In a study of the interaction of host discrimination and larval competition, Chow and Mackauer (1986) discovered that two stimuli, acting independently, deterred superparasitism by *Ephedrus californicus* Baker. The first, an external marker, perceived by experienced females only, achieved its greatest effect immediately after oviposition and declined after 10 hours. The effect of the second, an internal stimulus, increased gradually with time since oviposition. Detected by both naive and experienced females, it was speculated to be the result of host changes associated with the developing parasite egg or larva.

Thus, discrimination may be a complicated phenomenon to pin down, both as to the source of mechanisms that result in discrimination, and in their mode and duration of action.

D. Adaptive Reasons for Superparasitism:

Up to this point I have dealt with discrimination and superparasitism from a causal point of view, looking at proximate reasons why superparasitism may occur or be avoided. But an alternate, equally valuable, approach to understanding the existence and function of superparasitism is to investigate under what circumstances a female might choose superparasitism as her best strategy. Obviously, for a female parasitoid trying to maximize her fitness, finding and laying eggs only in unparasitized hosts would insure that the greatest proportion of her progeny would survive, and most authors agree that, when unparasitized hosts are available, a female should always reject already-parasitized hosts.

If healthy hosts are not readily available, decisions must be made whether or not to superparasitize. If a female is alone in exploiting a patch, any parasitized hosts she finds will contain her own eggs. If she were to superparasitize these hosts, only one of her eggs would survive per host, so there would always be wastage of eggs and time. Unless it somehow increases the probability of survival of one of the eggs (e.g. if superparasitism reduces the immune response of the host), "self-superparasitism" should be avoided and would be non-adaptive.

When conspecific eggs are already present in hosts, a second egg laid in it has a small chance of survival (because both larvae possess the same defence mechanisms and the second usually has a disadvantage). Bakker et al. (1985) showed (using wild-type and mutant strains of *Leptopilina heterotoma*) that eggs laid in hosts previously parasitized by a conspecific had an 8% chance of survival as compared a with 94% chance of survival in unparasitized hosts. If her egg does survive, the fitness a female gains is high, because she not only produces an offspring, but reduces the fitness of a competitor. Given the high risk involved in "conspecific superparasitism", however, the best strategy would involve trade-offs, taking into consideration the cost of "wasting" that egg vs the costs of producing further eggs, the probability of laying more eggs in the future, the risks of mortality if the female continues searching (Iwasa et al 1984), the energetic costs of travel and search, and inevitably, the probability of finding unparasitized hosts elsewhere (Charnov and Skinner 1984). In some situations her least costly option may be to deposit an egg and accept the small chance of its survival if other options do not promise a better "return" for her energy "investment".

Hosts parasitized by other species present her with varying probabilities of success, depending on such factors as the probability of her larva winning in larval competition, the time interval between the first and second oviposition, which could affect the outcome of larval combat, the relative ability of her egg to elude host defences, and so on. Laying her egg in these hosts (multiparasitism) would be adaptive under conditions similar to those proposed for "conspecific superparasitism". She should lay an egg only if the relative payoffs from multiparasitism are greater than for her other options.

It has been noticed by some authors that when a wasp is foraging in a group of conspecific adults, the tendency to superparasitize actually increases (Bakker et al. 1985, van Alphen et al. 1987). A mechanistic explanation was presented by Maindonald and

Markwick (1986), that the physical interference among the aggressive adults may result in more mature eggs in the female's ovaries and greater pressure to oviposit, leading to more superparasitism. Superparasitism in a group of conspecifics may also be an adaptive behaviour however; since the time between laying of the first egg and the second oviposition will be very small when conspecifics are searching the patch together, the advantage one egg would have over the other by hatching first is almost non-existant. In that case, then, both eggs have virtually an equal chance of survival, and the advantages of superparasitism increase greatly.

Van Alphen (1988) argues that when two parasitoids search a patch together, and the probability of finding other unexploited patches is low, a female increases the number of offspring she can obtain from this patch by staying longer than optimal foraging models would predict and laying additional eggs in parasitized hosts, in what he calls a "war of attrition".

There also exist rare situations in which a second egg has a better than even chance to survive. Van Alphen and Nell (1982) reported the case of *Pachycrepoideus vindemiae* Rondani, a pupal parasitoid of *Drosophila* whose female destroys the first egg she finds in a host before laying one herself, thereby increasing the survival chance of her own egg. If a host is able to encapsulate only one parasitoid egg (Bakker et al. 1985), then being the second egg "in" becomes an advantageous strategy as well. The probability of a second progeny in a host winning larval competition may be time-dependent if, for example, the mandibulate first instar progeny of the second female is able to attack and kill the second, non-mandibulate instar of the initial ovipositing female (Chow and Mackauer 1986).

Finally, the question of why inexperienced females tend to superparasitize (van Lenteren and Bakker 1975, van Lenteren 1976, Klomp et al. 1980) was re-examined by van Alphen et al. (1987). On the basis of work done with *Leptoplina heterotoma* and *Trichogramma evanescens* Westwood, they concluded that inexperienced females may superparasitize, not because they cannot discriminate, or have not learned to, but because it is their best strategy under the circumstances. Inexperienced wasps will only encounter the eggs of conspecifics. With no information about other possible patches or available hosts, their best strategy would seem to be to superparasitize the hosts they encounter (van Alphen and Nell 1982, Hubbard et al. 1987, van Alphen et al. 1987). Experienced wasps, on the other hand, with knowledge gained about other patches, may choose, in the presence of conspecific eggs, to leave.

Although the functional and causal approaches to studies of discrimination are quite different, they are, in fact, complementary, and necessary for a complete understanding of discrimination, clarifying not only what behaviours should evolve under various conditions, but what constraints determine the final strategies.

In nature, female parasitoids encountering and detecting parasitized hosts can either remain and superparasitize (or multiparasitize) or emigrate to another patch. The choice made will depend on the relative benefits and costs associated with that option. Some of the factors that may be taken into account include the parasitoid/host ratio on that patch, availability of her eggs, distance to and quality of another patch, the probability of future success, risk of predation, etc. If, for example, a habitat contains numerous patches nearby which, experience has led her to expect, contain a large number of hosts, a female is more likely to emigrate after a number of encounters with parasitized hosts than to remain and superparasitize.

Although patterns of superparasitism are found in laboratory studies of numerous parasitoids found in the field, there are few examples which describe the degree of superparasitism that exists in nature. This may demonstrate, not the lack of superparasitism in nature, but the difficulty in measuring it accurately . When collections are made from natural populations, because hosts have been parasitized over an unknown time period, there is no way of determining, for example, if supernumeraries may already have been destroyed.

If the distributions of hosts and patches in natural habitats are important in the decision to superparasitize, one would expect to find the greatest amount of superparasitism in habitats where host densities are low and where host patches are widely spaced or unpredictable. Waage and Godfray (1985) speculate that superparasitism should be common when recognition time is long, the rate of finding hosts is low, and fecundity of the parasitoids is high. Though we can speculate on the basis of laboratory investigations, about the role of superparasitism in natural populations, it is impossible to determine its actual impact without further field studies.

CHAPTER IV

PATCH TIME ALLOCATION OF *Opius dimidiatus*

A. Patch time Models:

As outlined in Chapter I, insect parasitoids are often viewed within the context of optimal foraging theory; they are assumed to be acting in a way that will maximize the rate at which they attack (oviposit in or on) hosts suitable for the survival of their offspring. If time is a limiting factor, they should be making the most efficient use of their foraging time on patches. A number of rules for determination of patch time by predators and parasitoids have been suggested in the literature:

(1) Fixed number of prey (Gibb 1962): This "hunting by expectation" model predicts that a predator learns to expect a certain number of prey in a "locality", and will leave after that number of prey have been captured. Gibb speculated that such a strategy would be used by predators that search for concealed prey and that can detect traces of previous attacks, conditions that apply to *O. dimidiatus*. However, van Alphen and Vet (1986) and Iwasa et al. (1981) both point out that this strategy could be adaptive only if there is low variation in host density between patches.

(2) Fixed time (Krebs 1973): Predators or parasitoids will leave after a fixed amount of time has elapsed. This strategy could be adaptive when variance in patch densities is small (Iwasa et al. 1984), and, because the rate of attack declines as host density increases, might sometimes explain inverse density-dependent patterns of parasitism.

(3) Threshold rate or GUT (Hassell and May 1974, Murdoch and Oaten 1975, Cook and Hubbard 1977, Krebs et al. 1974): The basis of models using a threshold rate is that predators or parasitoids will leave a patch when the prey (or host) capture rate falls below a certain value, the "threshold rate". Murdoch and Oaten (1975) predicted that this

rate was fixed and absolute. A later model by Cook and Hubbard (1977) tested Charnov's (1976) Marginal Value Theorem that predicts a threshold rate of capture equal to the average rate of capture for all patches in the habitat. They assumed that the threshold rate of capture is equal to the reciprocal of a parasitoid's "giving-up time" or GUT. (The GUT is defined in the literature for predators as the interval between "the last (prey) capture and leaving" (Krebs et al. 1974). A female parasitoid's GUT would be the interval between her last oviposition and her emigration from the patch.) They reasoned, therefore, that the GUT should be fixed for all patches regardless of host density, its value derived from the average profitability of all patches in the habitat. To describe the exploitation of patches, Cook and Hubbard used Roger's random parasite equation (Rogers 1972) and predicted the proportion of time that would be spent on patches of varying host densities. They suggested that the proportion of time spent on any patch would be related to its rank in the order of patches as compared with the average profitability of all patches, and predicted that as exploitation proceeded, the time budgets would become more equal (Cook and Hubbard 1977, Hubbard and Cook 1978). They tested this model using *N. canescens*, and found patch times which agreed with their predictions of a fixed GUT.

However, the idea of a fixed GUT has not received wide support. Van Lenteren and Bakker (1978) proposed a variable GUT based on the area of a patch. As the parasitoid searches, each encounter with a healthy host would extend its search time by a new longer GUT, so that the total search time is a function, first of the size of the patch, and ultimately, of the number of hosts encountered.

A density-dependent GUT model was proposed by McNair (1972) who suggested that in patches a parasitoid perceives as "good", it should be more persistant and use larger GUT's. He went on to suggest that the Marginal Value Theorem was, in fact, not designed to make predictions about GUT's, because the intake of energy and depletion of resources are random, point events, rather than continuous as assumed by the Marginal Value Theorem.

In several studies (Waage 1978, 1979; van Alphen and Galis 1983) the patch time of the female parasitoid has been shown to be the result of the interaction of a number of stimuli; some stimuli associated with the patch may affect the time until the first host encounter, and stimuli associated with the hosts may mediate the total search time by causing adjustments to the GUT.

In an often-cited series of experiments, Waage (1978, 1979) described a behavioural model that explains how the responses of *N. canescens* to a succession of such stimuli result in an adaptive apportioning of patch time. The wasp first responds to a kairomone produced by the host larvae which "arrests" it on the patch. In fact, a "patch" was defined by Waage as the "area containing a contact chemical produced by the host" because the "limits of the chemical describe the limits of host movement", and the parasitoids showed a distinct "arrestment response" to that chemical. The arrestment on the patch is manifested by a sharp "turning response" when the parasitoid reaches the edge of a patch, bringing it back onto the patch. This response gradually wanes until the parasitoid no longer turns back at the edge of the patch. Waage showed that the strength (i.e. duration) of this response is directly related to the concentration of host chemical (and by inference, the density of hosts that produce it). Whenever a host is found, oviposition delays the decay of the turning response by an "increment of responsiveness" that varies with the time since the last oviposition. As a result of responses to these two stimuli, patch times were longer when host densities were high, and increased when ovipositions occurred. This increment, by which the patch time was increased after an oviposition, decreased as the time between ovipositions got shorter. This suggests that at high host densities, when ovipositions would be expected to occur closer together, the new GUT's would be shorter and shorter. At low host densities, however, the longer interval between ovipositions would produce larger GUT's and therefore a longer possible search time for a lesser quality patch, a situation that would appear to be non-adaptive. In Waage's experiments, no evidence was found to indicate that the rate of rejection of parasitized hosts contributed to patch-leaving, though he did acknowledge the deterrent effect of an already-searched patch (Waage 1979, van Lenteren and Bakker 1978, Galis and van Alphen 1981).

Van Alphen and Vet (1986) speculated that encounters with parasitized hosts may indeed affect patch time, either because they impart information to the parasitoid about the increasing exploitation of healthy hosts, or because they decrease her "motivation" to continue searching. *Leptopilina heterotoma* showed a decrease in "motivation" after encounters with parasitized hosts, but no such tendency was demonstrated in *Asobara tabida* (Nees). However, *A. tabida* did increase both its total search time and its GUT with increasing host density (van Alphen and Galis 1983).

A variety of factors influence the foraging behaviour of a fruit parasite, *Rhagoletis pomonella* (Walsh), a tephritid fly which lays its eggs in the fruit of the hawthorn tree (Roitberg et al. 1982). Though *R. pomonella* flies initially use a fixed threshold rate of encounter with high quality (unmarked by host-marking pheromone) host fruit, "success-motivated search" resulting from oviposition increases residence time in fruit-containing trees, while encounters with marked or unsuitable host fruits caused them to emigrate sooner from trees.

It is clear that determination of patch time is the result of a complexity of behaviour cues and responses. In light of the varied patterns of patch time allocation presented, a study was undertaken to investigate those mechanisms used by *O. dimidiatus* in its search for leafminer larvae, and to compare its foraging behaviour with existing models.

B. Allocation of Search Time by *Opius dimidiatus*:

A key difference in the models to be tested is the extent to which information gathered and processed by the parasitoid could influence search effort. Fixed time and fixed number models, for example, do not permit a parasitoid to vary her patch residence time based on her perception of the richness of the patch. For this reason, it has been questioned whether such searching strategies would be adaptive where patch quality (i.e. density of available hosts) is variable (van Alphen and Vet 1986, Iwasa et al. 1981).

Other models (eg. variable GUT) emphasize the variation in patch quality and the differing payoffs they represent. An efficient parasitoid should, they suggest, vary her foraging effort, investing more energy in patches where payoffs are greater. In order to assess patch quality, a parasitoid female should use cues from the patch to estimate its potential profitability. Information may come from the size of the patch (van Lenteren and Bakker 1978), density of hosts (or concentration of host-produced stimuli) (Waage 1979, van Alphen and Galis 1983), direct encounters with hosts, and so on. Therefore, in investigating the foraging rules used by *O. dimidiatus*, a number of patch qualities, such as those mentioned above, were varied to determine their influence on a female's time budget. Variation in factors such as plant quality, parasitoid age and experience, and host age were minimized to reduce any confounding effects.

Methods and Materials:

Parasitoid females, 4-7 days old, were given abundant hosts for 24 hours prior to trials, to allow them to gain experience in oviposition and detection of parasitized hosts. Mated females were used in these experiments so that an adequate sample size could be ensured. (In previous studies, techniques necessary to provide unmated females imposed a high mortality rate, making it difficult to produce enough females when needed.) Just prior to each trial, the experimental female was observed ovipositing twice, to ensure that she was not physically hindered from tracking and parasitizing a host, and to reduce the possibility of any "oviposition pressure" that might affect her search effort.

In experiments in which mined leaves were used, all hosts were *L. trifolii* larvae in the late second or early third instar. Leaves were taken from broad bean plants; the petiole of each leaf was inserted into a vial filled with water which was then sealed with Parafilm^{TM}. The leaf was taped inside a large petri dish (14.5 cm diameter) which served as an experimental arena. Additional leaves were placed around the test leaf at a minimum distance of 2 cm between adjacent leaves. This was done to provide alternate patches to which the parasitoid could escape if she "decided" to leave the test area.

To transfer parasitoids to the arena, half a gelatin capsule containing the experimental female was inverted on the leaf over a central area which contained no mines. When the female walked onto the patch, the trial was begun. A video camera with time display was used to record all behaviours of the parasitoid during the trial. During the taping the parasitoid was observed through a stereo microscope, and a microphone used to record on the tape the precise instant a behaviour was observed to begin and end, to augment the information recorded on video tape. This "back-up system" proved to be extremely important in the accurate timing of this parasitoid's search behaviour. As described in Chapter II, a female following a mine searches in a very jerky manner, making it difficult to distinguish, for example, between a temporary hesitation and a resting behaviour, until several seconds had elapsed. Using the tape, it was possible to backtrack to find the precise second when a behaviour occurred. The continual voice-over gave the added advantage of accuracy, allowing the description and recording of subtle changes in behaviour that might have been missed on a video transcription.

The tapes were later reviewed and times recorded for the following behaviours:

1) active search time - as described in Chapter II

2) resting and grooming periods ("resting" periods were determined to be stops that lasted longer than 5 seconds).

3) ovipositions in hosts, from initial probe of host to withdrawal of ovipositor

4) rejection of hosts - as determined by rejection behaviour (see Chapter III). Dissections of hosts at completion of each trial verified oviposition or rejection.

Trials were conducted in an open arena and parasitoids were free to leave the "patch" either by flying off or by walking off. However, most parasitoids walked off to another patch.

In all experiments a leaf was considered to be a "patch", since it fulfilled the requirements of Waage's definition. As discussed in Chapter II, females are "arrested" on a mined leaf by components of the frass within the mines left by the host larvae. The area which contains, and defines the limits of, this attractant is the mined leaf. Furthermore, during numerous experiments, parasitoids were observed to turn sharply back onto the leaf when they had stepped off onto the filter paper lining the arena, the response described by Waage to a patch edge (Waage 1978).

In most studies of this kind, the currency of foraging effort is taken to be the total patch time, comprising both the active search time and time for non-search behaviours, including grooming, resting, handling of hosts, and so on. However, it was felt that non-search times were more likely to be affected by factors not involved with patch and host stimuli, and therefore less likely to be an accurate reflection of foraging effort under the experimental conditions. Though all behaviours on the patch were timed in each trial, it was the effect of patch experiences on the active search component which were examined in these analyses rather than total time. There is some support for the emphasis on active search time. Morrison (1986) believes that total time may be a poor predictor of spatial patterns of parasitism at the patch level and recommends the study of search times alone as a predictor of foraging effort, a view also expressed by Waage (1983). Thus, for all experiments, ANOVA's were used to compare the active searching components of the parasitoid's patch time.

Experiments 1 - 3 examine some of the qualities of a patch that might determine foraging effort, before the parasitoid contacts a host.

Experiment 1: Effect of Leaf Area

There exists the possibility that *O. dimidiatus* females may associate the richness of a patch with its size (i.e. leaf area) if, for example, *L. trifolii* distributed her eggs in a homogeneous manner over the habitat. If so, I would expect to find a relationship between leaf area and active search time. To test this possibility, parasitoids searched broad bean leaves containing no mines whose leaf areas varied between 5 - 30 cm^2.

Experiment 2: Effect of Leaf Area and Presence of Host Mines

Searching effort may be triggered by the presence of host mines. To test this, females were presented with leaves containing mines at a density of 1 mine per 5 cm^2. Larvae were carefully removed from the mines prior to the trial so that no host encounters were possible. Areas of leaves varied from 10 - 30 cm^2. (Mined 5 cm^2 leaves could not be found, since they would not have been opened 8 days prior to trials, when miner eggs were laid in leaves.) By comparing the active search times on each of these leaves, the effect of the area of mined patches could be tested. In addition, if the presence of host mines influences search effort, I would expect search times in this experiment to differ from those in Experiment 1.

Experiment 3: Effect of Host Density

In this experiment the variable to be tested is the density of hosts (assuming that the previous experiments demonstrate a response to host presence). To separate host density effects from those associated with host encounters, larvae were again removed just prior to trials, leaving the mines containing frass to provide host-related cues. Area of leaves was kept constant at 20 cm^2; density of mines varied from 2 - 18 mines per leaf. If host density is not a factor in determination of search effort, I would expect the search times to be the same regardless of changing host mine density.

The second set of experiments (Exps. 4 and 5) considers the possible influence of ovipositions in healthy hosts and encounters with parasitized hosts on further searching effort.

Experiment 4: Effect of Oviposition in Hosts

Females searched leaves containing host larvae and were allowed to oviposit in differing numbers of hosts. To ensure that only healthy hosts were encountered, while at the same time maintaining a constant density of available hosts, each host had to be replaced with a healthy one when it had been parasitized. Because larvae are contained within the mines, removal and insertion of hosts was impossible. A solution was achieved by using leaves containing 8 larvae, of which 4 were covered with tiny pieces of tape (approximately 3mm X 3mm.), after determining through observations that the presence of the tape did not alter a parasitoid's search behaviour. After a host was parasitized, and the female had left the area, the host was carefully covered, using fine forceps, with a piece of tape and another healthy larva was exposed to replace it. In this way, 4 hosts were always available, yet no encounters with parasitized hosts were permitted.

A maximum of only 4 hosts/leaf were available for two reasons: In pre-trials involving more than 4 host encounters, enough time elapsed for some parasitized larvae to tunnel from beneath the tape and become exposed again. In addition, the actual density of 8 mines/leaf (which allows up to 4 host encounters) corresponds to the mean density of mines/chrysanthemum leaf calculated from samples taken from infested greenhouse plants.

Parasitoid females were allowed to oviposit in a pre-determined number of hosts, ranging from 1 to 4. All hosts were then covered and the female continued to search with no further encounters until she left the patch.

If experiences with hosts do not influence time budgets, then search times even with ovipositions should not differ from a searching "bout" on a patch of similar mine density, where hosts are not encountered. Therefore, as a control, trials were conducted in which all healthy hosts on a leaf were covered, so that females searched patches containing the same density of mines without encountering any hosts.

In all experiments, the assumption was made that, because of the preparation of the leaf and the short time frame involved, there was no change in the quality of the leaf, as the parasitoid proceeded to search. If there is basis to the theory that parasitoids mark patches as they search (Price 1970, Waage 1979, van Lenteren and Bakker 1978), then there may be a perceived change in patch quality as she searches longer which is unavoidable. This theory and its implications will be discussed in the following chapter.

These procedures were carried out with as little disruption to the parasitoid as possible. Once a female is engaged in following a mine's stimuli, she is not easily distracted from her path, even when nudged by a small brush. While I did proceed with great caution, I was confident that females searched with a minimum of disturbance.

Experiment 5: Effects of Encounters with Parasitized Hosts

This experiment was similar to Experiment 4, except that all hosts had been parasitized by the same female just prior to the trial. A female could encounter 1 - 4 parasitized larvae before all hosts were covered. As a control, all parasitized hosts were covered so that no encounters were possible.

Results:

There was no difference in the mean times a female spent actively searching unmined leaves (Fig. 4.1) regardless of the area of that leaf (F=.12, p>0.95, df=5). Even when leaves contained mines (Fig. 4.2), the mean search times/patch were not different (F=.63, p>0.65, df=4). However, there was a significant difference in mean times between unmined and mined leaves (F=19.22, p<0.0001, df=4); females spent five times as much searching time on mined leaves, suggesting a response to the presence of mines or the host products associated with them.

Experiment 3 demonstrated a linear increase in searching time (Fig. 4.3) when host mine density increased. This interval of time spent searching a patch before abandoning it can be considered to be an "initial GUT", as defined by Morrison and Lewis (1981). It appears that for *O. dimidiatus* the "initial GUT" is determined by host density.

As shown in Figure 4.4, the total time spent searching increased with each oviposition. When no hosts were encountered, females searched for a mean of 110 seconds at this mine density before leaving. Each oviposition increased the total search time by an additional increment. Since handling time is not a factor in these calculations, the increment added was additional search time. (In fact, handling time per host did not change regardless of the number of hosts attacked in a searching "bout". (i.e. across treatment groups.) (F=.06, p>0.75))

To investigate the effect of each oviposition on the GUT, the observed period from the last encounter with a host until abandonment of the patch was used to represent a female's GUT after each trial. These intervals have been called "Terminal Search Intervals" (TSI) by Morrison and Lewis (1981), and can represent either the GUT after oviposition, or the search time spent after the last encounter with a parasitized host. When the TSI's were examined after encounters with healthy hosts (Fig. 4.5), they showed that TSI's increased over the "initial GUT" (when no hosts were encountered)

Fig. 4.1: Time spent actively searching on unmined leaves of different areas. (Means ± S.E.) Lines drawn from regression analysis; $y = .31x + 49.66$. ($n=50$, $r^2 = .01$, $p > 0.60$)

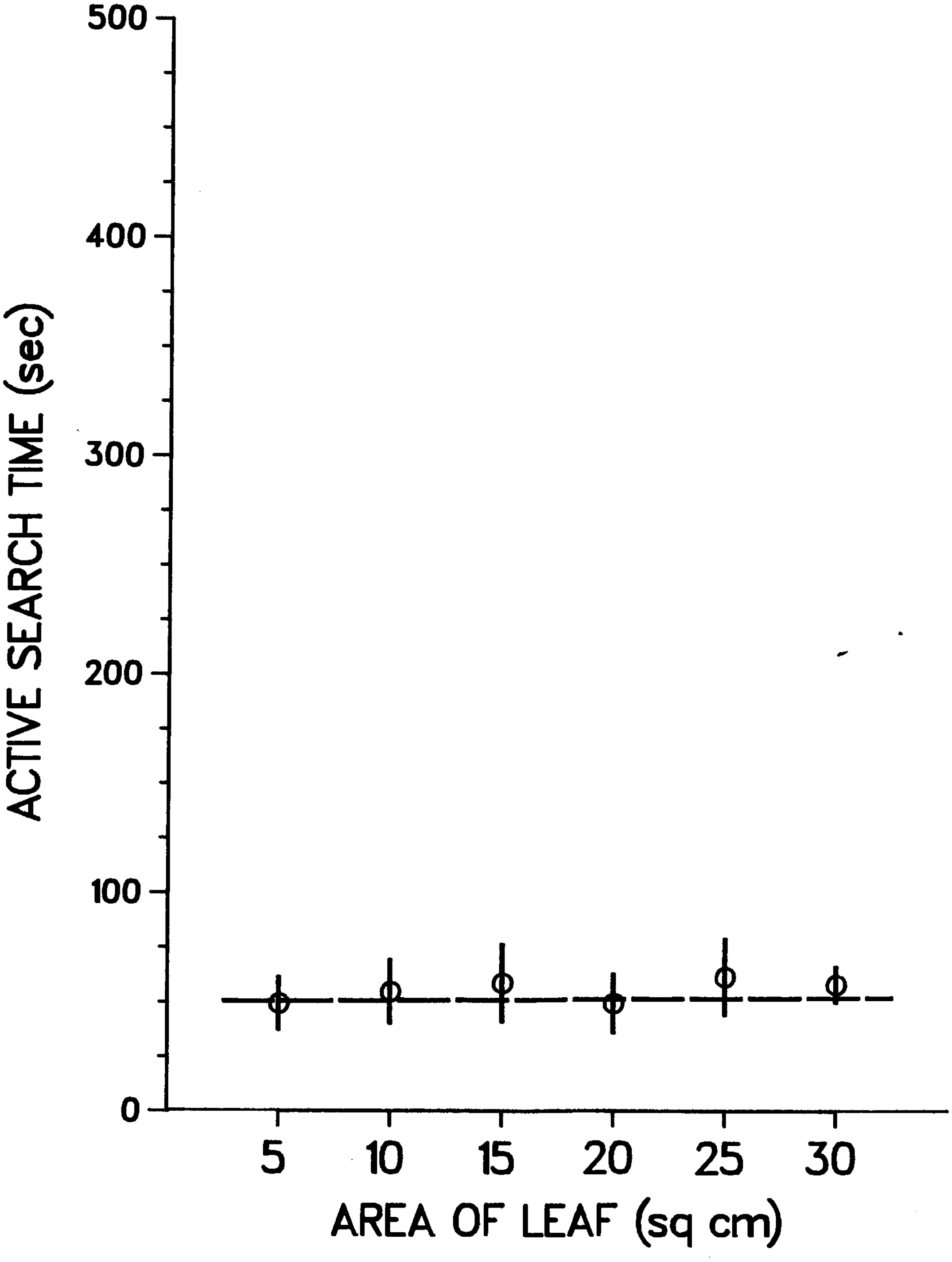

500
400
300
200
100
0
ACTIVE SEARCH TIME (sec)
5
10
15
20
25
30
AREA OF LEAF (sq cm)

Fig. 4.2: Time spent actively searching on mined leaves of different areas. Larvae were removed from mines prior to trials. Density of mines = 1 mine/ 5cm^2. (Means ± S.E.) Lines drawn from regression analysis; $y = -.32x + 268.84$ ($n=50$, $r^2=.01$, $p > 0.89$).

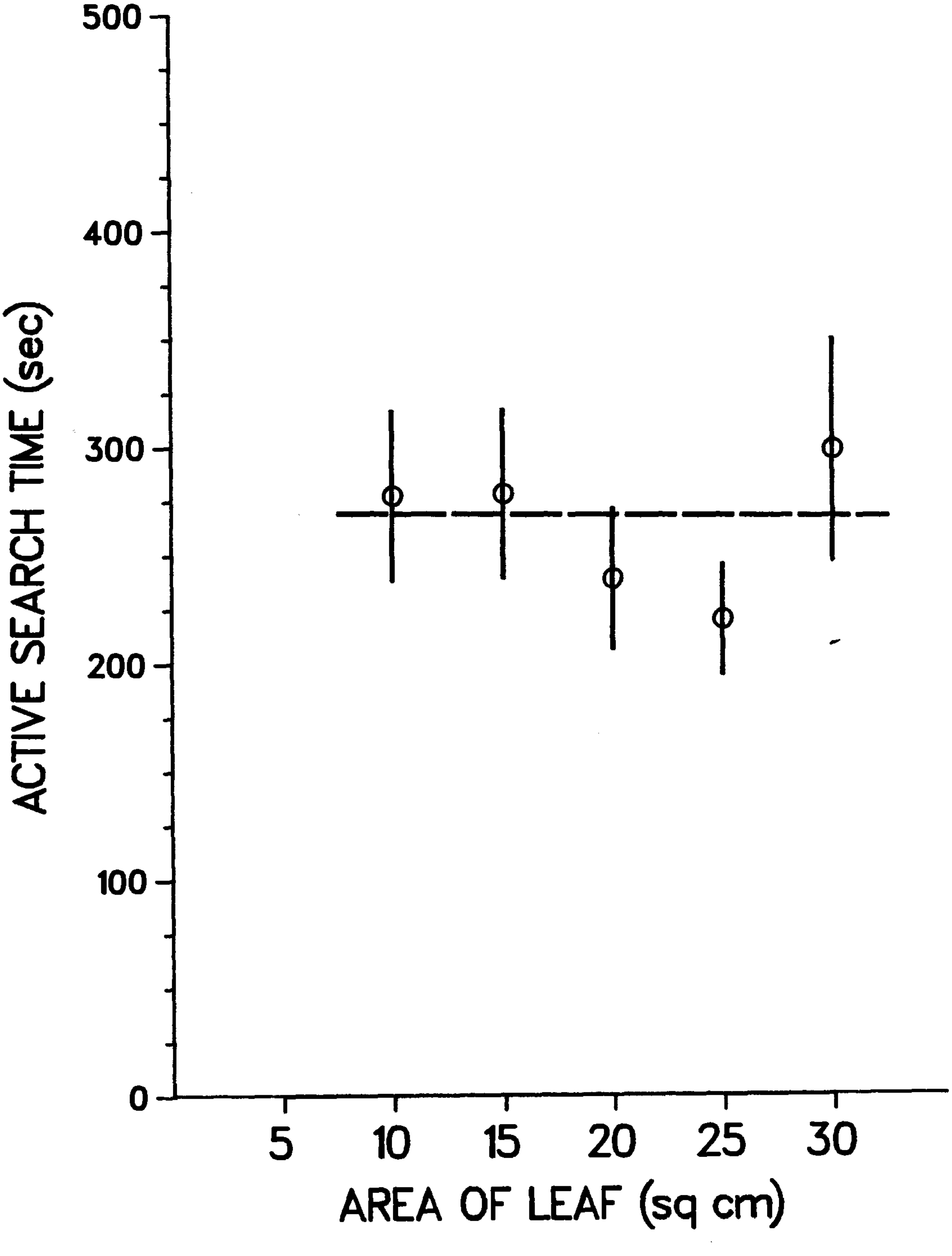

ACTIVE SEARCH TIME (sec)
500
400
300
200
100
0
5
10
15
20
25
30
AREA OF LEAF (sq cm)

Fig. 4.3: Time spent actively searching leaves containing different densities of mines. Larvae were removed from mines prior to trials. Area of leaves was 20 cm^2. (Means ± S.E.) Lines drawn from regression analysis; $y = 38.08x + 176.23$. ($n=50$, $r^2 =.58$, $p< 0.001$)

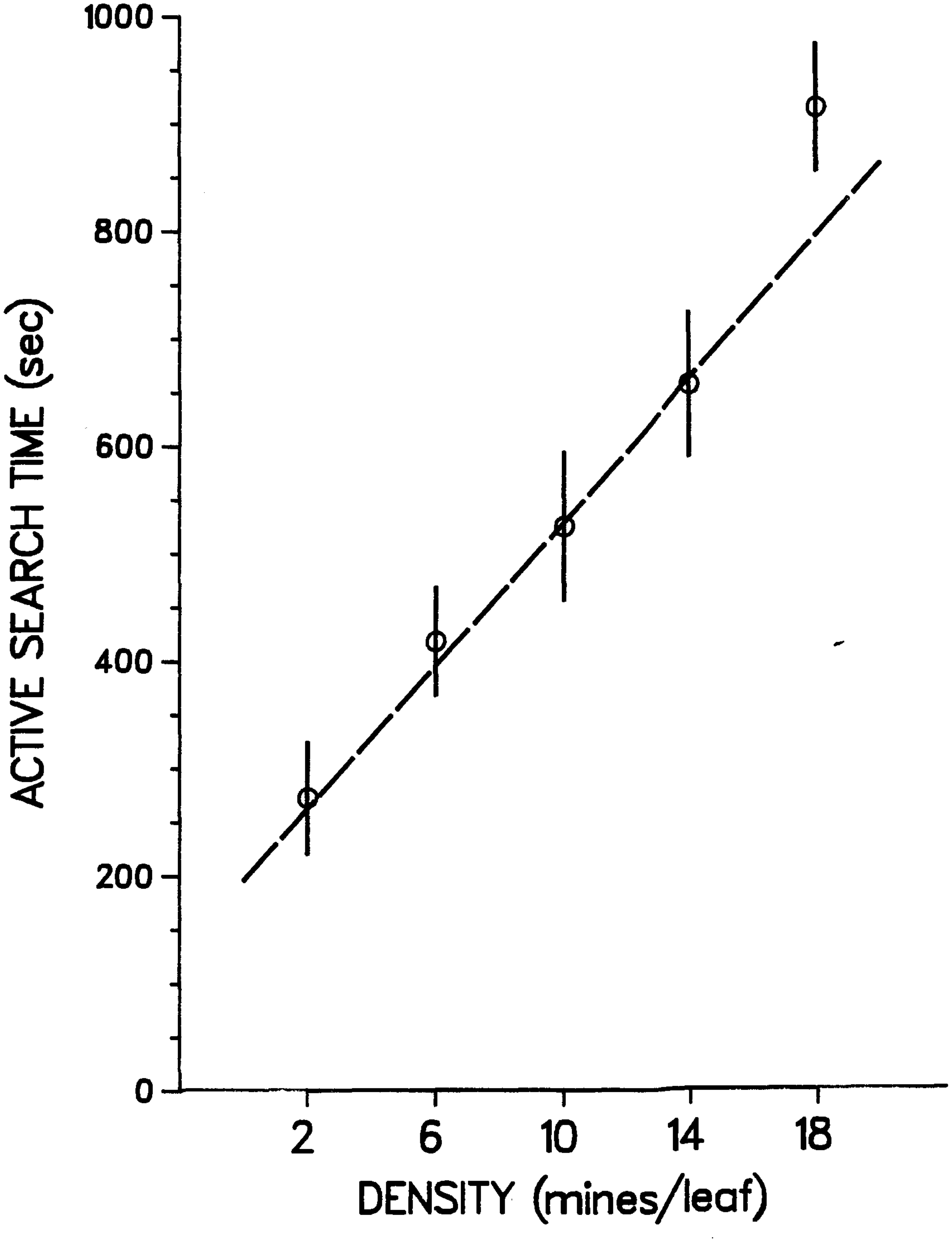

ACTIVE SEARCH TIME (sec)
1000
800
600
400
200
0
2
6
10
14
18
DENSITY (mines/leaf)

Fig. 4.4: Relationship between Total searching time and the number of healthy hosts attacked. (Means ± S.E.) Line drawn from regression analysis; $y = 95.26x + 132.24$. ($n=75$, $r^2=.35$, $p < 0.001$)

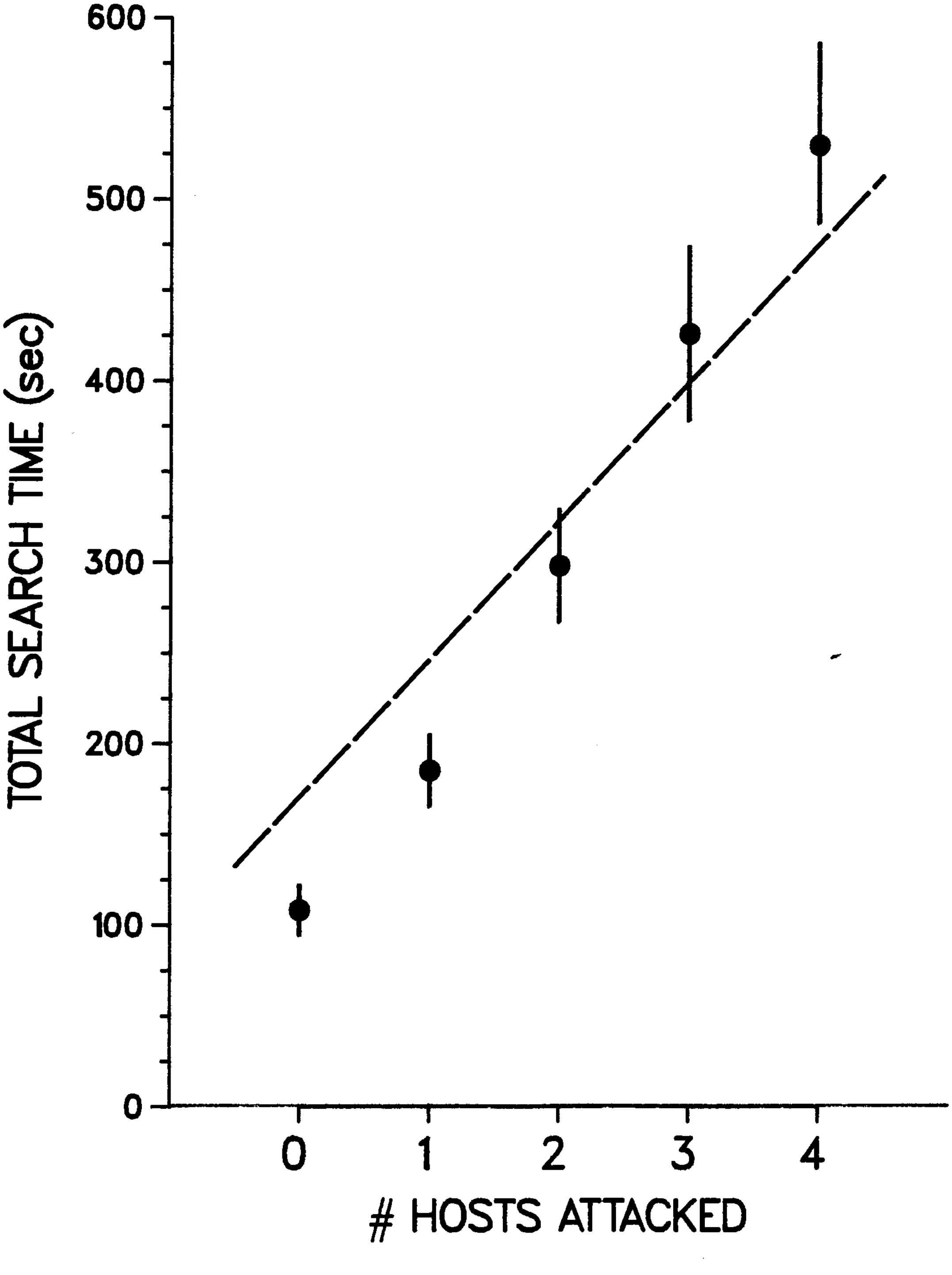
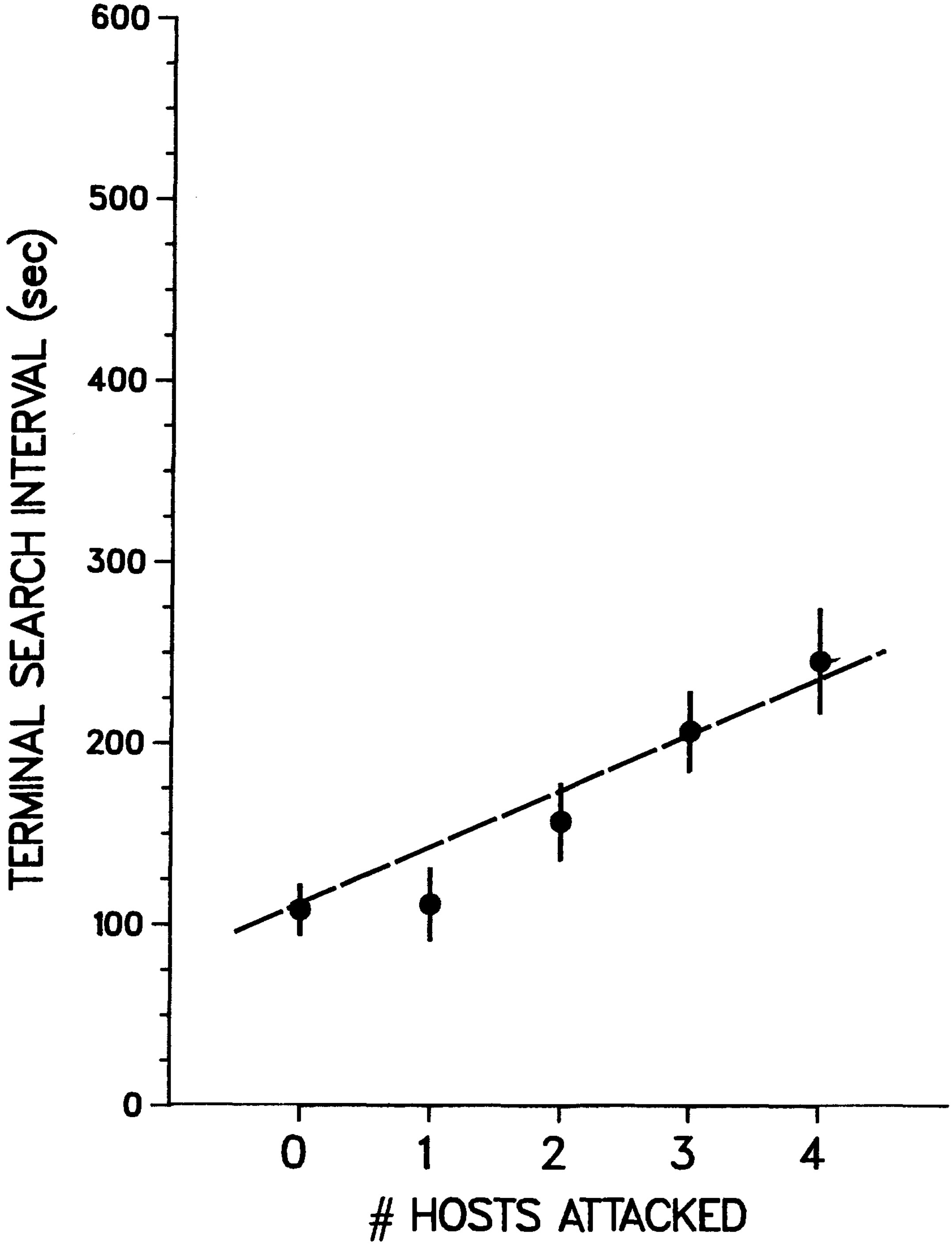

600
500
400
300
200
100
0
TOTAL SEARCH TIME (sec)
0
1
2
3
4
HOSTS ATTACKED

Fig. 4.5: Terminal search interval (TSI) as a function of the number of healthy hosts attacked. (Means ± S.E.) Line drawn from regression analysis; $y = 36.26x + 93.97$. ($n=75$, $r^2 =.28$, $p < 0.005$)


TERMINAL SEARCH INTERVAL (sec)
600
500
400
300
200
100
0
0
1
2
3
4
HOSTS ATTACKED

with ovipositions. An increasing number of ovipositions extended the TSI by a greater amount. These results suggest that oviposition does increase the GUT of *O. dimidiatus* at this host density, and that the new GUT becomes greater with each successive oviposition.

Waage's (1979) findings, that upon oviposition the GUT of *N. canascens* varied with the time since the last oviposition, were not supported for *O. dimidiatus*. The TSI's showed no relationship to the time interval since the previous oviposition (Fig. 4.6). Likewise, the TSI was not a function of the time since the last encounter with a parasitized host (Fig. 4.7). However, since these possibilities were not tested explicitly (by controlling the time since the last oviposition or encounter, and comparing TSI's), it may be premature to conclude that time since last oviposition and time since last encounter with a parasitized host are not important pieces of information.

When leaves containing only parasitized hosts were used (Fig. 4.8), the "initial GUT" (as represented by the control group) was about 70 seconds, lower than the "initial GUT" for patches containing healthy hosts (110 sec). This likely reflects the response of the females to a patch already searched and marked (Waage 1979, Galis and van Alphen 1981), or to the presence of some factor associated with parasitized hosts. With each parasitized host encountered, the total search time increased, but the slope of the regression line was much flatter than for healthy hosts (t-test, $p<0.001$). If hosts were encountered at random, this suggests that the total search time may be increased by a decreasing amount with each encounter; this prediction was proved correct when the TSI's were compared (Fig. 4.9). With each encounter with a parasitized host, the TSI dropped, until after 4 successive encounters, the mean increment added to the total search time was only 16 seconds.

Fig. 4.6: Terminal search interval as a function of the time since last oviposition.

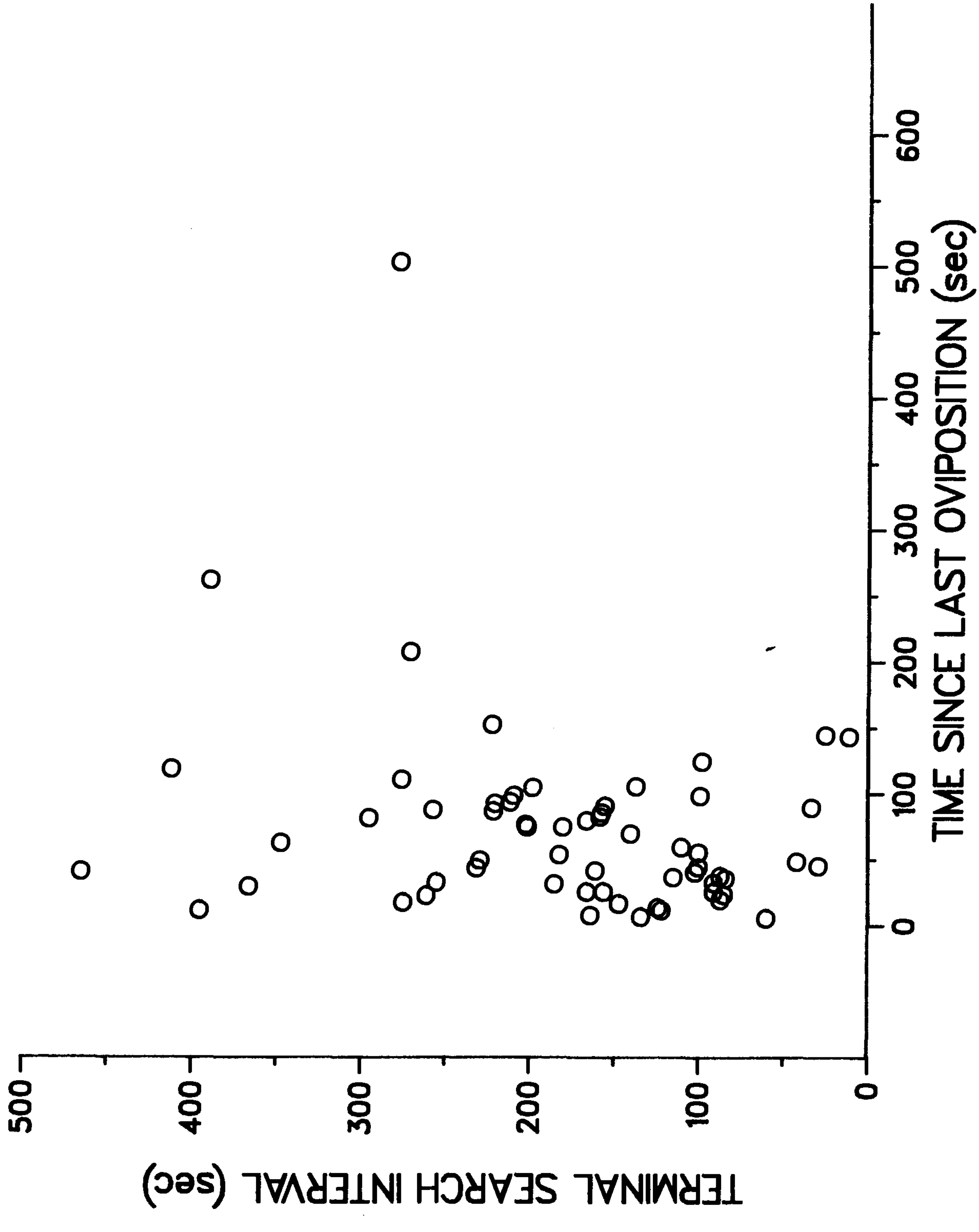

TERMINAL SEARCH INTERVAL (sec)
0
100
200
300
400
500
TIME SINCE LAST OVIPOSITION (sec)
0
100
200
300
400
500
600

Fig. 4.7: Terminal search interval as a function of the time since last encounter with a parasitized host.

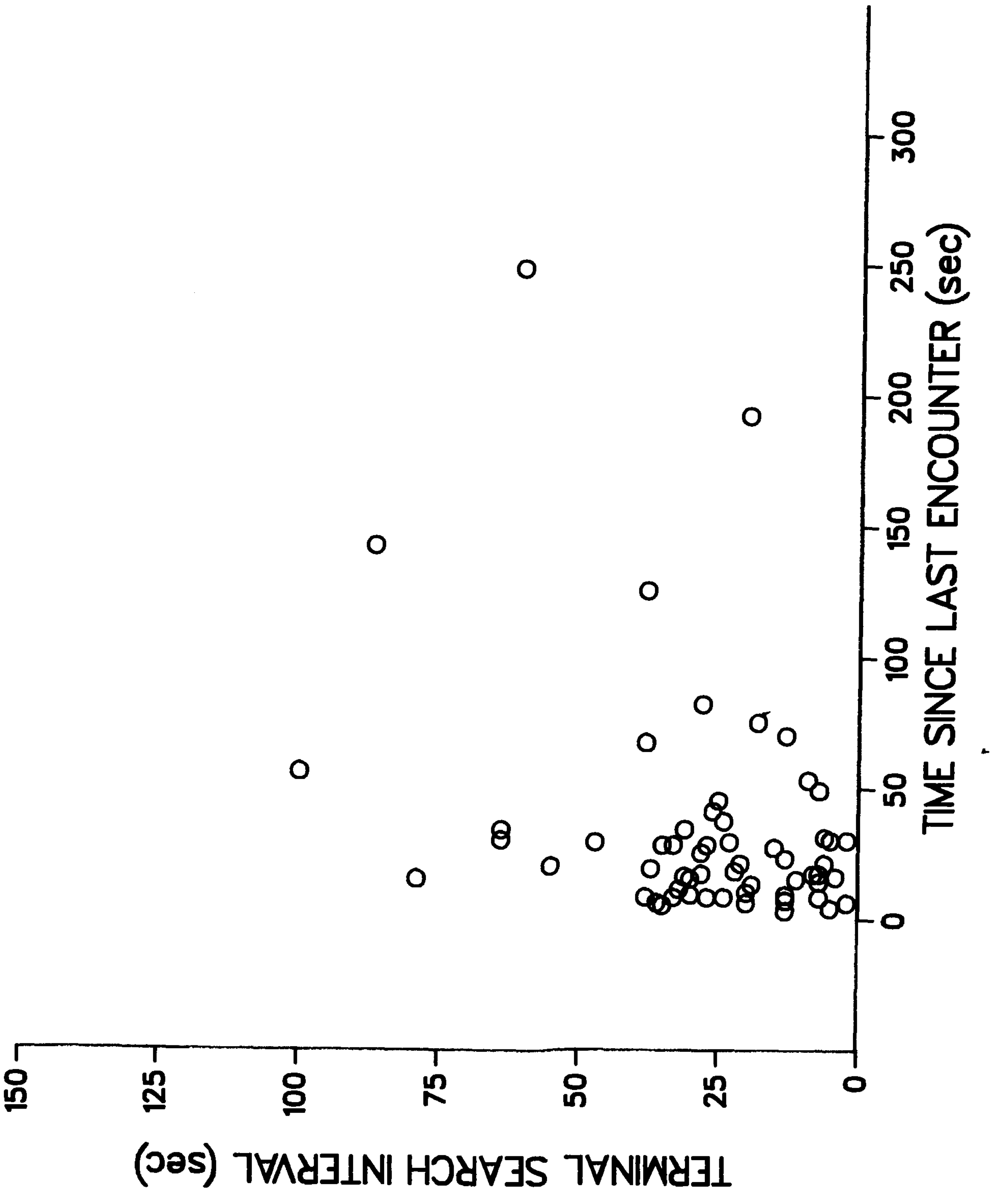

TERMINAL SEARCH INTERVAL (sec)
150
125
100
75
50
25
0
TIME SINCE LAST ENCOUNTER (sec)
0
50
100
150
200
250
300

Fig. 4.8: Relationship between total searching time and the number of parasitized hosts encountered. (Means ± S.E.) Line drawn from regression analysis; $y = 19.47x + 62.83$ ($n = 75$, $r^2 = .12$, $p < 0.005$)

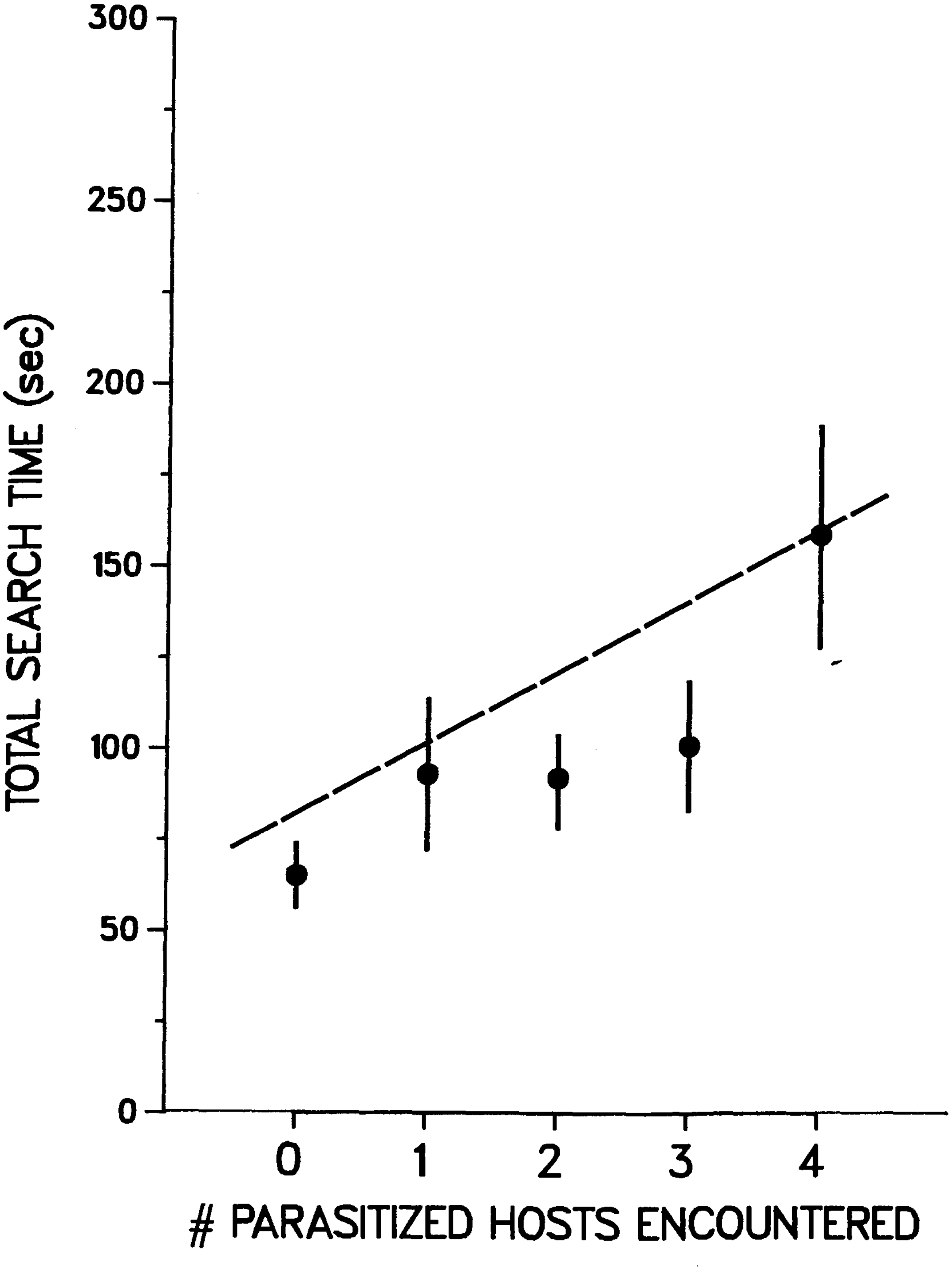
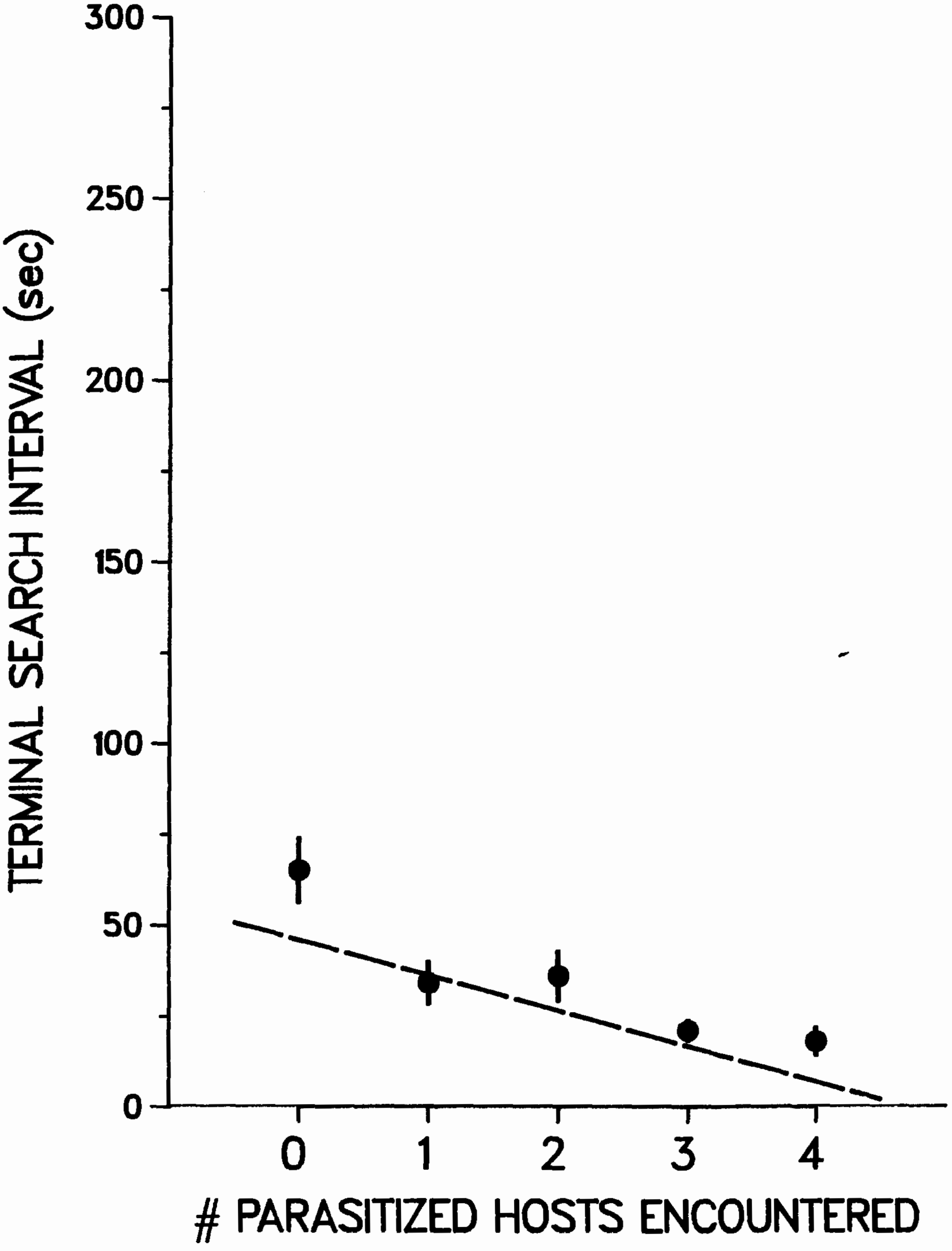

300
250
200
150
100
50
0
TOTAL SEARCH TIME (sec)
0
1
2
3
4
PARASITIZED HOSTS ENCOUNTERED

Fig. 4.9: Terminal search interval as a function of the number of parasitized hosts encountered. (Means ± S.E.) Line drawn from regression analysis; $y = -10.79x + 56.23$ ($n = 75$, $r^2 = .29$, $p < 0.001$)


TERMINAL SEARCH INTERVAL (sec)
300
250
200
150
100
50
0
0
1
2
3
4
PARASITIZED HOSTS ENCOUNTERED

Discussion

The results of these experiments do not support the predictions of a "fixed number" hypothesis (Gibb 1962). In fact, they showed a density dependent response to host numbers (Exp.3) which is not compatible with a "searching by expectation" strategy. Since *L. trifolii* larvae vary greatly in their distribution among leaves of a plant (Lynch and Johnson 1987, Parrella and Jones 1984), this would be an inefficient way to search. At host densities lower than a parasitoid's expectation, a female would remain forever or superparasitize; at densities higher than her expected number, the patch would not be effectively exploited.

Since active search times varied with a change in host density (Exp. 3), and with host encounters (Exps. 4 and 5), the "fixed time" hypothesis (Krebs 1973) was also unsupported. Because variance in *L. trifolii* distributions is not small, this was not expected to be an efficient searching strategy for *O. dimidiatus*.

Results of Experiments 4 and 5 do not fit the "fixed GUT" model (Murdoch and Oaten 1975, Cook and Hubbard 1978, Roitberg et al. 1982). Giving-up times, as estimated by the terminal search interval, varied with patch experiences. The "initial GUT" is also not determined by the area of the patch, as proposed by van Lenteren and Bakker (1978). As demonstrated in Experiment 3, the "initial GUT" of *O. dimidiatus* increases with the density of hosts perceived by the parasitoid as she begins to search the patch. The behaviour of *O. dimidiatus* fits into the "variable GUT" hypothesis proposed by McNair (1982) and Waage (1978,1979), in which the initial influence on foraging effort is the density of hosts. It appears that a female arriving in a patch reacts to the stimuli associated with hosts or host mines (possibly kairomone concentration or rate of contact with mines), and "sets" a giving-up time that reflects her perception of the profitability of the patch. Encounters with hosts are random events with no guarantee that hosts will be discovered at a predictable short-term rate. However, if a female has a high "expectation" of finding larvae, she should be able to invest more time and energy, remaining long enough that they will be found. Thus, the higher her "expectation" of encountering hosts, the longer is the time she will initially search without finding a host.

Conversely, if she perceives the patch to be of poor quality and the probability of finding healthy hosts low, she should be able to invest less time in a losing cause and emigrate more quickly.

The behavioural mechanisms which result in the initial search time appear, from observations, to match Waage's (1978) behavioural model of a waning "turning-back response" to the patch edge (previously described in this chapter). If no host is found within that "initial GUT", the female leaves the patch.

Her search time is further modified by both ovipositions into healthy hosts (Exp. 4) and encounters with parasitized hosts (Exp. 5). Upon encountering, and ovipositing in, a healthy host, a new GUT is set that exceeds the previous one, and the female renews her search until another host is found, or the new GUT is reached. These ovipositions may be a comfirmation of the patch's profitability, perhaps an indication that other hosts are available. When hosts are highly clumped, as in the *A. tabida - L.heterotoma* system (van Lenteren and Bakker 1978), an increase in search time after an oviposition is considered to be an adaptation to host distribution. Though *L. trifolii* larvae do not appear to be clumped in a patch, *O. dimidiatus* acts to increase her search effort. How this increase is acheived is unknown; possible mechanisms may involve a change in locomotory response, or a change in the rate of habituation to the patch edge (as suggested by Waage (1978) for *N. canescens*).

When a parasitized host is encountered, the female "resets her clock" to a new but lower GUT and continues to search. Encounters with parasitized hosts may allow the parasitoid to perceive the level of exploitation of that patch by giving information about either the decreasing availability of healthy hosts, or the increasing proportion of parasitized hosts. Again, the mechanism that results in a lower GUT is unstudied. After a number of parasitized hosts have been encountered and no ovipositions have occurred, the new GUT approaches zero. This suggests that a mechanism is in place that reduces the amount of time and energy a parasitoid will invest in a patch which, information indicates, is of little value to her.

The possibility was considered that an encounter with a parasitized host might affect the searching time in a more drastic way, decreasing the "initial GUT". If encounters with parasitized hosts indicate to a female the declining profitability of a patch, it was expected that she would spend less time in a losing cause, and decrease her total searching time with each oviposition. Van Alphen and Bakker found that *Leptopilina heterotoma* searched less total time in patches containing parasitized hosts than in patches from which the parasitized hosts had been removed (in van Alphen and Vet 1986). They interpreted this as a decreasing motivation to search after parasitized host encounters. However, it appears that an encounter with any host can cause *O. dimidiatus* to invest additional time in searching the patch (Fig. 4.8). For this species, it appears that the increasing rate of encounters with parasitized hosts is more influential in causing abandonment of a patch than is the decreasing rate of oviposition in healthy hosts.

So far, the influences of ovipositions and encounters with parasitized hosts on search time have been seen in isolation. In the real foraging world of a parasitoid, it is expected that she will encounter both healthy and parasitized hosts, in proportions that vary as she exploits the patch. To build a more realistic foraging scenario, the interactions of these influences should be considered. It is clear from the results that a time budget calculated from information about healthy hosts alone is unrealistic. *O. dimidiatus* increases her GUT with each oviposition, regardless of the time interval between ovipositions (unlike *N. canescens*, (Waage 1979)). If the number of encounters with healthy hosts were the only criterion of patch time, her increased exploitation of a patch would result in longer and longer periods being spent there, while the patch becomes less and less profitable. The fact that this does not happen is due to the counteracting effect of encounters with parasitized hosts which, in some way augment her continuing perception of the patch. I would expect, therefore, that the searching time allocation by *O. dimidiatus* will be a function of some interaction of the two experiences.

The rules of thumb she uses in a mixed patch may involve, for example, the sequence in which these encounters occur. Roitberg and Prokopy (1984) determined that, though visits to parasitized hosts caused a decline in the allocation of search time of *R. pomonella*, an additional oviposition in an unparasitized host cancelled out the effect caused by those parasitized hosts. They suggested, therefore, that the sequence of host encounters is a determining factor in the time budget of *R. pomonella.*

A further possibility involves a ratio of encounters which would be updated with each new encounter, and allow her to respond to the current patch quality. Morrison and Lewis (1981) proposed that the terminal ratio of encounters with parasitized hosts to the number of hosts attacked provided the GUT for *Trichogramma pretiosum* Riley. These two studies suggest that, rather than treating ovipositions and encounters with parasitized hosts as independent components of foraging models, we should be examining the possibility that interactions between the two may produce a more accurate and realistic result.

Furthermore, a number of other factors have been found to contribute to, or modify, patch times and the migration from patches. These include:

(1) Effects of previously-searched patches (Greany and Oatman 1972, Galis and van Alphen 1981).

(2) Prior experience on other patches (Waage 1979, van Lenteren and Bakker 1978).

(3) Distance between patches and interpatch travel (Roitberg and Prokopy 1982).

(4) Encounters with non-hosts (Roitberg et al. 1982).

(5) Encounters with other parasitoids (Beddington 1975).

(6) Encounters with unsuitable stages of hosts (van Alphen and Vet 1986).

(7) Risk of mortality (Charnov and Skinner 1985).

A number of these points will be discussed in the next chapter. The relative importance of these factors and the way they interact should not be overlooked in models of patch time.

Before performing these experiments, it was necessary to choose an experimental patch in which to test the searching effort of *Opius dimidiatus*. The term "patch" is usually used for a discrete area in which a parasitoid searches for hosts. Realistically, it is what is perceived as a "patch" by the parasitoid, and so it should be defined by her behaviour as she searches. Van Alphen and Vet (1986) point out that, when chemical cues restrict the female's search within a certain area, it is easy to identify that area as a "patch". When no clear boundaries exist (e.g. when search areas are contiguous or overlap), it is more difficult to define.

In these experiments, a leaf containing hosts was chosen as a "patch" because observations suggested that *O. dimidiatus* females were "arrested" on leaves that contain host-produced stimuli, turning back into the leaf when they reached the leaf edge. (see Waage's (1978) definition of "patch".) As indicated by their behaviours in these experiments, females appear to perceive a "patch" as an area containing the host presence rather than as a unit of the plant. When leaf areas were changed, they were not treated by females as different size patches; search effort remained the same regardless of leaf size. Yet when density of hosts (and degree of host stimulus) varied, the searching behaviour of a female varied.

A leaf which contains the hosts is discrete; leafminer larvae do not, under normal conditions, move into the petioles or stem of the plant, nor do leaves overlap, so their presence is limited to the leaf area. (In fact, if only the precise area containing host-produced stimuli were considered, the "patch" would contain only the mines and the leaf around the larva which transmits mouthhook vibrations. As the mines fill the leaf surface, the entire leaf becomes the "patch".)

Therefore, a leaf containing hosts can be considered as a patch, at least on one scale. This is not to say that an entire plant may not also be considered to be a "patch". Heads and Lawton (1983) discuss the concept of "fine-grained" vs "coarse-grained" patches and suggest that parasitoids respond to "patches" by showing "density-dependent, enemy-induced mortality". While this definition is consistent with a leaf, it may also apply to a chrysanthemum or bean plant. However, observations so far have not confirmed that these parasitoids move within a plant before migrating to another. On the contrary, they appear to move horizontally, flying from one leaf to another leaf on a nearby plant.

A final observation: females actively searching a "patch" for hosts do so by walking over the leaf surface; they fly only when migrating from one patch (leaf) to another. Thus, there appears to be strong circumstantial evidence that parasitoids, by changes in their behaviour, are treating the leaf as a separate entity

Though preliminary tests were not done to compare responses at different patch sizes, I feel confident, on the basis of these behavioural observations and the correspondence with existing, working definitions, that the choice of a leaf as a "patch" was valid.

This study began with a consideration of optimal foraging models. Model development provides a conceptual way of looking at animal behaviour and a method of organizing details of our observations into a logical pattern. They give us a starting point from which to investigate our own questions about what we observe, and suggest ways to proceed. The evolution of models often show the progression of thought from a general idea, often derived from logical inference, to a more detailed and realistic picture of how animals perform in nature. As models become more "finely-tuned", their applicability becomes less general and they may fit specific situations. They become valuable, not as dogma, but as tools for further exploration.

In examining patch time allocation, for example, I used existing foraging models as a starting point to suggest a logical way to design my experiments and elements of parasitoid behaviour to examine. The results of these experiments showed similarities and differences in comparison with both conceptual and empirical models, but the models provided a framework on which to hang, and make sense of, my observations.

This study has not attempted either to add to the body of foraging theory, or to validate existing models. The intent has been to describe the behaviour of *O. dimidiatus* within a theoretical context.

In summary, *O. dimiatus* females initially employ a variable GUT rule to set their foraging time on a patch. The time they spend searching a patch is dependent initially on the density of host mines or the strength of some stimulus associated with the mines, which allows them to set an "initial GUT" that reflects their perception of the potential of the patch. An encounter with and oviposition in a healthy host results in a resetting of the GUT to a value that increases with each successive oviposition. Conversely, an encounter with a parasitized host results in a new lower GUT that decreases in duration with each successive encounter with parasitized hosts.

CHAPTER V

FINAL DISCUSSION

For many parasitoids, simple foraging models such as "fixed number", "fixed time" and "fixed GUT" may not adequately describe the behaviours observed. Many of these models are designed to maximize some component of a forager's fitness across the habitat, and therefore often require a prior knowledge of habitat quality. Examination of the behaviour of some species of insect parasitoids have, instead, revealed that their search strategies tend to optimize their use of each patch within the habitat (van Alphen and Galis 1983, Waage 1979). The "decisions" made by these parasitoids are based on information that can easily be obtained. The patch searched by *O. dimidiatus*, for example, is small and discrete, and the hosts within it are sedentary, making information about the patch and its hosts easily accessible.

That is not to say that the treatment of one patch must be completely independent of other patches in the habitat. There is some indication that a female's perception of a patch's quality, and the subsequent setting of the initial GUT may be influenced by her prior experience on a similar patch. Waage (1979) found that when patches of the same host density were visited by *N. canescens* females, the second patch received a shorter visit. His explanation was that the female may have become habituated to the concentration of kairomone produced by that density of hosts.

No evidence exists, however, that parasitoid females "weight" their initial GUT upon arrival on a patch, relative to their success on previous patches. This "learning" approach to optimal foraging as presented by McNamara and Houston (1985) suggests that an animal may learn about the environment as it forages and use what it learns to modify its behaviour, eventually approaching optimal exploitation of its environment.

A foraging strategy based on information gained from a patch permits a parasitoid to be more flexible in response to conditions she finds and therefore more efficient in using that patch. Whereas some "fixed" models (eg. fixed number) do not allow for stochasticity in the foraging process, a variable GUT strategy such as described for a number of parasitoids enable the female to adjust her behaviour as experiences on the patch unveil a more complete picture of its quality.

Of course, these models are simplistic in the way they view a parasitoid's behaviour: the rules of thumb followed are likely to be more complex than described, but a number of common "threads" seem to weave through the models which show the different levels of information available to a parasitoid, and how each directs her further searching effort.

In most cases, there is a long range attraction to host or host plant stimuli, often the response to a kairomone. Such an attractant, though likely to be imprecise in the information it imparts, should still indicate the presence of hosts. If the parasitoid associates the attractant with host density, she may respond by investing more time in an initial search of that area.

In Waage's (1979) model, *N. canescens* locates patches of hidden hosts by the volatiles the hosts produce. She associates the concentration of volatiles with density of hosts, and so sets her "initial GUT" in response to the level of kairomone. This response insures that her initial investment is greater where the probability of finding hosts is greater.

The next major level of information appears to be related to encounters with and ovipositions in healthy hosts. If hosts are clumped, each oviposition should indicate to the parasitoid the availability of other hosts nearby. They should, then, be expected to increase their patch residence time after ovipositions.

There is different information to be gained from encounters with parasitized hosts, however. Whereas ovpositions provide information about the probability of finding more hosts in a patch, encounters with parasitized hosts should give some measure of the quality of the hosts in the patch, indicating either the increasing proportion of parasitized hosts or the decreasing proportion of healthy hosts.

In the Roitberg and Prokopy (1984) model, flies locate their host fruit by short range sight. Therefore, when they have located a tree, and before they are able to detect fruit, they will initially spend a fixed amount of time searching. If host fruit are present, their density will determine the probability that they will be found within that time. In this way, females are more likely to continue searching high density patches. Each oviposition results in "success-motivated search", retaining them in patches where they are more likely to encounter more fruit. The rate at which unparasitized fruit is encountered may give an indication of the probability of finding more within that area. Thus, the time between successive ovipositions becomes an important factor in determining a fly's further residence time. As intervals between ovipositions lengthen, leading her to expect a depleted supply of hosts, the added time she is willing to invest decreases. As *Rhagoletis* flies encounter parasitized hosts as well as healthy hosts, the rate and sequence of these events allow them to assess the extent to which the patch is becoming exploited, and reduce their patch residence time accordingly. Information gained from encounters with parasitized hosts is important to *Rhagoletis* because they are egg-limited (Roitberg et al. 1982); each egg is valuable so that time spent in low quality patches means wasted chances in better patches.

In Waage's (1979) model, the patch time of *N. canescens* is determined by the timing of all ovipositions on a patch. However, as the rate of ovipositions decreases (as indicated by longer periods between ovipositions), her time on the patch actually increases. As mentioned before, it is difficult to understand how such a response is adaptive. For example, if ovipositions come quickly, then the increments added to her total residence time will be very small, and the amount of time she will spend on these patches is only slightly more than if no hosts were found. In addition, if ovipositions occur at the end of her searching bout, the amount of time remaining may be too small to allow her to exploit the patch further. One explanation for this strategy may be that, if the parasitoid "knows" how many hosts to expect, then a number of quick ovipositions mean that fewer will be left, and she will not need to invest much additional time. Conversely, she may be willing to spend a longer time searching when ovipositions have been rare, with the "expectation" that most hosts are still available. This might explain why encounters with parasitized hosts are unnecessary to *N. canescens* in determining patch depletion. Basing one's assessment of patch potential on the availability of remaining hosts is misleading, however, since the remaining hosts may already be parasitized.

Morrison and Lewis (1981) described the "initial GUT" of *T. pretiosum* on *Heliothis zea* eggs as "fixed". Parasitoids will search leaves more intensely if they contain the kairomone produced by adult *H. zea* scales, but they do not correlate this kairomone with egg density. During this fixed initial search period, there is a greater chance of discovering hosts if density is high, so the probability of further search time is a function of the density of hosts. Ovipositions are responsible for the parasitoid's perception of the host density and result in greater patch time being allotted. However, as the attack rate increases, the probability of finding parasitized hosts also increases, indicating the depletion of the patch. Therefore, the ratio of re-encounters with parasitized hosts to attacks on healthy hosts (the "terminal ratio") is used by the parasitoid to adjust her further searching effort. The result of *T. pretiosum*'s strategies is that at low host densities, patch residence times will be low, because of the low probability that hosts will be found within the fixed initial search period. At higher densities, each oviposition increases the chances that she will find another host. However, the hosts remain, so that it is the decreasing quality of the patch, as indicated by the "terminal ratio" that ultimately determines the residence time.

Van Lenteren and Bakker (1978) describe a strategy in which *P. bochei* females are attracted initially to the size of the deteriorating spot on a fruit in which their hosts, *Drosophila* larvae, are feeding, and so relate their "initial GUT" to the size of the patch. However, host density is not correlated with the size of spots, so an "initial GUT" based on patch size would not be functional.

The behaviours shown by *O. dimidiatus* appear to follow a multi-level strategy. Females are attracted to leaves from a long distance, so will search any leaf for a fixed, limited time, as denmonstated in Fig. 4.1. The presence of hosts, however, as indicated by mines and their stimuli, allow her to assess potential host density and set a searching period that will permit her to exploit this density of hosts. This information sets an initial limit to the amount of time (and therefore energy) that she need invest in that patch. It can be considered only a "rough approximation" of patch quality, however. Cues relating to mines and frass may present misleading information about host availability; some mines may contain parasitized hosts while others may be empty, the larvae having recently left to pupate. As she begins to encounter hosts she is able to "fine-tune" her original assessment, based on the knowledge she gains from patch experiences and direct host encounters, and she can then employ other "decision rules" to enable her to use the patch more efficiently.

After an oviposition, *O. dimidiatus* females intensify their searching effort, each successive oviposition increasing the time they will search by an increasing amount. It is obvious that there must be a "damping" of this increasing effect of successive ovipositions, and this is provided by encounters with parasitized hosts. It is probable that some combination of the effects of ovipositions and parasitized host encounters are responsible for determing the total residence time for this species.

Unlike the Roitberg and Prokopy model, however, the GUT of *O. dimidiatus* actually increases after an encounter with a parasitized host (Fig. 4.8) though by a decreasing amount, as indicated by the declining TSI (Fig. 4.9). Encounters with parasitized hosts may not, in fact, be as important to *O. dimidiatus* as for *R. pomonella* since *O. dimidiatus* is not egg-limited and can afford to use less valuable (i.e. already-parasitized) hosts if conditions require such a strategy. The fact that she is willing to add to her search time even after such encounters may also reflect the non-clumped distribution of her hosts.

In theory, a patch that has been previously searched and exploited should represent a lower quality patch, since there is a diminished probability of finding hosts that will support the complete development of her offspring. Hosts that have already been parasitized will still be available, but their value to her will be reduced in terms of the fitness she will gain / egg laid. If a parasitoid female can detect prior exploitation of a patch, it should cause her to adjust her assessment of its profitability and reduce her searching effort to a degree that reflects the reduced payoff from this patch. If, on the other hand, she can increase the chances of the surviving larvae being hers by laying more eggs per host, she should stay longer (van Alphen 1988).

There is some speculation in the literature that parasitoids may be able to detect "patch marks" left behind by a previously-searching female. Both Waage (1979) and van Lenteren and Bakker (1978) found that a second visit by parasitoid females to a patch they themselves had previously searched was shorter than the first, and suggested that a response to patch marking was the explanation. Price (1970) found evidence that females of a number of parasitic insect genera recognize and avoid trails left by individuals of the same species, of different species in the same genera, and of different genera. In my experiments, *O. dimidiatus* females showed a lower GUT when arriving on a patch containing hosts previously parasitized by themselves. This was considered to be a response to stimuli associated with parasitized hosts, but it is possible that the response might have resulted from her perception of cues that indicate a previously-searched patch. This could be determined by comparing search times on (1) unsearched leaves from which hosts had been removed, (2) searched leaves (hosts removed), and (3) searched leaves from which parasitized hosts had been removed. A response to "searched" patches would cause treatments (2) and (3) to differ from (1); a response to cues associated with parasitized hosts would cause treatments (1) and (2) to differ from (3).

There are interesting implications to the theory that females mark patches as they search. A marking female is assumed to be laying down the mark to provide information for herself, rather than for the benefit of other females. However, since the mark is being laid down as she forages, the probability increases with her time on the patch that she will begin to encounter her own mark. If she responds to this cue as though the patch had been exploited, she may emigrate from the patch without having encountered hosts.

Encounters with other adult females on a patch may also indicate exploitation of a patch and affect patch time. Beddington (1975) reported that interference by adult females searching on the same patch caused them to leave sooner than if they had searched alone. It is possible that this is a response to the probability that the first female will have used the available hosts. It is tempting to speculate that if this idea is valid, she might leave more readily if the other female were conspecific. (For a contrasting view of the outcome of adult interference, see van Alphen 1988.)

Direct evidence of previous patch use can be derived if the parasitoid is able to discriminate between parasitized and healthy hosts. In these experiments, *O. dimidiatus* has been shown to discriminate hosts previously parasitized by herself and can respond by avoiding superparasitism. It seems reasonable, based on the theory of varying returns from parasitized hosts, that parasitoids should develop the ability to discriminate hosts parasitized not only by themselves, but by conspecifics and females of other species.(For further discussion see chapter III.) Though some studies have suggested that interspecific discrimination is possible (Bakker et al. 1985, Vet et al. 1984), very little evidence has been found that a parasitoid can detect the difference between parasitism of hosts by conspecifics and by themselves. (For one example, see Hubbard et al. 1987.)

Even armed with knowledge about the quality of a patch she is presently searching, a female may still remain if the chance is even less that she will find a better quality patch without expending too much energy in transit. Roitberg and Prokopy (1982) pointed out the importance of interpatch distance on the foraging effort of ***Rhagoletis*** flies. The flies invested less searching effort in a patch when neighbouring patches were nearby and cost of interpatch travel less, than when they were further away. Ultimately, the decision to leave is likely to be the result of combining probabilities inherent in a great many factors.

Though these represent only a few of the pieces of information that may be available to a searching female, and which may influence her subsequent behaviour, they serve to illustrate the complexities involved in trying to determine "optimum" behaviour, and the importance of examining the rules of thumb which make it possible for a forager to adjust to variation in its foraging environment.

In investigating these decision rules, it is important to consider the constraints that may play a role in a parasitoid's ability to gather and act on information, since these constraints may ultimately determine which rules of thumb are employed. In a mixed patch containing high numbers of healthy and parasitized hosts, for example, it is difficult to accept that a female could retain the memory of all host encounters and "count" them. It is more likely that a simple decision rule is used that incorporates the most recent experiences. One example of this is the "sliding window" model (Roitberg and Mangel 1989) in which, as each new experience is added to the memory, an earlier one is lost. If, for example, a parasitoid can retain the memory of the past five encounters with healthy (H) and parasitized (P) hosts, then her memory window at a particular instant may include "H P H P H" (from most distant to most recent experience). An additional encounter with a parasitized host (P*) would cause the window to slide to the right, resulting in a memory of P H P H P*. Her decisions would then be made as a result of the most recent updating of information. As a patch becomes exploited, the ratio of "P"'s to "H"'s in her memory window would increase. The manner in which the information gained from a "sliding window" would translate into optimal behaviour, is dependent on the nature of the rules of thumb which have evolved to use this information. Roitberg and Mangel (1989, In Press) showed how optimal oviposition decisions vary with the parasitoid's information state.

Other constraints on a parasitoid's performance might include a variation in ability to perceive small changes in kairomone concentration, limits to visual perception, inability to discriminate between healthy and parasitized hosts, failure to detect non-moving hosts, and so on. The decision rules that evolve will ultimately be shaped by such physiological and behavioural limitations.

It remains only to examine how these decision rules are implemented, what mechanisms have evolved to direct a parasitoid's behaviour. For example, how does a trail's stimulus lead to the finding of a host? Is the female moving in the direction of higher concentrations of frass volatiles, or is she mechanically responding to an increasing mine width? How does the perception of parasitized hosts cause a parasitoid to avoid superparasitism? Is she responding to a cue that blocks her ability to bring an egg into position, or perhaps to a stimulus that causes the withdrawal of the ovipositor? Or is she simply missing the stimulus that triggers oviposition? What is the mechanism that causes the increased GUT after an oviposition? Is there an increase in responsiveness to the patch edge, as suggested by Waage (1979), or does each oviposition result in a change in her searching pattern? This last question is of particular interest to me as a result of my studies of the allocation of searching time of this species, and because adjustments to search patterns have been observed. An increased GUT would result if, after an oviposition, the female engaged in "area-restricted search", reducing her rate of speed, and/or increasing her turning rate. Such behaviour would delay her reaching the edge of the patch, and thereby increase her patch residence time. Conversely, by increasing her rate of speed and/or decreasing the rate at which she turns after encountering a parasitized host, a female would be brought to the patch edge more frequently and would have greater opportunity to leave.

Waage's (1979) description of the foraging behaviour of *N. canescens* has been widely quoted, not only because of the elegance of his experiments, but because he ties together the predictions of optimal foraging models, the rules of thumb which implement an optimal strategy, and the behavioural mechanisms which make possible the foraging behaviour. His study provides an example of the many levels at which one may investigate animal behaviour. The ways in which behavioural ecologists approach foraging decisions are much like the ways three different professionals might see the same beautiful building. The planner will focus on how the entire structure has been designed to achieve a particular concept. The architect can envision how the plans were formulated to execute the idea. The carpenter imagines how the internal pieces of the structure were pieced together to express the architect's drawings and the planner's dream. Foraging behaviour is most often seen in terms of the "grand design", what an animal's behaviour should be trying to achieve. More recent studies (including this one) are beginning to examine the behavioural rules animals use to arrive at optimal solutions. Very few investigations have pinpointed the proximate mechanisms involved in behaviours. Each of these approaches has its own value and offers its own unique insights. Together they complement each other, adding greater depth to our picture of the behavioural aspects of foraging.

LITERATURE CITED

Alphen, J.J.M. van. 1980. Aspects of the foraging behaviour of *Tetrastichus asparagi* Crawford and *Tetrastichus* spp. (Eulophidae), gregarious egg parasitoids of the asparagus beetles *Crioceris asparagi* L. and *C. duodecimpunctata* L. (Chrysomelidae). 1. Host species selection, host stage selection and host discrimination. Neth. J. Zool. 30(2):307-325.

Alphen, J.J.M. van 1988. Patch-time allocation by insect parasitoids: superparasitism and aggregation. *In* Population Genetics and Evolution. G. de Jong (ed), Springer-Verlag, Berlin.

Alphen, J.J.M. van, M.J. van Dijken, and J.K. Waage. 1987. A functional approach to superparasitism; host discrimination needs not to be learnt. Neth. J. Zool. 37(2):167-179.

Alphen, J.J.M. van and R.A.B. Drijiver. 1982. Host selection by Asobara tabida Nees (Braconidae:Alysiinae) a larval parasitoid of fruit inhabiting Drosophila species. Neth. J. Zool. 32(2):215-231.

Alphen, J.J.M. van and F. Galis. 1983. Patch time allocation and parasitization efficiency of *Asobara tabida*, a larval parasitoid of *Drosophila*. J. Anim. Ecol. 52:931-952.

Alphen, J.J.M. van and H.W. Nell. 1982. Superparasitism and host discrimination by *Asobara tabida* Nees (Braconidae:Alysiinae), a larval parasitoid of Drosophilidae. Neth. J. Zool. 32(2):232-260.

Alphen, J.J.M. van and L.E.M. Vet. 1986. Parasitoid host finding and selection. *In* Insect Parasitoids. 13th Symposium of the Royal Entomological Society of London. J.K. Waage and D. Greathead (eds.) Academic Press, London.

Bakker, K., J.J.M.van Alphen, F.H.D. van Batenburg, N. vanderHoeven, H.W. Nell, W.T.F.H. van Strien-van Liempt and T.C.T. Turlings. 1985. The function of host discrimination and superparasitism in parasitoids. Oecologia 67:512-516.

Bakker, K., S.N. Bagchee, W.R. van Zwet and E. Meelis. 1967. Host discrimination in *Pseudeucoila bochei* (Hymenoptera:Cynipidae). Ent. exp. and appl. 10:295-311.

Beckage, N.E. 1985. Endocrine interactions between endoparasitic insects and their hosts. Ann. Rev. Entomol. 30:371-413.

Beddington, J.R. 1975. Mutual interference between parasites or predators and its effect on searching efficiency. J. Anim. Ecol. 44: 331-340.

Bradley, W.G. and K.D. Arbuthnot. 1938. The relation of host physiology to development of the braconid parasite, *Chelonus annulipes* Wesmael. Ann. Ent. Soc. Amer. 31:359-365.

Chandler, L.D. and F.E. Gilstrap. 1987. Seasonal fluctuations and age structure of *Liriomyza trifolii* (Diptera:Agromyzidae) larval populations on bell peppers. J. Econ. Entomol. 80:102-106.

Charnov, E.L. 1976. Optimal foraging, the marginal value theorem. Theoret.Popul. Biol. 9:129-136.

Charnov, E.L. and S.W. Skinner. 1984. Evolution of host selection and clutch size in parasitoid wasps. Fla. Entomol. 67(1):5-21.

Charnov, E.L. and S.W. Skinner. 1985. Complementary approaches to the understanding of parasitoid oviposition decisions. Environ. Entomol. 14:383-391.

Chow, F.J. and M. Mackauer. 1986. Host discrimination and larval competition in the aphid parasite *Ephedrus californicus*. Entomol. exp. appl. 41:243-254.

Cloutier, C., L.A. Dohse, and F. Bauduin. 1984. Host discrimination in the aphid parasitoid *Aphidius nigripes*. Can. J. Zool. 62:1367-1372.

Cook, R.M. and S.F. Hubbard. 1977. Adaptive searching strategies in insect parasites. J. Anim. Ecol. 46: 115-125.

Cowie, R.J. and J.R. Krebs. 1979. Optimal foraging in patchy environments. *in* Population Dynamics. R.M. Anderson, B.D. Turner and R.L. Taylor (eds.), Blackwell Scientific Publications, Oxford.

Fisher, R.C. 1961. A study in insect multiparasitism. 1. Host selection and oviposition. J. Exp. Biol. 38:267-275.

Fisher, R.C. 1963. Oxygen requirements and the physiological suppression of supernumerary insect parasitoids. J. Exp. Biol. 40:531-540.

Flanders, S.E. 1950. Regulation of ovulation and egg dispersal in the parasitic Hymenoptera. Can. Ent. 82:134-140.

Force, D.C. and P.S. Messenger. 1965. Laboratory studies on conpetition among three parasites of the spotted alfalfa aphid *Therioaphis maculata* (Buckton). Ecology 46:853-859.

Galis, F. and J.J.M. van Alphen. 1981. Patch time allocation and search intensity of *Asobara tabida* nees (Braconidae), a larval parasitoid of *Drosophila*. Neth. J. Zool. 31(3):596-611.

Gibb, J.A. 1962. Tinbergen's hypothesis of the role of specific searching images. Ibis 104:106-111.

Glas, P.C.G. and L.E.M. Vet. 1983. Host-habitat location and host location by *Diachasma alloeum* Muesbeck (Hym:Braconidae) a parasitoid of *Rhagoletis pomonella* Walsh (Dipt:Tephritidae). Neth. J. Zool. 33(1):41-54.

Greany, P.D. and E.R. Oatman. 1972. Analysis of host discrimination in the parasite *Orgilis lepidus* (Hymenoptera:Braconidae). Ann. Ent. Soc. Amer. 65:377-383.

Greany, P.D., J.H. Tumlinson, D.L. Chambers and G.M. Roush. 1977. Chemically mediated host finding by *Biosteris (Opius) longicaudatus*, a parasitoid of tephritid fruit fly larvae. J. Chem. Ecol. 3:189-195.

Green, R.F. 1984. Stopping rules for optimal foragers. Amer. Natur. 123(1):30-43.

Harrison, E.G., R.C. Fisher and K.M. Ross. 1985. The temporal effects of Dufour's gland secretion in host discrimination by *Nemeritis canescens*. Entomol. exp. appl. 38:215-220.

Hassell. M.P. and R.M. May. 1974. Aggregation in predators and insect parasites and its effect on stability. J. Anim. Ecol. 43:567-594.

Heads, P.A. and J. H. Lawton. 1983. Studies on the natural enemy complex of the holly leaf miner: the effects of scale on the detection of aggregative response and the implications for biological control. Oikos 40:267-276.

Hubbard, S.F. and R.M. Cook. 1978. Optimal foraging by parasitoid wasps. J. Anim. Ecol. 47:593-604.

Hubbard, S.F., G. Marris, A. Reynolds and G.W. Rowe. 1987. Adaptive patterns in the avoidance of superparasitism by solitary parasitic wasps. J. Anim. Ecol. 56:387-403.

Iwasa, Y., M. Higashi and N. Yamamura. 1981. Prey distribution as a factor determining the choice of optimal foraging strategy. Amer. Natur. 117(5):710-723.

Iwasa, Y., Y. Suzuki and H. Matsuda. 1984. Theory of oviposition strategy of parasitoids. 1. Effect of mortality and limited egg number. Theoret. Pop. Biol. 26:205-227.

Jervis, M.A. and N.A.C. Kidd. 1986. Host-feeding strategies in hymenopteran parasitoids. Biol. Rev. 61:395-434.

Jones, V.P., M.P. Parrella, and D.R. Hodel. 1986. Biological control of leafminers in greenhouse chrysanthemums. Calif. Agric. 40(1&2):10-12.

Klomp, H., B.J. Teerink and W. C. Ma. 1980. Discrimination between parasitised and unparasitised hosts in egg parasite *Trichogramma embryophagum*: a matter of learning and forgetting. Neth. J. Zool. 30(2):254-277.

Krebs, J.R. 1973. Behavioural aspects of predation. *In* Perspectives in Ethology. P.P.G. Bateson and P.H. Klopfer (eds.), Plenum Press, N.Y.

Krebs, J.R. and R.H. McCleery. 1984. Optimization in behavioural ecology. *In* Behavioural Ecology. An Evolutionary Approach. 2nd ed. J.R. Krebs and N.B. Davies (eds.), Blackwell Scientific Publications, Boston.

Krebs, J.R., J.C. Ryan, and E.L. Charnov. 1974. Hunting by expectation or optimal foraging? A study of patch use by chickadees. Anim. Behav. 22:953-964.

Krebs, J.R., D.W. Stephens and W.J. Sutherland. 1983. Perspectives in optimal foraging. *In* Perspectives in Ornithology. A.H. Brush, G.A. Clark Jr. (eds.), Cambridge Univ. Press, USA.

Lathrop, F.H. and P.C. Newton. 1933. The biology of *Opius melleus* Gahan, a parasite of the blueberry maggot. J. Agric. Res. 46:143-160.

Lawrence, P.O. 1981. Host vibration - a cue to host location by the parasite, *Biosteris longicaudatus*. Oecologia (Berl) 48:249-251.

Lawrence, P.O. 1982. *Biosteres longicaudatus*: developmental dependence on host (*Anastrepha suspensa*) physiology. Exp. Parasitol. 53:396-405.

Lawrence, P.O., R.M. Baranowski and P.D. Greany. 1976. Effect of host age on development of *Biosteres (=Opius) longicaudatus*, a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa*. Fla. Entomol. 59(1):33-39.

Legaspi, B.A. 1986. Host discrimination in two species of ichneumonid wasps, *Diadegma* spp. attacking larvae of *Plutella xylostella*. Entomol. exp. appl. 41:79-82.

Lema, K-M. and S.L. Poe. 1979. Age specific mortality of *Liriomyza sativae* due to parasitization by *Opius dimidiatus* and *Chrysonotomyia formosa*. Environ. Entomol. 8:935-937.

Lenteren, J.C.van. 1976. The development of host discrimination and the prevention of superparasitism in the parasite *Pseudeucoila bochei* Weld (Hym:Cynipidae). Neth. J. Zool. 26:1-83.

Lenteren, J.C.van. 1981. Host discrimination by parasitoids. *In* Semiochemicals. D.A. Norland, R.L. Jones and W.J. Lewis (eds), John Wiley and Sons Inc.

Lenteren, J.C.van and K. Bakker. 1975. Discrimination between parasitised and unparasitised hosts in the parasitic wasp *Pseudeucoila bochei*: a matter of learning. Nature 254:417-419.

Lenteren, J.C. van and K. Bakker. 1978. Behavioural aspects of the functional responses of a parasite (*Pseudeucoila bochei* Weld) to its host (*Drosophila melanogaster*). Neth. J. Zool. 28:213- 233.

Lynch, J.A. and M.W. Johnson. 1987. Stratified sampling of *Liriomyza trifolii* spp. (Diptera:Agromyzidae) and associated parasites on watermelon. J. Econ. Entom. 80(6):1254-1261.

Mackauer, M. 1986. Growth and developmental interactions in some aphids and their hymenopterous parasites. J. Insect Physiol. 32(4):275-280.

Maindonald, J.H. and N.P. Markwick. 1986. The avoidance of superparasitism in four species of parasitic wasp - mathematical models and experimental results. Res. Popul. Ecol. 28(1):1-16.

McClanahan, R.J. 1974.Notes on the vegetable leafminer, *Liriomyza sativae* (Diptera:Agromyzidae), *In* Ontario. Proc. Entomol. Soc. Ontario. 105:40-44.

McNair, J.N. 1982. Optimal giving-up times and the marginal value theorem. Amer. Natur. 119:511-529.

McNamara, J.N. and A.J. Houston. 1985. Optimal foraging and learning. J. Theor. Biol. 117:231-249.

Minkenburg, O.P.J.M. and J.C. van Lenteren. 1986. The leafminers, *Liriomyza bryoniae* and *L. trifolii* (Diptera:Agromyzidae), their parasites and host plants: a review. Agric. Univ. Wageningen Papers. 86(2):1-50.

Morrison, G. 1986. Stochastic aggregative responses and spatial patterns of parasitism in patchy host-parasitoid interactions. Oecologia 70:402-410.

Morrison, G. and W.J. Lewis. 1981. The allocation of searching time by *Trichogramma pretiosum* in host-containing patches. Ent. exp. and appl. 30:31-39.

Murdoch, W.W. and R. Oaten. 1975. Predation and population stability. Adv. Ecol. Res. 9:2-13.

Parrella, M.P. 1987. Biology of *Liriomyza trifolii*. Ann. Rev. Entomol. 32:201-224.

Parrella, M.P. and V.P. Jones. 1984. Coping with the leafminer crisis. Calif. Agric. 38(9):17-19.

Pemberton, C.E. and H.F. Willard. 1918. A contribution to the biology of fruit fly parasites in Hawaii. J. Agr. Res. 15:419-466.

Petitt, F.L. 1984. Oviposition behavior of *Opius dissitus* and its uses for management of *Liriomyza sativae* on greenhouse tomatoes. *In*: Proc. 4th Ann. Ind. Conf. Leafminers. S.L. Poe (ed), Sarasota. Florida:81-86.

Price, P.W. 1970. Trail odors: Recognition by insects parasitic on cocoons. Science 170:546-547.

Pyke, G.H. 1984. Optimal foraging theory: a critical review. Ann. Rev. Ecol. Syst. 15:523-575.

Richards, O.W. and R.G. Davies. 1977. Imms' General Textbook of Entomology. 10th ed.(vol. 1), pp 77-78. Chapman and Hall, London.

Rogers, D.J. 1972. The ichneumon wasp *Venturia canescens*; oviposition and avoidance of superparasitism. Entomol. exp. appl. 15:190-191.

Rogers, D.J. and M.P Hassell. 1974. General models for insect parasite and predator searching behaviour: interference. J. Anim. Ecol. 43:239-253.

Roitberg, B.D., J.C. van Lenteren, J.J.M. van Alphen and R.J. Prokopy. 1982. Foraging behaviour of *Rhagoletis pomonella*, a parasite of hawthorn (*Crataegus viridus*), in nature. J. Anim. Ecol. 51:307-325.

Roitberg, B.D. and M. Mangel. 1988. On the evolutionary ecology of marking pheromones. Evol. Ecol. (In Press).

Roitberg, B.D. and M. Mangel. 1989. Dynamic information and host acceptance by a tephritid fruit fly. Ecol. Entomol. (In Press).

Roitberg, B.D. and R.J. Prokopy. 1982. Influence of intertree distance on foraging behaviour of *Rhagoletis pomonella* in the field. Ecol. Entomol. 7:437-442.

Roitberg, B.D. and R.J. Prokopy. 1984. Host visitation sequence as a determinant of search persistence in fruit parasitic tephritid flies. Oecologia 62:7-12.

Roitberg, B.D. and R.J. Prokopy. 1987. Insects that mark host plants. Bioscience 37(6):400-406.

Salt, G. 1934. Experimental studies in insect parasitism. II Superparasitism. Proc. R. Soc. London. Sec. B. 122:57-75.

Schmidt, J.M. and J.J. Smith. 1987. Short interval time measurement by a parasitoid wasp. Science 237:813-952.

Strien-van Liempt, W.T.F.H.van 1983. The competition between *Asobara tabida* Nees van Esenbeck, 1834 and *Leptopilina heterotoma* (Thomson, 1862) in multiparasitized hosts. 1. The course of competition. Neth. J. Zool. 33(2):125-163.

Trumble, J.T. 1985. Planning ahead for leafminer control. Calif. Agric. 39(7&8):8-9.

Vet, L.E.M., M. Meyer, K. Bakker and J.J.M. van Alphen. 1984. Intra- and interspecific host discrimination in *Asobara* (Hymenoptera) larval endoparasitoids of Drosophilidae: comparison between closely-related and less closely-related species. Anim. Behav. 32:871-874.

Vinson, S.B. 1976. Host selection by insect parasitoids. Ann. Rev. Entomol. 21:109-133.

Vinson, S.B. and F.S. Guillot. 1982. Host marking: source of a substance that results in host discrimination in insect parasitoids. Entomophaga 17:241-245.

Waage, J.K. 1978. Arrestment responses of the parasitoid, *Nemeritis canescens*, to a contact chemical produced by its host, *Plodia interpunctella*. Physiol. Entom. 3:135-146.

Waage, J.K. 1979. Foraging for patchily-distributed hosts by the parasitoid, *Nemeritis canescens*. J. Anim. Ecol. 48:353-371.

Waage, J.K. 1983. Aggregation in field parasitoid populations: foraging time allocation by a population of *Diadegma* (Hymenoptera: Ichneumonidae). Ecol. Entomol. 8:447-453.

Waage, J.K. 1986. Family planning in parasitoids: adaptive patterns of progeny and sex allocation. *In* Insect Parasitoids. J.K. Waage, D. Greathead (eds.), Academic Press, London.

Waage, J.K. and H.J.C. Godfray. 1985. Reproductive strategies and population ecology of insect parasitoids. *In* Behavioural Ecology: Ecological Consequences of Adaptive Behaviour. R.M. Sibley, R.H. Smith (eds.), Blackwell Scientific Publications, Oxford.

Wellington, W.G. 1977. Returning the insect to insect ecology: Some consequences for pest management. Environ. Entomol. 6(1):1-8.

Wharton, R. 1984. The status of certain Braconidae (Hymenoptera) cultured for biological control programs, and descriptions of a new species of *Macrocentrus*. Proc. Entomol. Soc. Wash. 86(4):902-912.

Wylie, H.G. 1970. Oviposition restraint of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) on hosts parasitised by hymenopterous species. Can. Ent. 102:886-894.

Ydenberg, R.C. 1984. Great tits and giving-up times: decision rules for leaving patches. Behav. 90(1-3):1-24.