

TR 85-10
✓

THE HOST-SEARCHING BEHAVIOUR OF COCCOPHAGUS ATRATUS COMPERE
(APHELINIDAE: HYMENOPTERA)

by

MAXWELL MAITLAND CLARK

THESIS SUBMITTED TO RHODES UNIVERSITY
IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF THE
DEGREE OF MASTER OF SCIENCE

Department of Zoology and Entomology
Rhodes University
Grahamstown
South Africa

December, 1984.

FRONTISPIECE



A female Coccophagus atratus parasitoid examining a scale insect before she oviposits. The actual size of the parasitoid is indicated by the black spot in the lower right hand corner. Photograph of a painting by J.S. Donaldson.

CONTENTS

ACKNOWLEDGEMENTS.....	1
RÉSUMÉ.....	2
1. INTRODUCTION.....	3
2. CLASSIFICATION AND BIOLOGY OF <u>C. atratus</u> AND ITS HOST INSECTS.....	8
2.1 Classification of <u>C. atratus</u>	8
2.2 Classification of host insects.....	9
2.3 Mating behaviour of <u>C. atratus</u>	10
2.4 Oviposition and adult feeding behaviour of <u>C. atratus</u>	10
2.5 Preoviposition period.....	13
3. GENERAL MATERIALS AND METHODS.....	17
3.1 Scale insect collections.....	17
3.2 Scale insect culture.....	17
3.3 Culturing <u>C. atratus</u> parasitoids.....	19
3.4 Olfactory experiments.....	20
3.4.1 Experimental methods.....	22
3.4.2 Control experiments.....	23
3.4.3 Limitations to the use of the olfactometer.....	24
3.5 Statistical analyses.....	25
4. HOST HABITAT LOCATION.....	26
4.1 Hosts of <u>C. atratus</u>	27
4.2 Plant species associated with <u>C. atratus</u> ' host insects.....	27
4.3 Distribution of <u>C. atratus</u>	32
4.4 Role of plants in host habitat location by <u>C. atratus</u>	34
4.5 Hypothesis of host habitat location.....	35
5. HOST LOCATION.....	41
5.1 Response of <u>C. atratus</u> to odours from unparasitised scale insects.....	42
5.2 Response of <u>C. atratus</u> to odours from parasitised scale insects.....	45
5.3 Response to a choice of odours.....	46

5.4 Influence of plants on the attractiveness of honeydew odour	48
5.5 Activity index of virgin and mated females.....	51
5.6 Modified hypothesis for host location.....	53
6. BEHAVIOUR OF <u>C. ATRATUS</u> IN THE FIELD.....	56
6.1 Effect of plants on searching behaviour in the field.....	56
6.2 Effect of reproductive status on host-searching behaviour..	59
6.3 Modified hypothesis for host location.....	62
7. HOST RECOGNITION.....	64
7.1 Stage in searching behaviour at which hosts are recognised.	64
7.2 Cues used to identify hosts suitable for male eggs.....	70
7.3 Hypothesis for host-acceptance behaviour.....	80
8. DISCUSSION.....	84
8.1 Host-searching behaviour.....	84
8.1.1 <u>C. atratus</u>	84
8.1.2 General host-searching behaviour.....	86
8.2 Interpretation of <u>C. atratus</u> sex ratios.....	87
8.3 Proposed sequence for the evolution of heteronomous parasitoids.....	88
8.4 Use of heteronomous hyperparasitoids in biological control.	90
SUMMARY.....	92
REFERENCES.....	94

ACKNOWLEDGEMENTS

I am grateful to Professor V.C. Moran and Mr G.H. Walter for their stimulating supervision.

The help and advice of Mr J.S. Donaldson, who also studied aphelinid biology, is gratefully acknowledged. I thank Dr P.E. Hulley for constructive criticism during the project.

I would like to thank the following people for their assistance: Professor E. van der Merwe and Mr H.G. Robertson for help with statistical analyses; Dr S. Naser for unpublished information; Dr G.L. Prinsloo and Dr D.J. Williams for insect identifications; Dr A. Jacot Guillardmod for plant identifications; Mr D.H. Forsyth for technical assistance; and Mrs G. Walter for drawing many of the figures.

The financial support of the Council for Scientific and Industrial Research is acknowledged. Research was also funded by a grant from the Department of Agriculture and the Council of Rhodes University to Mr G.H. Walter.

Finally, I would like to thank my parents for their support and encouragement.

RÉSUMÉ

The host-searching behaviour of the parasitoid Coccophagus atratus Compere was investigated. C. atratus parasitoids have unusual host relationships. Female offspring develop in scale insects but male offspring develop hyperparasitically on their conspecific females, or on other parasitoid species. C. atratus females, therefore, must locate, identify and oviposit into two different types of hosts.

A primary aim of this thesis, was to identify when and how the behaviour of a female, searching for hosts suitable for female offspring, differed from that of a female searching for hosts suitable for male offspring. This was done by investigating and comparing the behaviour of virgin and mated females. Virgin females can lay only male eggs while mated females can lay both male and female eggs.

The role of plant odours and host odours in attracting C. atratus females to the host habitat and to their scale insect hosts was examined with the aid of an olfactometer.

Field observations, to test the validity of results obtained in laboratory experiments, indicated that C. atratus females do not search initially for their hosts' food plants, but search directly for hosts.

Only when hosts were physically located did the behaviour of virgin and mated females differ. Recognition cues used by the females to distinguish between the two types of hosts were identified.

Finally, the implications of results obtained were discussed in relation to ecological and evolutionary aspects of heteronomous parasitoid biology.

CHAPTER 1: INTRODUCTION

Aphelinids are tiny parasitic wasps often less than 1mm in length (Viggiani, 1981; Hayat, 1983) and many species have been used in biological control programmes (Clausen, 1977; Rosen & DeBach, 1979). Species in several aphelinid genera are unusual because the host relationships of the males differ from those of their conspecific females. These species have been called heteronomous parasitoids (Walter, 1983a). Many species of heteronomous parasitoids have males that develop hyperparasitically, but females that are primary parasitoids. Species with these host relationships are called heteronomous hyperparasitoids (Walter, 1983a).

Because aphelinids, like most other Hymenoptera, are arrhenotokous, virgin females lay only male (haploid) eggs, while mated females, except for a few species (see Walter, 1983b), lay male (haploid) and female (diploid) eggs. Mated females store sperm in their spermathecae and they can therefore deposit either fertilised or unfertilised eggs by selectively releasing or withholding sperm (Zinna, 1962; Flanders, 1969). Heteronomous hyperparasitoid females are able to locate, identify and oviposit in the host-type appropriate for the development of each sex.

As mated female heteronomous hyperparasitoids lay eggs of the appropriate sex in the correct host type it has been suggested that they have two separate behaviours to enable each type of host to be located (Walter, 1983b). This suggestion raises questions about the 'decisions' made by female heteronomous hyperparasitoids when they search for hosts (Walter, 1983b). However, the specific details of their host searching behaviour can be dealt with only after the general theory of host-searching behaviour in parasitoids has been discussed.

To reproduce successfully, female parasitoids require suitable hosts in which to oviposit, and they usually need to search actively for them. Salt (1935; 1938) was the first to consider host-searching behaviour as a series of processes that restrict the number of potential host species of a parasitoid. A number of authors have subsequently followed Salts' (1935; 1938) suggestions, and have divided the behaviour that

leads to successful parasitisation of hosts into 5 steps, (1) host-habitat location, (2) host location, (3) host acceptance, (4) host suitability and (5) host regulation (Flanders, 1953; Doutt, 1959; 1964; Vinson, 1975; 1976). Host suitability and host regulation, reviewed by Vinson & Iwantsch (1980a and 1980b respectively), are not considered in this study as they are processes that occur after the host has been located and parasitised and are not part of what is generally considered as host-searching behaviour.

The three initial steps in host-searching behaviour were studied in a heteronomous hyperparasitoid, Coccophagous atratus Compere, to answer the following questions about host-searching behaviour in these unusual parasitoids. Firstly, do females, when they search for hosts suitable for male offspring, respond to different cues from those used when they seek hosts suitable for female offspring? Secondly, how do C. atratus females locate and identify suitable hosts for development of male and female offspring? Answers to these questions would help identify the stage in the sequence of host-searching behaviour at which the cues used for parasitisation of 'male' hosts differ from the cues used for parasitisation of 'female' hosts. Does this 'difference' (or dichotomy) present the ovipositing female with a choice, or does she simply respond to those stimuli that are present? The results of this study have implications for the study of sex ratios of heteronomous hyperparasitoids and for the interpretation of the steps proposed for the evolution of heteronomous parasitoids by Walter (1983a).

C. atratus was chosen for this study because it is a heteronomous hyperparasitoid with males that develop hyperparasitically on hymenopterous parasitoids and females that develop as primary endoparasitoids of scale insects, and because they were commonly found in the study area. The hypothesis tested (Fig. 1.1) was derived from current theory on parasitoid host-searching behaviour (DeBach, 1964; Vinson, 1975; 1976; 1981; Weseloh, 1981) and predicts that mated and virgin C. atratus females will locate the same host habitat, because both types of hosts may be found together. The dichotomy in host-searching behaviour (Fig. 1.1) was expected to occur during host location because mated C. atratus females are expected to respond to cues from both types of hosts, and virgin females are expected to

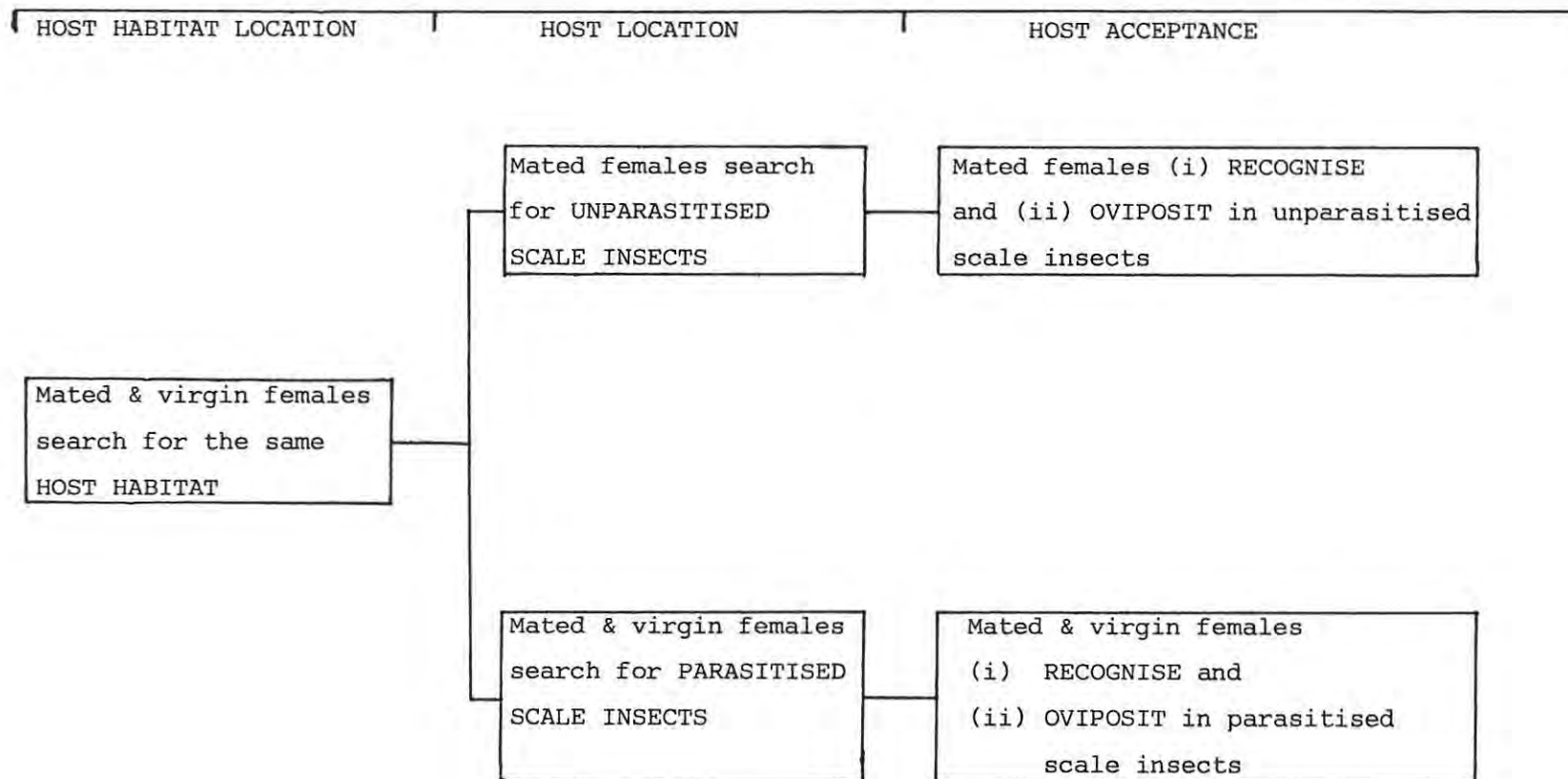


Fig. 1.1 The hypothetical host-searching behaviour of virgin and mated Coccophagus atratus females.

respond only to cues from hosts suitable for males. Virgin females were therefore more frequently used in experiments to determine how C. atratus females locate hosts. Mated females were used only in selected experiments to compare their behaviour with that of virgin females so that the point at which their host-searching behaviour differs, could be identified.

Before starting with the study on host-searching behaviour, the basic biology of C. atratus had to be determined so that the precise host relationships were known (Chapter 2). The parasitoid and its host could then be cultured in the laboratory and the appropriate experiments could be designed (Chapter 3).

The behaviour associated with host-habitat location was the first aspect of host-searching behaviour studied (Chapter 4). The geographical distribution of C. atratus, the species of hosts attacked and the plant species on which the hosts feed was examined in an attempt to identify the host habitat for which virgin and mated C. atratus females were expected to search. The role of plant odours in host-habitat location was then investigated in detail with the aid of an olfactometer.

Host location was the following stage of host-searching behaviour to be studied (Chapter 5). The responses of virgin and mated C. atratus females to odours from hosts suitable for male eggs and hosts suitable for female eggs was determined in the olfactometer. Results in the previous section had suggested that different plant species may have different effects on the host-searching behaviour of C. atratus females. To ascertain whether chemicals in the plants, to which C. atratus responded, were taken up by the hosts and excreted in their honeydew, the hosts were reared on different plant species. The scale-insect honeydew was collected and the response of C. atratus females was observed in the olfactometer.

Interpretation of the results obtained in the olfactometer experiments was restricted by the various limitations of the apparatus and by experimental design. Field studies (Chapter 6) were therefore undertaken to observe what virgin and mated females actually do in the

field and to establish how realistic the results of laboratory experiments were.

Finally, host recognition behaviour was studied to identify what cues C. atratus females use to differentiate between hosts suitable for male eggs and hosts suitable for female eggs (Chapter 7).

In the discussion (Chapter 8), these sections were summarised and the implications of the results in this thesis, for various ecological and evolutionary aspects of heteronomous parasitoid biology, are discussed. Many of the results obtained are relevant to the use of parasitoids in biological control, and suggestions are made as to how parasitoids can be manipulated to improve their ability to locate hosts, thereby improving their efficiency in biological control.

CHAPTER 2: CLASSIFICATION AND BIOLOGY OF C. atratus AND ITS HOST INSECTS

The parasitoid species chosen for study was C. atratus, which parasitises the soft scale insect Filippia gemina De Lotto (Coccidae). C. atratus was chosen as an experimental insect to test the hypothesis developed in chapter 1 because its host relationships are suitable for this purpose, and because it is a common parasitoid species in Grahamstown. (33° 18' S; 26° 32' E). Females develop in unparasitised scale insects and males develop ectoparasitically on parasitoids within scale insects. Similar species to both host and parasitoid exist locally, so the identity of these species had, initially, to be confirmed. Aids to the rapid identification of living material were also required. In addition, the basic biology of both parasitoid and host has not been reported and some fundamental aspects are covered here. Some of the information in this chapter was collected in collaboration with Donaldson (1984), who studied the sex ratios of C. atratus.

2.1. Classification of C. atratus.

Before commencing with this study the identity of C. atratus had to be confirmed to avoid confusing it with morphologically similar species, such as C. anthracinus Compere. The latter has been collected in the Cape Province from the same host species parasitised by C. atratus (Annecke & Insley, 1974). The morphological characters required to identify C. atratus are extremely small, therefore slide mounted material had to be used. Specimens were mounted on microscope slides using the methods described by Prinsloo (1980) and Noyes (1982). The parasitoids were identified using Hayat's (1983) key to genera of the Aphelinidae and then to species level using Annecke & Insley's (1974) key to Coccophagus Westwood. Identified specimens were then compared with named material held in the National Collection of Insects, Pretoria. Slides were prepared regularly during this study to confirm the identity of the insects being used.

2.2. Classification of host insects.

Scale insect hosts of C. atratus were identified to establish the host range of this parasitoid species in the field. To classify scale insects to species level the specimens must be cleared, stained and mounted on microscope slides so that taxonomically important morphological characters may be seen. The following method of preparing slide mounted specimens was modified from that described by Cilliers (1967).

The coccids were first macerated in 10% KOH to remove the body contents. They were then placed in distilled water where the remaining body contents were gently squeezed out of a small hole in the cuticle. The hole was made with a fine-pointed pin. Tracheae were hooked and pulled through the hole with the aid of a bent-tipped minuten pin. Specimens were transferred to a second bath of distilled water for 15 minutes to ensure that all the KOH had been removed. Before staining, the specimens were placed for 15 minutes in distilled water to which a few drops of 15% acetic acid had been added. The specimens were then stained overnight in a lactophenol stain mixture described by Cilliers (1967). After the specimens had been stained, they were transferred to glacial acetic acid for 15 minutes to fix the stain and to replace excess water. Xylene was used to clear the specimens prior to mounting them on microscope slides in Canada Balsam.

The most common host of C. atratus in the field was identified as Filippia gemina. Firstly, Steinweden's (1929) key to coccid genera was used, and specimens ran to Lichtensia Signoret in the key. Steinweden (1929) concluded that Lichtensia is a subjective synonym of Filippia Targioni Tozzetti. De Lotto (1974) also recognised this synonymy and included species of Lichtensia in his key to Filippia. De Lotto's (1974) key was therefore used to identify F. gemina to species. Specimens were also compared with type material housed in the National Collection of Insects, Pretoria. The identification was later confirmed by D.J. Williams (in litt., 12/10/1984) at the British Museum (Natural History).

2.3. Mating behaviour of *C. atratus*.

Mating behaviour was studied for two reasons. Firstly, when mated females were used in experiments, it was necessary to recognise that mating had been successful. Copulation was therefore observed to determine at what stage in courtship behaviour insertion of the aedeagus occurred. Secondly, *C. atratus* males are indistinguishable from males of other *Coccophagus* Westwood species and no taxonomic key is available to separate them. However, *C. anthracinus* males did not mate with *C. atratus* females, therefore mating behaviour could be used to separate these species.

To observe *C. atratus* mating behaviour, a single male and virgin female were placed together in a glass vial. Males usually appeared excited and ran rapidly around inside the vial, apparently searching for the female. This behaviour may be elicited by a female-produced sex pheromone, which virgin females emit. Mated females do not elicit such behaviour. On encountering a virgin female, the male heads her off by "herding" her, using his antennae spread widely apart to block her path. If the female becomes quiescent the male walks sideways around her, facing her all the time, and then mounts her from behind. This precopulatory behaviour usually lasted from 2 to 10 seconds. Copulation took from 1 to 2 seconds (N=15), occasionally slightly longer. Immediately after copulation, the male climbed off the female; neither sex displays post-copulatory behaviour as described by Walter (1984) for *Coccophagus bartletti* Annecke & Insley and for *Coccophagus lutescens* Compere. The courtship behaviour of *C. atratus* is simpler than that of the latter two species, possibly because pheromones may play a greater role in *C. atratus* courtship behaviour. *C. atratus* females mate only once in their lifetime, but males were observed to mate frequently; one male mated with 17 females.

2.4. Oviposition and adult feeding behaviour of *C. atratus*.

The size of hosts chosen by *C. atratus* females for oviposition was determined so that the parasitoids could be presented with the appropriate hosts in later experiments. Also, the normal sequence of behavioural events that occur during oviposition had to be known before experiments on host-acceptance criteria could be interpreted.

Host insects suitable for the oviposition of diploid eggs are unparasitised soft scales of either sex, in their late second or early third instar (Fig. 2.1). The size of scale insects acceptable for diploid egg deposition was $1.7 \pm 0.03\text{mm}$ long and $0.8 \pm 0.02\text{mm}$ wide ($\bar{x} \pm 1 \text{ S.E.}$; $N=130$).



Fig. 2.1. Unparasitised Filippia gemina scale insects that are suitable for the oviposition of female eggs by mated Coccophagous atratus females.

On locating a suitable unparasitised host, a mated C. atratus female proceeds to examine the host and, if it is found suitable, oviposits an egg into it. This oviposition behaviour can be divided into four distinct and sequential steps. Firstly, the female taps the host with her antennae. Then she climbs onto the host and continues to tap it with her antennae. Next, the female displays distinct turning movements, rotating rapidly through 180° several times, pausing between each turn to tap the host with her antennae. Finally, the female adopts an oviposition posture, drills through the host cuticle with her ovipositor and lays a single diploid egg either in the host's

haemolymph or in the midgut. Other Coccophagus species are more selective in choosing a site for oviposition and may lay only in the suboesophageal ganglion (Flanders et al., 1961) or only in the Malpighian tubules of their host (Flanders, 1973). Drilling and oviposition by C. atratus lasted from 3 to 10 seconds ($\bar{x} \pm 1 \text{ S.E.} = 5.9 \pm 0.16\text{s}$; $N = 100$).

Host-feeding by C. atratus females was never observed. Many parasitoid species are known to feed on the body fluids of their hosts (van Lenteren et al., 1976; Nell et al., 1976; Dowell et al., 1981; Walter, 1984). Some parasitoid species do not host feed (Askew, 1971; Dowell et al., 1981) and these species presumably obtain sufficient nutrients during their larval development or from other sources, such as plant exudates, nectar or honeydew.

C. atratus females obtain honeydew from their host scale insects by tapping the coccid's anal plates with their antennae. In response to this stimulation, scale insects usually excrete a small drop of honeydew. Sometimes the scale insect forcibly ejects the honeydew, which may strike the parasitoid's face and cause it to leave.

When the female parasitoid larva hatches and develops, it causes its host to swell outwards, thus giving the scale insect a humped appearance. At the same time the scale dorsum darkens, eventually turning a characteristic black colour. Once the entire contents of the host are consumed, the scale insect forms a dry, hollow, mummy in which the larva voids its meconium and pupates. Parasitoids in this stage (Fig. 2.2) are suitable for the development of C. atratus males.

Inspection, by C. atratus females, of a scale insect that contains a well-developed parasitoid larva or pupa is similar to the behaviour of a female inspecting an unparasitised scale insect. However, differences in oviposition behaviour do occur after the ovipositor penetrates the host's cuticle. Haploid eggs are laid onto final-instar larvae or prepupae inside the scale insect mummy. Drilling and oviposition of a haploid egg lasted between 12s and 13 min. ($\bar{x} \pm 1 \text{ S.E.} = 63 \pm 11.3\text{s}$; $N = 100$). The haploid egg is attached to the parasitoid pupa by a stalk. No specific area on the parasitoid pupa was preferred for attachment of the egg. Other heteronomous hyperparasitoids with ectoparasitic males



Fig. 2.2. Parasitised Filippia gemina scale insects that are suitable for the oviposition of male eggs by virgin and mated Coccophagous atratus females.

also lay haploid eggs on the late larval or prepupal stages of their hosts (Broodryk & Douth, 1966; McDaniel and Moran, 1972; Wilk & Kitayama, 1981), and attach their eggs to the body of their host by means of a short stalk. Coccophagoides similis (Masi) and some Phycus species appear to be exceptions because they usually fasten male eggs onto the inner body wall of the scale mummy and not onto the host (Flanders, 1959; Fisher, 1961; Zinna, 1962; Williams, 1972). The egg stalk, which is formed during oviposition of the egg (Flanders, 1936; 1937), holds the egg firmly to the host, and has been observed on ectoparasitic haploid eggs of other heteronomous species as well as on both haploid and diploid eggs of ectoparasitic parasitoids (reviewed by Walter, 1983b). The oviposition behaviour of C. atratus females laying male and female eggs is summarised in Fig. 2.3.

2.5. Preoviposition period.

Newly-emerged females in some parasitoid species are not in an appropriate physiological state to search for hosts and oviposit in

OVIPOSITION BEHAVIOUR

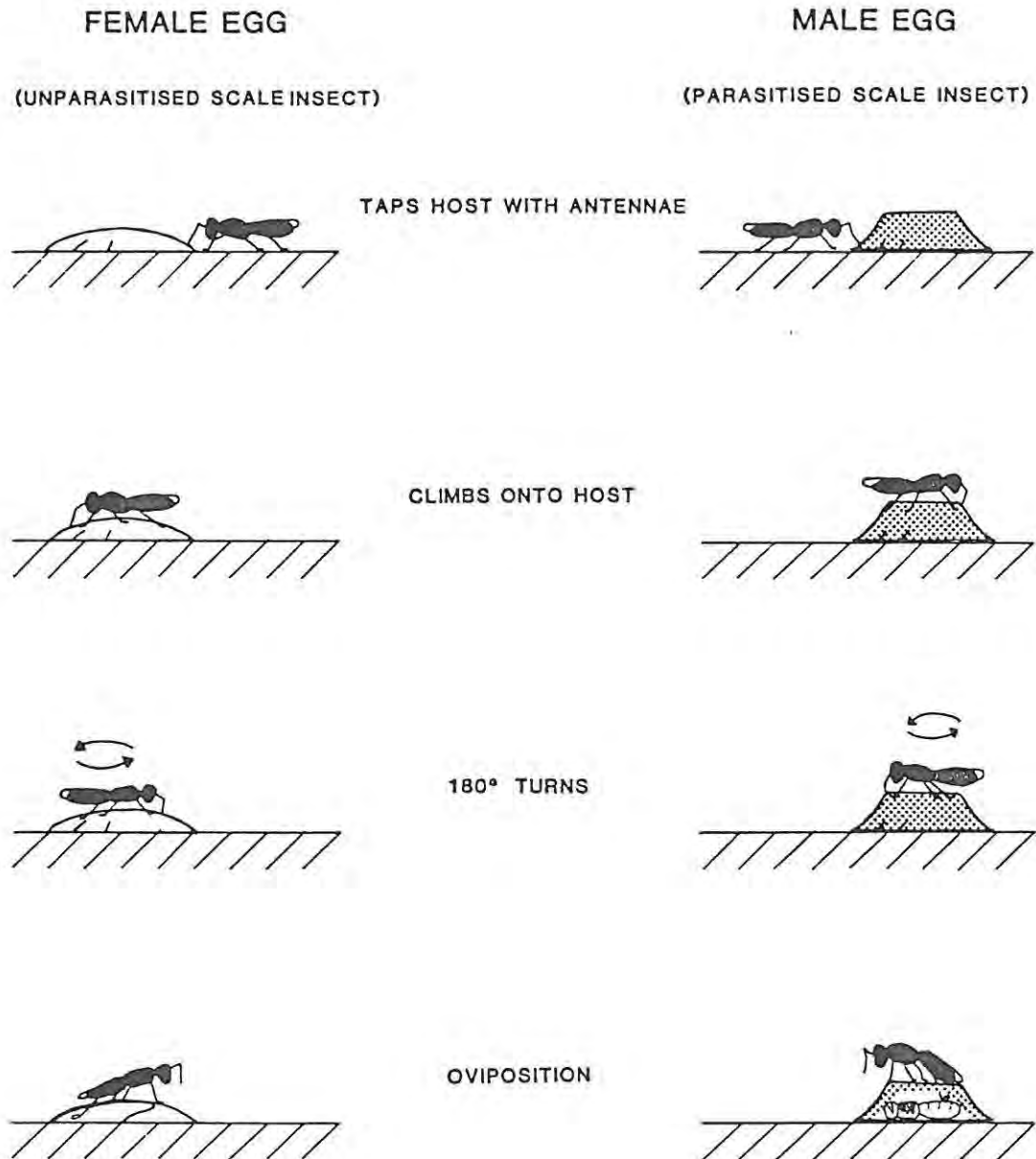


Fig. 2.3. Oviposition behaviours of Coccophagous atratus females for ovipositing female and male eggs.

them (Vinson, 1981). The duration of this preoviposition period differs between species and is associated with the nutritional requirements of the adult females (Doutt, 1964). If a parasitoid used in experiments on host-searching behaviour is not in the correct physiological state to oviposit, then it probably will not search for hosts. Therefore results obtained would be inappropriate to an interpretation of host-searching behaviour. Opius fletcheri Silvestri (Braconidae) females for example, are not attracted to the host's food plant during the first three days after emergence (Nishida, 1956). Females of the tachinid Eucarcelia rutilla Villeneuve were attracted to the odour of oak trees at the beginning of their preoviposition period, but were repelled by the odour of oak at the end of this period and were attracted to pine trees where their hosts occurred (Herebout & van der Veer, 1969).

C. atratus females were inactive and reluctant to oviposit during the first 24h after emergence. Donaldson (1984) showed that the initial inactivity in this species was correlated with the number of mature eggs present in the females' ovarioles. Immediately after emergence, females had only 1 or 2 mature eggs, but 24h old females had an average of 18 eggs and oviposited readily if presented with hosts (Donaldson, 1984). Although adult females do not host-feed but they do drink honeydew obtained from unparasitised scale insects. Honeydew of hosts of C. atratus was not examined but honeydew from other species contains sugars (Mittler, 1958), protein (Maltaise & Auclair, 1952), minerals and vitamins (Saad & Bishop, 1976). Amino acids present in honeydew include some of those essential for insects (Maltaise & Auclair, 1952), so honeydew is clearly a good food source for the parasitoids. Coccophagus scutellaris Dalman females emerge with their ovaries full of mature eggs (Cendaña, 1937; Jarraya, 1975). They, however, also do not oviposit during the first 24h after emergence (Jarraya, 1975). C. atratus females used in experiments in this study were between 24h and 7 days old, had been fed honey and had not oviposited previously. They therefore had a full complement of mature eggs.

The information presented in this chapter enabled a system to be developed for the culture of C. atratus. In addition, information on scale insect sizes suitable for female egg deposition, and age of parasitoid larvae appropriate for male egg deposition, ensured that

the correct hosts were always presented to females during observations on host-searching behaviour. The study of oviposition behaviour enabled the results on host acceptance behaviour (Chapter 7) to be interpreted. Finally, all females used in experiments were in a physiologically appropriate condition to search for hosts and to oviposit.

CHAPTER 3: GENERAL MATERIALS AND METHODS

In this chapter, the methods used to collect parasitoids from the field and to establish a laboratory culture of C. atratus are described. Details of the biology and host relationships of C. atratus (Chapter 2) were used to work out culture techniques for C. atratus and its host F. gemina. The general materials and methods used in experimental work are also described. More specific details are provided in the relevant sections because the results of some experiments had to be known before the following experiments could be designed.

3.1. Scale insect collections.

During the study period, collections of scale insects were made in the field, firstly to obtain C. atratus parasitoids for culture and secondly to add to the host records of C. atratus recorded in the literature.

Plant material infested with scale insects was placed into cardboard emergence boxes (Fig.3.1). Adult parasitoids were attracted to the light that shone through a glass vial fixed over a hole at one end of the emergence box. Parasitoids could be removed from the detachable glass vial. The scale insects and their host plants were identified for records.

3.2. Scale insect cultures.

F. gemina was the most common host insect of C. atratus in the field, and was commonly found infesting Chrysanthemoides monolifera Norlind plants in the vicinity of Grahamstown. Mature F. gemina scale insects produce an egg-filled ovisac covered with white wax for protection (Fig. 3.2). Ovisacs were collected from C. monolifera plants in the field and placed on potted C. monolifera plants in a controlled environment room. The conditions in the environment room were 12h light ($26 \pm 2^{\circ}\text{C}$; $60 \pm 5\%$ RH) and 12h dark ($18 \pm 2^{\circ}\text{C}$; $80 \pm 5\%$ RH). When the eggs in the ovisacs hatched, the F. gemina crawlers dispersed onto the plants. Scale insects were allowed to reproduce continuously, thus ensuring a continual supply of scale insects in all stages of development. Scale insects required for culturing C. atratus or for use in experiments were obtained from this culture. When scale insect



Fig. 3.1. Cardboard emergence box used to collect adult parasitoids from parasitised scale insects collected in the field.



Fig. 3.2. A *Filippia gemina* ovisac on a *Chrysanthemoides monolifera* leaf. Yellow crawlers have hatched from the eggs in the ovisac and have settled on the leaf.

numbers were depleted, ovisacs were obtained from the field to replenish the culture.

3.3. Culturing *C. atratus* parasitoids.

The laboratory culture of *C. atratus* was started with adult parasitoids obtained from parasitised *F. gemina* scale insects collected on *C. monolifera* plants.

The parasitoid culture was housed in a different controlled environment room from that used for scale insect cultures, although conditions in the two rooms were identical. Potted *C. monolifera* plants with suitable scale insect infestations were transferred from the scale insect culture to a muslin cage in the second environment room. *C. atratus* parasitoids were released on the plant and allowed to parasitise the scale insects.

When *C. atratus* females were required for experiments, parasitised scale insects, identified by their humped shape and black dorsum, were carefully removed from the plants and placed in gelatin capsules which ensured that the emerged females were unmated. Adult females were transferred on the day of emergence to muslin-topped glass vials and supplied with a drop of honey for food. If not fed honey the parasitoids rarely survived for longer than 48h. The average life span of *Coccophagoides utilis* Doult females increased from 4 days, if they were unfed, to 20 days if fed honey (Broodryk & Doult, 1966). *C. atratus* males were obtained by the exposure of parasitised scale insects to virgin parasitoid females, which laid male eggs on to the parasitoid pupae within the scale insect mummies.

All *C. atratus* females used in experiments were reared on *F. gemina* that were feeding on *C. monolifera*. This avoided possible affects on host-searching behaviour due to the parasitoids being reared on different host species and on different plant species. There are several examples of parasitoids becoming conditioned to their hosts (Taylor & Stern, 1971; Legner & Thompson, 1977) although it does not appear to occur in all parasitoid species (Salt, 1935; and see Arthur, 1966). In some experiments, however, the scale insects were reared on different plant species for a specific purpose and this will be reported in the appropriate section.

3.4. Olfactory experiments.

Olfactory cues are used by many parasitoids to locate their hosts (Vinson, 1981; Weseloh, 1981). An airflow olfactometer described by Vet et al. (1983) was constructed to determine the response of C. atratus females to various odours.

The olfactometer, shown in Fig. 3.3, incorporates four distinct odour fields that do not mix along their boundary layers. This system has the advantage over T- and Y- tube olfactometers used in the past (Monteith, 1955; Read et al., 1970; Rotheray, 1981; Shahjahan, 1974) because the wasps can walk freely from one odour field into another without hinderance. This is not possible in Y- and T- tubes because air turbulence at the junction of their arms causes odours to mix (Vet et al., 1983). The problem is eliminated in the olfactometer used in this study because it has distinct odour fields with no air turbulence. The sharp boundaries between the odour fields are due to both the symmetrical design of the system and to the sensitive airflow control system (Vet et al., 1983).

The olfactometer constructed was a slightly modified version of the one described by Vet et al. (1983) because certain materials were unavailable. One of the design changes affected the depth of the exposure chamber, which was 13mm instead of 10mm, and the narrowest width across the exposure chamber was 108mm instead of 110mm. These alterations meant that the optimal air flow rate in the system, required to produce the distinct boundaries between odour fields, differed from the 300ml min per arm used by Vet et al. (1983). To determine the optimal airflow rate in the system, NH_4OH and HCL were mixed in each of the four catching vials to form white NH_4Cl smoke. A flow rate of 150 ml min^{-1} through each arm produced distinct air fields with no mixing or air turbulence observed (Fig. 3.4).

The airflow in each arm was regulated by individual flowmeters (Aarlborg FM112-02G) which could be finely adjusted. Total airflow from all four arms was controlled by a larger flowmeter (Aarlborg FM 082-03ST) connected between the exposure chamber and the vacuum pump.

Three glass vials were connected to each of the four arms. The vial nearest to the chamber serves to catch wasps that may walk down the

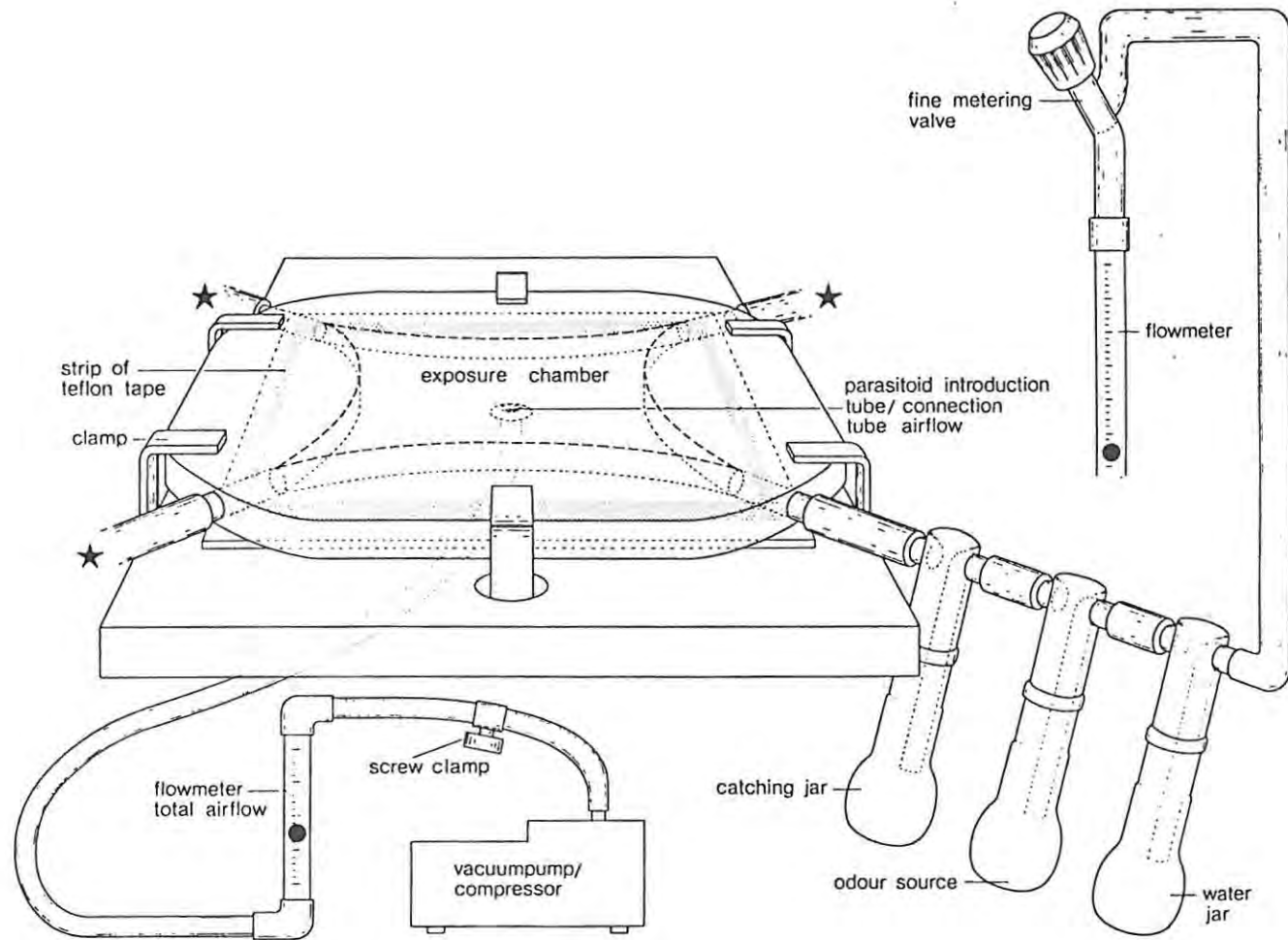


Fig. 3.3. Perspective view of the olfactometer. Reproduced from Vet et al. (1983). Only one arm is shown, the rest are represented by stars.

tube towards the odour source in the second sample vial. The third vial contains distilled water. Incoming air passes over the water and creates a uniform humidity.

The whole system was surrounded by white cardboard walls to prevent the parasitoid under observation being disturbed by movement of the observer. Light was provided by a single fluorescent tube placed 60cm above the exposure chamber. Wasp behaviour was observed by looking through a gap between the cardboard walls and the fluorescent tube.

The olfactometer was operated in a windowless room to ensure that no light could distract the wasps as they are positively phototactic. To ensure that the air was not recirculated through the olfactometer, a rubber pipe leading out of the room was attached to the vacuum pump outlet. Room temperature was maintained at $24 \pm 2^{\circ}$ C.

3.4.1. Experimental methods.

At the start of each experiment the odour source was placed into one of the four sample vials. Next, the odour fields were set up by starting the airflow and checking the flow rate. Parasitoids were introduced into the exposure chamber by disconnecting the extractor tube. Generally the parasitoids walked up the tube into the chamber. The extractor tube was reconnected to restore airflow.

At first parasitoids were tested individually but in later experiments groups of 4 parasitoid females were used but this had no effect on the results. The number of females tested in each trial was 4 unless stated otherwise.

To determine which, if any, odour field was preferred by the parasitoids, records were taken of which odour field the parasitoid was in every 30s for a total of 15 minutes. The initial choice made by the parasitoid as it entered the exposure chamber was also recorded. Thus a total of $15 \times 2 + 1 = 31$ observation points was recorded for each parasitoid in 1, 2, 3 or in all 4 odour fields, depending on the amount of movement of the parasitoid.

After each trial the parasitoids were removed by dismantling the exposure chamber. Absolute ethanol was used to swab out the exposure



Fig. 3.4. Photograph of the olfactometer with NH_4Cl 'smoke' being sucked through the system. Two concentrations of 'smoke' was used to show the four air fields clearly. A white deposit, seen in two of the air fields, was caused by the higher 'smoke' concentration in these air fields.

chamber between trials. After every 4 trials, or when the type of odour being tested was changed, the entire apparatus was cleaned, first in hot water and detergent, then in 95% ethanol. This successfully prevented contamination of the olfactometer by odours derived from the parasitoids or with volatile chemicals from the odour sources used. Such chemical contamination was a potential source for the introduction of a bias into the system. A lack of bias is clearly shown by the control experiments reported in the next section. Apart from these control experiments, other controls were carried out at regular intervals to ensure that no bias developed in the olfactometer during the course of an experiment.

3.4.2. Control experiments.

To test for bias in the olfactometer, control experiments were done without introducing test odours into the system. When presented with 4 blank 'odour' fields the parasitoids were expected not to show a preference for any particular field. A preference would indicate a bias in the system. In later experiments virgin and mated C. atratus females were tested separately to compare their behaviour. Virgin and mated females were consequently tested separately in the control experiments.

The results indicate that neither the virgin nor mated females displayed any significant bias towards any one of the four fields of airflow in the olfactometer (Table 3.1). This table provides an example of the information recorded during a typical experiment. In later experiments the results are presented graphically as percentage time spent in each odour field, but statistical analyses were performed on the raw data.

If movement of the wasp in the olfactometer was random, a score of 193.75 would be expected because it represents 25% of the total experimental time of 15 minutes. A score greater than 193.75 (or 25%) for an odour field, and which is significantly different statistically is interpreted as the parasitoids' preference for the odour source. Similarly, a lower score may be interpreted as a repulsion from the tested odour.

Table 3.1 : Control experiments for virgin and mated Coccophagus atratus females in the olfactometer using four 'blank' odour fields. Each point scored in an odour field represented time spent in that odour field.

<u>C.ATRATUS</u>		SCORE IN EACH ODOUR FIELD				
<u>FEMALES</u>	<u>N</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>P</u>
VIRGIN	25	187	174	209	205	P > 0.25
MATED	25	192	188	215	180	P > 0.25

3.4.3. Limitations to the use of the olfactometer.

There may be limitations in extending observations made on parasitoids in the olfactometer to parasitoid behaviour in the field. This is mainly a function of the artificial environment within the exposure chamber of the olfactometer. Firstly, odour concentrations are probably much higher than those encountered by parasitoids in the field and high odour concentration may even retard movement because the insect believes it is near the source (Farkas et al., 1974). If the females usually respond only to a limited range of odour concentration, which they would encounter at a particular distance from the odour source, then they may not respond to the odour concentration offered in the olfactometer.

Secondly, a problem may arise if a hierarchy of behavioural responses occurs. For example, at high odour concentrations the parasitoid may require a visual cue before normal searching behaviour is stimulated. In the tachinid Drino bohémica Mesnil, odour perception stimulated the parasitoid to orientate visually to movement (Monteith, 1956). Also, several insect species require combinations of volatile chemicals in order to find hosts (Roelofs & Carde, 1977; Tamaki, 1977).

Finally, in the field, C. atratus searches for hosts and responds to odours whilst flying. The exposure chamber of the olfactometer restricts the normal activity of the parasitoids, forcing them to walk, or, at the most jump, but prevents them from flying. Insects may not

respond to a stimulus in the same way when walking or when flying (Kennedy, 1977a). Because this situation is artificial, no attempt has been made to analyse the mechanism by which the parasitoids respond to odours (eg. anemotaxis or chemotaxis). Analysis of the mechanism of orientation is usually difficult in most types of olfactometer designs (Kennedy, 1977a; 1977b). The olfactometer experiments in this study were designed to identify odours to which C. atratus females respond, and which they are likely to use in host location. They were not intended to analyse how the females use the odour to locate hosts. A wind tunnel may provide a better means of determining how C. atratus uses olfactory cues to locate hosts. Problems may arise when designing wind tunnel experiments because C. atratus is an active flier, so the apparatus would have to be large and this would make observation of such a small insect difficult.

In this thesis, the terms attraction and repulsion are used with respect to the parasitoid's response to the odour, and does not imply an attraction to the source of the odour. This distinction is necessary because, as already stated, the mechanism by which the parasitoids orientate to the odour source was not determined.

At no time during the experiments did a C. atratus female walk down an arm of the olfactometer into the catching vial. This appeared to be because the stainless steel tube was dark inside, and as the parasitoids are strongly attracted to light, they were reluctant to enter the tube. This olfactometer may be more appropriate in behavioural observations on wasps that search in dark crevices for hosts like stemborers.

3.5. Statistical analyses.

The G-test was applied to test for goodness of fit (2x2 contingency tables and RxC test of independence) instead of the traditional chi-square test. The advantage of the G-test is that it is more robust and it has been recommended on theoretical grounds (Sokal & Rohlf, 1981). The G-test was routinely applied with Williams' correction (Williams, 1976) which results in a more conservative test. When the number of observations in any particular category was less than 5, Fischers' exact test was applied (Siegel, 1956; Sokal & Rohlf, 1981).

CHAPTER 4: HOST HABITAT LOCATION

The initial step performed by a parasitoid in search of a host is said to be location of the habitat in which the host lives (Salt, 1935; Flanders, 1953; Vinson, 1975; 1976; 1981). In spite of the suggested importance of this process, "...little is known about habitat location by parasitoids" and "...there has been relatively little research as to the stimuli involved" (Vinson, 1981).

It is generally acknowledged that the term habitat is difficult to define (Udvardy, 1959; Whittaker et al., 1973; Pimm & Lawton, 1980). Andrewartha & Birch (1954), after long discussion, decided that "a place in which to live" is the most appropriate description. Although it is often difficult to identify the habitat of the host, it may be simple in certain cases. For example, the habitat of carrion-feeding insects or pests of stored products is relatively easy to define. In contrast, the habitat of phytophagous insects may be difficult to define because phytophagous insects may be found in association with several plant species and with several vegetation types. There are only a few examples of parasitoids that locate their hosts' habitat when the hosts are not present. Alysia manducator Panzer (Braconidae) and Nasonia vitripennis Walker (Pteromalidae) were attracted to meat (Laing, 1937) but the result obtained for Nasonia is somewhat contentious (Jacobi, 1939; Wylie, 1958). Venturia canescens Gravenhorst (Ichneumonidae) which was first attracted to the hosts' food of oatmeal (Thorpe & Jones, 1937); Pseudeucoila bochei Wild (Cynipidae) locates the larval food of its drosophilid hosts in the absence of the host insects (van Lenteren & Bakker, 1978); and Biosteres (Opius) longicaudatus Ashmead which locates rotting fruit irrespective of the presence or absence of tephritid fruit fly larvae (Greany et al., 1977).

The habitat of the scale insects parasitised by C. atratus may be described as the plants on which they feed. Plants are important in influencing host-searching behaviour (Salt, 1935; Zwölfer & Kraus, 1957) and several parasitoids have been shown to respond to plant volatiles from their hosts' food plant (Thorpe & Caudle, 1938; Nishida, 1956; Arthur, 1962; Camors & Payne, 1972; 1973; Shahjahan & Streams, 1973; Elzen et al., 1983). Generally however there has been little work

done that helps explain the factors important in host habitat location (Vinson, 1981). The hosts of C. atratus are phytophagous so the plants on which the hosts feed may be identified as the hosts habitat by the parasitoids.

C. atratus has two types of hosts, parasitised and unparasitised scale insects, and both types are often located on the same plant, even next to each other. Virgin and mated females are therefore expected to respond to identical cues in their search for their hosts' habitat. Before identifying possible cues it is essential to know which host species are attacked by C. atratus, which plant species the scale insects feed upon, whether these plants occur in a single vegetation type or not and whether the distribution of C. atratus coincides strongly with any particular plant or vegetation type.

4.1. Hosts of C. atratus.

C. atratus has been recorded parasitising at least 22 species of scale insects and mealybugs belonging to 6 coccoid families (Table 4.1). These are all records from field-collected material. This distinction is necessary because parasitoids may attack host species presented to them in the laboratory but which they do not attack in the field (Salt, 1975; 1976). To determine whether any consistent pattern occurred as to the host plants inhabited by these scale insects, a list of their host plants was also compiled.

4.2. Plant species associated with C. atratus' host insects.

Plant species on which scale insects parasitised by C. atratus have been collected are listed in Table 4.2. Thirty-three plant species in 18 families are represented. It seems unlikely that C. atratus females could identify each of these plants individually so the plants may be expected to have some feature in common. There is no taxonomic similarity between the plant species listed in Table 4.2. Moericke et al. (1975) showed that plant colour, size and form are important cues used by apple maggot flies, Rhagoletis pomonella (Walsh), to locate their host plants. The plants listed in Table 4.2 differ widely in their height, size, shape and colour, which suggests that vision may not be useful in locating any particular plant species. Therefore

Table 4.1. List of Coccoidea parasitised by Coccophagous atratus. A:- Annecke & Insley, (1974); N:- S. Naser in litt. (1984); T:- This study.

FAMILY	SPECIES	SOURCE		
Cerococcidae	<u>Cerococcus</u> sp	A	N	
Coccidae	<u>Avricus</u> sp		N	
	<u>Ceroplastes</u> sp	A	N	
	<u>C. elytropapi</u> Brain	A		
	<u>Cerostegia rufa</u> (De Lotto)	A	N	
	<u>Cryptinglisia</u> sp		N	
	<u>C. elytropappi</u> (Brain)		N	
	<u>Coccus annecke</u> i De Lotto	A		
	<u>C. hesperidum</u> Linnaeus	A		
	<u>Filippia gemina</u> De Lotto		N	T*
	<u>Gascardia</u> sp	A	N	T
	<u>G. destructor</u> (Newstead)		N	T
	<u>G. rustica</u> (De lotto)	A	N	
	<u>G. tachardiaformis</u> (Brain)	A	N	
	<u>Saissetia coffeae</u> (Walker)		N	T
	<u>S. oleae</u> (Olivier)	A	N	
	<u>S. somereni</u> (Newstead)		N	T
Lacciferidae	<u>Tachardina</u> sp		N	
Lecanodiaspididae	<u>Lecanodiaspis</u> sp		N	
	<u>L. ?erica</u> Hodgson		N	
Pseudococcidae	Mealybug		N	
	(genus & species under study)			
	<u>Octococcus</u> sp		N	

* Of the scale insect species sampled during this study, Filippia gemina was the species most commonly parasitised and yielded both male and female C. atratus. Mostly males were obtained from the other scale insect species.

TABLE 4.2 : List of plant species on which host insects of Coccophagus atratus feed. A: Annecke & Insley (1974); N: S. Nesar (in litt., 1984); T: This study.

FAMILY	SPECIES	FYNBOS	TYPE OF PLANT	SOURCE
AIZOALEAE	Mesembryanthemum sp.		Herb	N A
ANCARDIACEAE	<u>Carissa bispinosa</u> (L.) Desf. ex Brenan		Shrub	N A
	<u>Ozoroa argentina</u> (Thunb.) Meisn		Shrub	N
	<u>Rhus</u> sp.		Tree	N
	<u>R. chirindensis</u> Bax. F. Forma Legatii (Schonl) R. and A. Fernandes		Tree	N
	<u>R. quenzii</u> / <u>viminialis</u>		Tree	N
	<u>R. schlechteri</u> Diels		Tree	N A
APOCYNACEAE	<u>Nerium oleander</u> L.	(exotic)	Shrub	N
COMPOSITAE	<u>Chrysanthamoides monilifera</u> (L.) T. Norl.	*	Shrub	N T
	<u>Elytropappus gnaphaloides</u> (L.) Levyns		Shrub	N
	<u>E. rhinocerotis</u> (L.F.) Less.		Shrub	N A
	<u>Metalasia gnaphaloides</u> (Thunb.) Druce	*	Shrub	N
	<u>Stoebe cinerea</u> Thunb.		Shrub	N A

CONTINUED

TABLE 4.2 CONTINUED

FAMILY	SPECIES	FYNBOS	SIZE OF PLANT	SOURCE
ERICACEAE	<u>Erica</u> sp	*	Herb	N
	<u>E. caffra</u> L.	*	Shrub	N
FABACEAE	<u>Acacia karroo</u> Hayne		Tree	T
	<u>A. mearnsii</u> De Wild		Tree	N
	<u>Aspalathus attenuata</u> Dahlg.	*	Herb	N A
LILIACEAE	<u>Protasparagus capensis</u> (L.) Oberm.	*	Herb	N A
	<u>P. racemosus</u> (Willd.) Oberm.		Herb	N A
MELIACEAE	<u>Trichilia emetica</u> Vahl		Tree	T
MORACEAE	<u>Ficus carica</u> L.		Tree	N
MYRTACEAE	<u>Psidium guajava</u> L.	(exotic)	Tree	N A
POACEAE	<u>Aristida junciformis</u> Trin. and Rupr.	*	Herb	N

CONTINUED

TABLE 4.2 CONTINUED

FAMILY	SPECIES	FYNBOS	SIZE OF PLANT	SOURCE
ROSACEA	<u>Cliffortia</u> sp.	*	Shrub	N A
	<u>C. strobilifera</u> Mutt.	*	Shrub	N A T
RUTACEAE	Citrus	(exotic)	Tree	N A
SANTALACEAE	<u>Thesium aggregatum</u> A.W. Hill	*	Herb	N
SAPOTACEAE	<u>Sideroxylon inerme</u> L.		Tree	N
SELAGINACEAE	<u>Selago corymbosa</u> L.	*	Herb	N A
SOLANACEAE	<u>Lycium</u> sp.		Shrub	N A
	<u>Solanum tomentosum</u> L.		Herb	N
THYMELAEACEAE	<u>Passerina vulgaris</u> Thodat	*	Shrub	N

odours seem the most likely source of cues that C. atratus females may respond to during location of their hosts' habitat. The responses of C. atratus females to odours from some of these plant species were determined in a later experiment.

4.3. Distribution of C. atratus.

Although C. atratus attacks many scale insect species (Table 4.1) on many plant species (Table 4.2), its principal insect host in Grahamstown is Filippia gemina, and both are most commonly found on Chrysanthemoides monolifera. Therefore the geographical distribution of C. atratus was plotted to determine whether it coincided with the distribution of its principal host F. gemina or with the distribution of the common food plant of this scale insect, C. monolifera. If there is any coincidence this may provide a clue as to how the parasitoid identifies its hosts' habitat. The geographical distribution of C. atratus was drawn up using information gathered from Annecke (1964), Annecke & Insley (1974), and from collections made during this study. The distribution of C. atratus is limited to the coastal regions of the western and eastern Cape Province (Fig. 4.1). Subba Rao & Rai (1969) reported finding C. atratus in India, but according to Annecke & Insley (1974) their material "...apparently bears no relation to C. atratus, and probably does not represent a species of Coccophagus."

The distribution of C. atratus is not linked with that of its host insect species. Species of Gascardia and Saissetia, for example, have a world-wide distribution. F. gemina, the species most commonly parasitised by C. atratus, has been found at St Lucia Lake in northern Natal (De Lotto, 1974) which is well outside this parasitoids' recorded distribution. In Fig. 4.1 the distribution of C. monolifera, a plant species commonly found with C. atratus-infested scale insects, is plotted, and shows that its range is far greater than that of C. atratus.

The recorded distribution of C. atratus overlaps, to a large extent, with the distribution of fynbos vegetation (Fig. 4.1). However only 12 plant species listed in Table 4.2, including C. monolifera form part of fynbos (Acocks, 1975). Fynbos is a heathland (Moll & Jarman, 1984) comprising mainly low-growing shrubs, and it is possible that C. atratus prefers searching in this type of open veld.

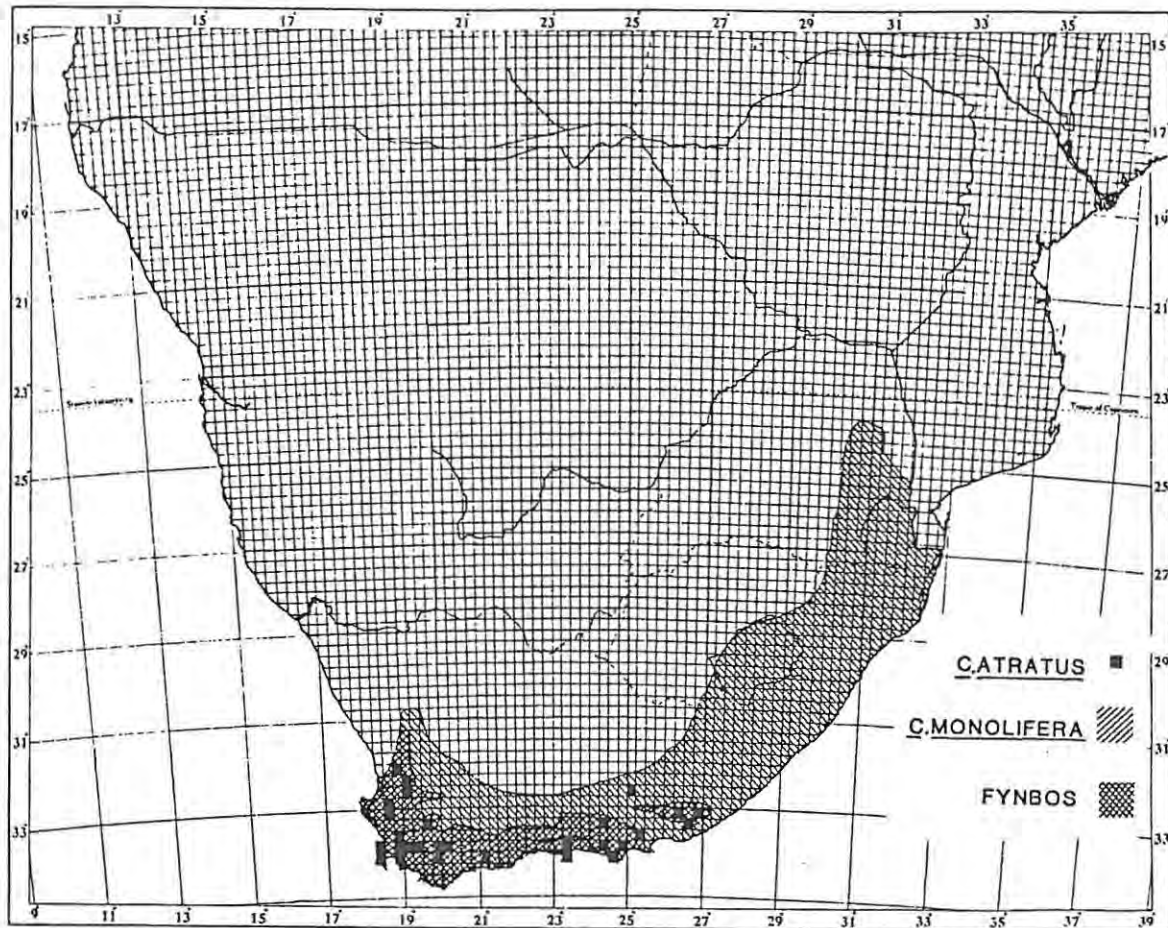


Fig. 4.1. Map of southern Africa showing the distribution of Coccophagous atratus parasitoids, Chrysanthemoides monolifera plants and fynbos vegetation.

Even if C. atratus does prefer searching for hosts in fynbos vegetation, this does not solve the problem of how the parasitoid locates its hosts' habitat. Fynbos vegetation covers an area of $4.14 \times 10^4 \text{ km}^2$ (Kruger, 1979) which is still a vast area for the parasitoids to cover in search of host insects.

The role of plants in the host habitat location behaviour of C. atratus is uncertain. Experimental evidence is required to determine how, if at all, C. atratus responds to plants. Therefore the responses of C. atratus females to plant odours was investigated to determine whether odours are used by the parasitoid as a means by which the host habitat can be located.

4.4. Role of plants in host habitat location by *C. atratus*.

Odour may provide a directional stimulus (Kennedy, 1977a) enabling a parasitoid to make oriented movements to its source (Shorey, 1977). To obtain an indication of the possible role of plant odours in the location of the hosts' habitat by *C. atratus*, females were exposed to odours from one of several plant species, in the olfactometer. Six plant species were chosen from Table 4.2. Three of them; *Chrysanthemoides monolifera*, *Trichilia emetica* Vahl and *Cliffortia strobilifera* Mutt., are species on which *C. atratus*-infested scale insects were regularly found. The other three; *Carpobrotus edulis* (L.), *Dodonaea viscosa* Jacq. and *Olea europaea* L. subsp. *africana* (Miller), are found within the geographical range of *C. atratus*, and regularly harbour suitable scale insects, but they are species from which *C. atratus* has never been found, despite the collection of numerous scale insect samples from them.

Fresh, unblemished leaves from these plant species were used as the odour source in the olfactometer. Leaves were checked for the presence of honeydew, insects or fungus before being used in experiments. To standardise odour concentration the leaf weight of the odour source used in each experiment was arbitrarily chosen as 1,4g (wet weight). Leaves from the plants were placed in the olfactometer to determine the parasitoids' response. The effect of odours from six plant species were tested individually on virgin females. Mated females were tested with only one plant species from each group to compare their behaviour with that of virgin females.

The results, presented in Fig. 4.2, show that virgin and mated females were attracted to odours from plant species on which *C. atratus*-parasitised scale insects have been found in the field. Therefore, plant odour may be used by both virgin and mated females to locate plant species on which to search for hosts. Females were repelled by odours produced by *C. edulis*, *D. viscosa* and *O. europaea*. Repulsion from these plants may explain why *C. atratus* does not attack suitable scale insect hosts present on these plants in the field. A female flying nearby one of the 'repellant' plants would not be attracted to the plant, if not repelled by it, and would not have an opportunity to locate hosts on the plant. Mated females tended to be slightly more

attracted to C. monolifera and more repelled by O. europaea odour than virgin females, but this difference is not statistically significant.

The results obtained in this chapter indicate that the prediction of the original hypothesis (Fig. 1.1), that C. atratus would locate all plant species fed on by their hosts, is incorrect and the hypothesis needs to be altered to fit the observations reported in Fig. 4.2.

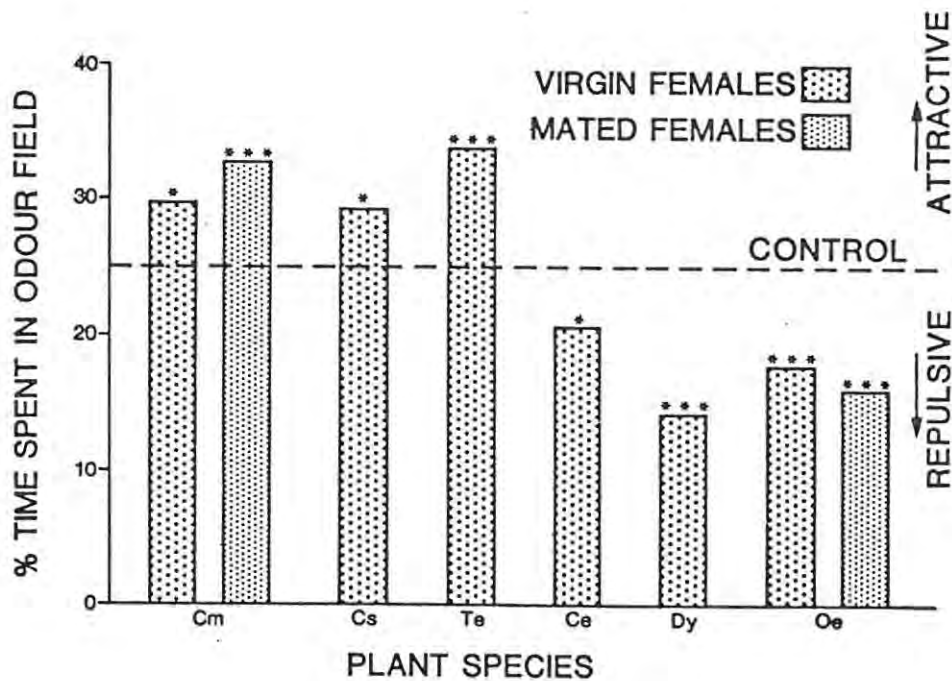


Fig. 4.2. Response of virgin and mated Coccophagous atratus females to odours from various plant species. N=25 in each case. (Cm: Chrysanthemoides monolifera; Cs: Cliffortia strobilifera; Te: Trichilia emetica; Ce: Carpobrotus edulis; Dy: Dodonaea viscosa; Oe: Olea europaea). Levels of significance are represented by asterisks on the top of each histogram bar; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

4.5. Hypothesis for host habitat location.

The role of the host habitat in host selection behaviour appears to be more complex than originally predicted (Fig. 1.1). In the original hypothesis, C. atratus females were first expected to search for the hosts' habitat before searching for hosts. This does not seem to be

correct (Fig. 4.3). Parasitoids search for host plant species upon which the host is often found. However, odour from some species of plants that harbour suitable hosts (including F. gemina) are repulsive to C. atratus females. Plants that attract female parasitoids are here called acceptable plants, whereas those that repel parasitoids are called unacceptable plants.

The observations reported in this chapter indicate that the parasitoid does not locate the entire habitat of the host species, but finds certain portions of it. The term host habitat location is therefore misleading, and it is more accurate to identify the habitat, for which the parasitoid initially searches, as the plants which it finds acceptable.

The effect of unacceptable plants on the behaviour of C. atratus in the field may seem to be similar to the effect, shown by Monteith (1960), of non-food plants masking the odours of host larvae and their food plants from the tachinid parasitoid Drino bohémica (Mesnil).

There are several examples of parasitoids that are affected by the plants on which their hosts feed. Table 4.3 provides examples of parasitoid species that prefer to attack a host on one plant species rather than on another plant species. Also, hosts on certain plant species may not be attacked at all. The results obtained for C. atratus may explain these observations. Parasitoids may be more attracted to certain plant species than others, and may also be repelled by some plant species. This effect may influence the range of host species parasitised and this is discussed in the following chapter.

Results presented in this chapter show that C. atratus parasitises a large number of host species, which, in turn, feed on many plant species. Both virgin and mated females are attracted to odours from acceptable plant species, as was expected, but they were repelled from odours of other plant species, which they seem to find unacceptable.

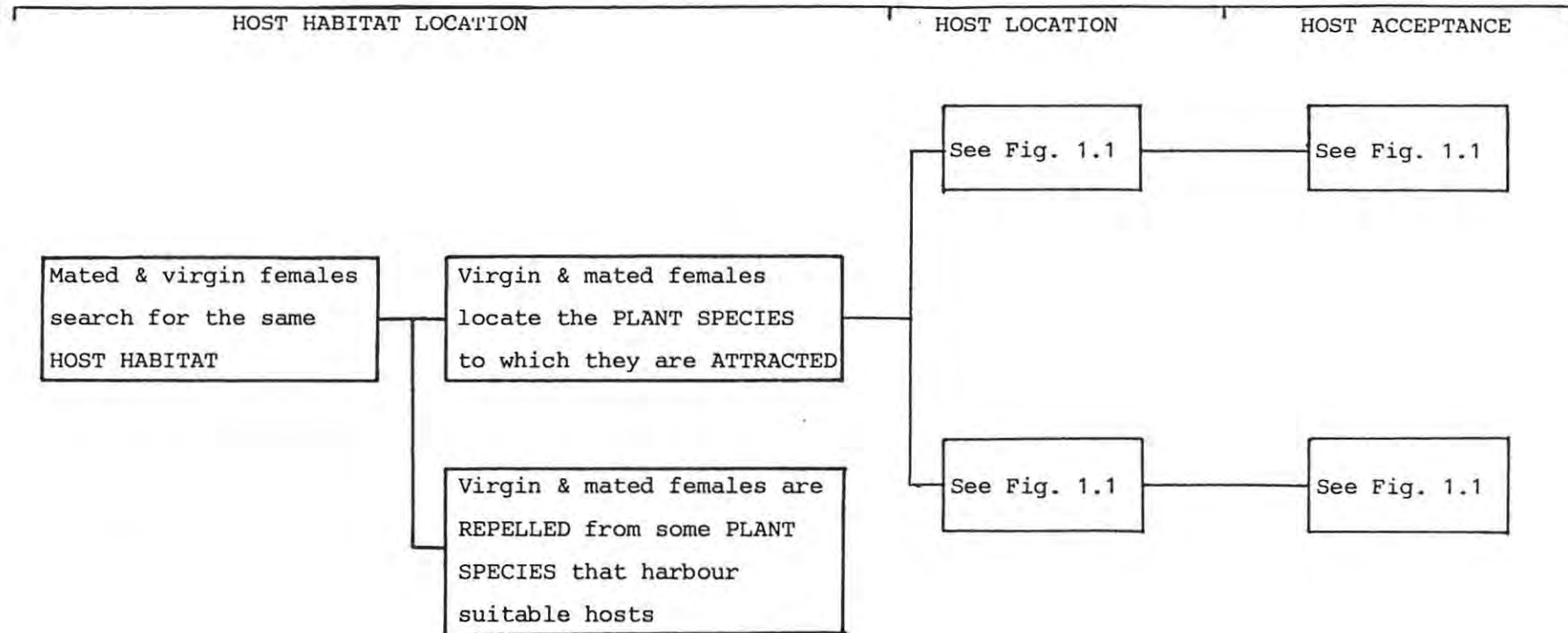


Fig. 4.3. Modified hypothesis to explain host-habitat location by Coccophagus atratus females. The hypothesis has been modified from Fig. 1.1. Host location and host acceptance behaviour remains as postulated in Fig. 1.1.

TABLE 4.3 : Examples of parasitoids that prefer attacking hosts on particular plant species.

< or > = greater or less preference to the particular plant species.

PARASITOID SPECIES	HOST SPECIES	PLANTS PREFERRED BY THE PARASITOID	PLANTS ON WHICH HOST IS NOT PARASITISED	AUTHORS
<u>Microbracon brevicornis</u> Wesm.	<u>Heliothis armigera</u> (Hubner)	<u>Antirrhinum</u> sp.	'Other plant species'	Taylor 1932
<u>Cardiochiles nigriceps</u> Viereck	<u>Heliothis</u> sp.	<u>Nicotiana tabacum</u> Linnaeus	<u>Arachis hypogaea</u> Linnaeus	Snow et al. 1966
<u>Apanteles glomeratus</u> (Linnaeus)	<u>Pieris brassilae</u> Linnaeus	<u>Brassica</u> sp.	<u>Cakile maritima</u> Scopoli <u>Capparis spinosa</u> Linnaeus	Salt 1958
<u>Leiphron pallipes</u> Curtis	<u>Lygus lineolaris</u> (Palisot de Beauvois)	<u>Erigeron</u> spp	-	Streams et al. 1968
<u>Peristenus pseudopallipes</u> (Loan)	<u>Lygus lineolaris</u> (Palisot de Beauvois)	<u>Erigeron</u> spp	-	Shahjahan 1974
<u>Diadromus pulchellus</u> Wesmeal	Microlepidoptera	Plants containing sulphur compounds	-	Lecomte & Thibout 1984

Table 4.3 CONTINUED

PARASITOID SPECIES	HOST SPECIES	PLANTS PREFERRED BY THE PARASITOID	PLANTS ON WHICH HOST IS NOT PARASITISED	AUTHORS
<u>Itoplectis conquisitor</u> (Say)	<u>Rhyaciona buoliana</u> (Schiff.)	<u>Pinus silvestris</u> > Linnaeus <u>P. resinosa</u> Aiton		Arthur 1962
<u>Ephialtes (Apechthis)</u> sp.	<u>Choristineura</u> sp.	<u>Quercus</u> sp.	<u>Abies</u> sp.	Zwolfer & Krous 1957
<u>Collyria calcitrator</u> Gravenhorst	<u>Cephus pygmaeus</u> Linnaeus	<u>Hordeum</u> sp. > <u>Triticum</u> sp.		Walker 1940
<u>Telenomus coelodasis</u> Ashmead	<u>Heterocampa</u> <u>guttivitta</u> (Walker)	<u>Fagus</u> sp. > <u>Acer saccharum</u> Marsh		Allen 1972
<u>Drino bohémica</u> Mesnil	Sawfly	<u>Pinus resinosa</u> Aiton > <u>P. strobus</u> Linnaeus > <u>P. sylvestris</u> Linnaeus > <u>P. banksiana</u> Lambert		Monteith 1955

Host habitat location is followed by host location (Fig. 1.1) How does C. atratus find its hosts? The host species listed in Table 4.1 are structurally diverse. There is little visual similarity between a soft scale insect, a waxy scale insect and a mealybug. The parasitoids may rely mainly on odour to locate their hosts, and this, too, was investigated with the aid of the olfactometer.

CHAPTER 5: HOST LOCATION

After a parasitoid has located a plant that potentially harbours suitable scale insects, it must then find the hosts (Fig. 1.1). Weseloh (1981) defined host location "...as the perception and orientation by parasitoids to their hosts, from a distance, by responses to stimuli produced or induced by the hosts or its products." The types of stimuli that C. atratus females are likely to receive from their scale insect hosts are olfactory and visual. Other parasitoid species respond to different stimuli, for example, substrate-borne vibrations (DeLeon, 1935; van den Assem & Keunen, 1958; Ryan & Rudinsky, 1962), sounds produced by the hosts (Cade, 1975; Soper, et al., 1976) and even infrared radiation is possibly used as a cue (Richerson & Borden, 1972), but none of these stimuli are likely to be produced by the sedentary scale insect hosts of C. atratus.

Sight may be important in the host-finding behaviour of C. atratus, but it is probably a close-range stimulus and will be discussed in the next chapter. Odour may be used, by orientating insects, as a long-range and/or a short-range cue (Vinson, 1976; 1981; Weseloh, 1981), and, host-derived odours have been shown to influence the host-finding behaviour of several parasitoid species in an olfactometer (Thorpe & Jones, 1937; Williams, 1951; Monteith, 1955; 1958; Starks & Schuster, 1974). Some parasitoids of scale insects, for example Aphytis melinus DeBach and A. coheni DeBach, appear to be attracted by the sex pheromones of their coccoid hosts (Sternlicht, 1973), but this must still be confirmed in laboratory experiments. Odour seems to be the most likely cue to which scale insect parasitoids would respond and it is used by other parasitoid species to locate their hosts.

The term kairomone, coined by Brown et al., (1970), is often used to describe odours by which parasitoids locate their hosts (for examples see Weseloh, 1981). However, there is some controversy as to the use of this term. Pasteels (1982) has argued against its use mainly because the definition implies that a kairomone is nonadaptive to the transmitter. If maladaptive it should, theoretically, be eliminated through natural selection. Pasteels' lead has been followed here and the term kairomone is not used. Host odours or host-derived odours are terms that are preferred.

C. atratus has two types of hosts; unparasitised scale insects in which mated females lay diploid eggs, and parasitoid pupae within scale insects, on which mated and virgin females lay haploid eggs. In the following experiments virgin and mated females were tested separately in the olfactometer. Each female was exposed to odour from either unparasitised scale insects or from parasitised scale insects. In a further experiment, females were presented with a choice of the two odours.

The host species used as the odour source in these experiments was F. gemina because it was found to be the commonest host of C. atratus in the field. The scale insects were reared on C. monolifera plants. C. atratus specimens used in all experiments had been reared on F. gemina, thus preventing problems with conditioned responses that might occur if another plant species had been used.

5.1. Response of C. atratus to odours from unparasitised scale insects.

Although only mated C. atratus females can deposit eggs in unparasitised scale insects, both virgin and mated C. atratus females were tested, to compare their behaviour. Virgin females were not expected to respond to odours from unparasitised scale insects (Fig. 1.1).

The response of C. atratus females to three odours, presented independently, were tested in the olfactometer.

- (1) Unparasitised F. gemina scale insects, in which only female C. atratus larvae can develop, were used in initial tests.
- (2) Unparasitised F. gemina were left on a C. monolifera leaf to determine if there was an increased response by the females to a combination of leaf and host odour.
- (3) Honeydew from F. gemina was tested to determine whether female wasps respond to honeydew or to the host itself. A response to honeydew would indicate that the stimulus would not be a sex pheromone.

Honeydew was obtained from F. gemina scale insects feeding on C. monolifera plants. The honeydew was collected in a glass petri-dish held close to the infested leaf by a retort stand (Fig. 5.1). The scale insects propelled the honeydew droplets away from themselves, and

these droplets stuck to the petri-dish. When sufficient honeydew had been collected, the petri-dish was removed and weighed. Then 2 ml of distilled water was poured into the petri-dish to dissolve the honeydew. The solution was poured into a container and the petri-dish was weighed again to calculate the weight of the honeydew in solution. The concentration of honeydew was then determined.



Fig. 5.1. Method of honeydew collection. The bottom half of a glass petri-dish was held close to a Filippia gemina-infested Chrysanthemoides monolifera plant to catch honeydew falling from the plant.

Odour concentration was standardised by using 15 hosts in each experiment. The concentration of honeydew in distilled water was 7.8g per litre.

Unmated females were strongly attracted to all three odours (Fig. 5.2), and the level of response in each case was found to be significantly greater than values obtained in control experiments. Controls run at the same time as the experiments showed no bias in the responses of the females to the apparatus (Chapter 3). Mated females

were tested only on scale insect odour and not in combination with leaves, as their behaviour is not expected to differ from that of virgin females.

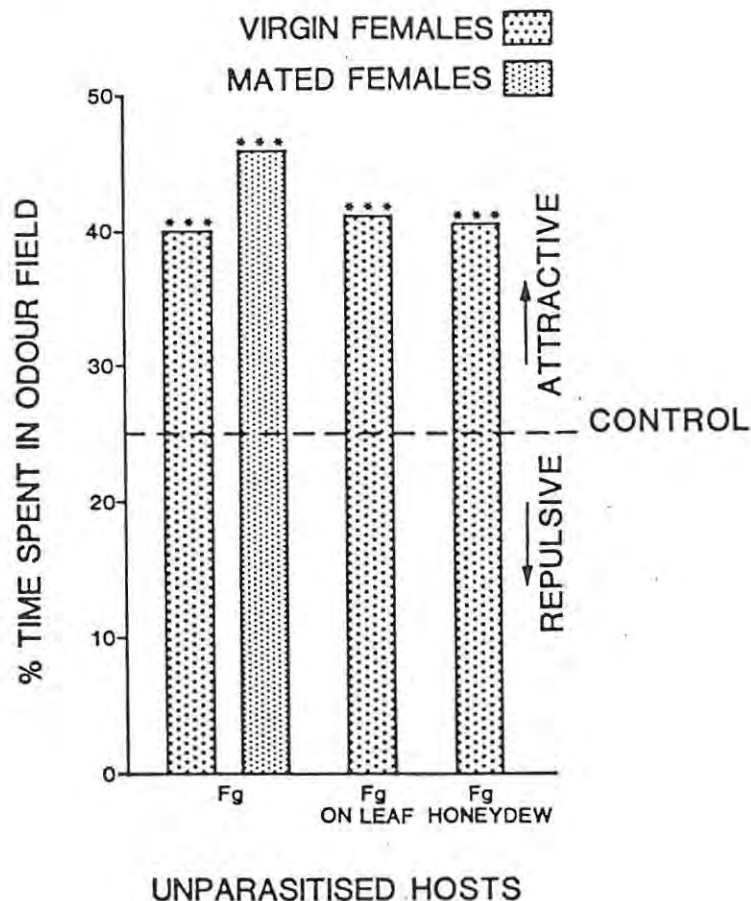


Fig. 5.2. Response of virgin and mated *Coccophagus atratus* females to odours from unparasitised *Filippia gemina* (Fg) scale insects which are suitable only for the development of female eggs. N=25 in each case. Levels of significance are represented by asterisks on the top of each histogram bar; ***: $p < 0.001$.

The response of virgin females to odour from unparasitised scale insects was not expected because they cannot oviposit in unparasitised scale insects. The combined odour of hosts plus *C. monolifera* leaves did enhance the attraction of *C. atratus* to the host scale insects. In addition, honeydew alone elicited as strong a response as the other test odours (Fig. 5.2). Honeydew is possibly the common denominator in all treatments, because the scale insects could have been producing honeydew during the experiments. Therefore honeydew may be the main

cue attracting females to unparasitised hosts. Additional tests would have to be conducted to establish whether honeydew could be the principal attractant.

Quednau & Hübsch (1964) and Vinson *et al.* (1978) showed that honeydew was important in host location by *Aphytis* and *Metaphycus* species, although their experiments allowed the parasitoid to contact the honeydew. They did not fully determine the response of the parasitoids to honeydew odour as a long-range stimulus.

The response obtained from virgin females was unexpected. What is their response to odours from parasitised scale insects?

5.2. Response to odours from parasitised scale insects.

Virgin and mated *C. atratus* females were tested in the olfactometer with odours from parasitised *F. gemina* scale insects that contained mature larvae or prepupae of *C. atratus* females. As both virgin and mated females can oviposit in these hosts, their behaviour was expected to be the same. Virgin females were also tested with odour from parasitised *F. gemina* scale insects still feeding on a *C. monilifera* leaf to observe whether the combined odours enhanced any response to the parasitised scale insects. The response to honeydew was tested in the previous section, and mummified scale insects do not produce honeydew anyway.

Virgin and mated females were both found to be significantly attracted to parasitised hosts (Fig 5.3). The combined odour of parasitised scale insects and a leaf did not influence the strength with which the females were attracted. A comparison of the results presented in Fig. 5.2 with those depicted in Fig. 5.3 shows that the attraction of both virgin and mated females to odour from unparasitised scale insects was much greater than to parasitised scale insects ($G = 8,41$; $P < 0.01$).

In the field, females will probably be exposed simultaneously to odours from both parasitised and unparasitised hosts. It is thus artificial or unnatural to provide the females with odour from one host type only and this may account for the unexpected attraction of virgin females by unparasitised scale insects. In other words they may have responded to scale insect odour only because "clean" air was less acceptable to

them. Therefore virgin and mated females were provided with a simultaneous choice, in the olfactometer, between odours from parasitised and from unparasitised scale insects.

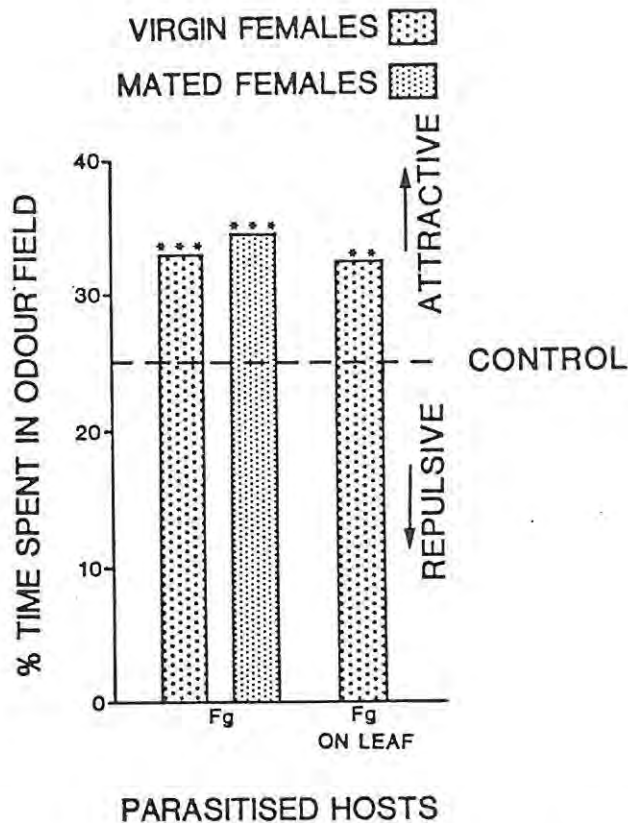


Fig. 5.3. Response of virgin and mated *Coccophagous atratus* females to odours from parasitised *Filippia gemina* (Fg) scale insects which are suitable for the development of male eggs. N=25 in each case. Levels of significance are represented by asterisks on the top of each histogram bar; **: $P < 0.01$; ***: $P < 0.001$.

5.3. Response to a choice of odours.

Virgin and mated females were tested separately in the olfactometer so that their behaviour could be compared. Females were provided with a simultaneous choice between parasitised and unparasitised scale insects, which were placed into adjacent arms of the olfactometer. A direct comparison of the two odours by the wasps was therefore possible.

Even when provided with a choice, virgin females were more attracted to unparasitised scale insects (Table 5.1) and not, as expected, to parasitised hosts. In fact their response to parasitised hosts did not differ significantly from their response to the two blank odour fields, indicating that hosts for males were ignored under these experimental circumstances.

TABLE 5.1 : Response of virgin Coccophagus atratus females when provided with a choice between odours of parasitised and unparasitised Filippia gemina in an olfactometer (N = 25).

VIRGIN <u>C. ATRATUS</u> FEMALES	ODOUR FIELDS			
	<u>FILIPPIA GEMINA</u> UNPARASITISED	<u>FILIPPIA GEMINA</u> PARASITISED	CONTROL BLANK	CONTROL BLANK
SCORE FOR EACH ODOUR FIELD	279	159	160	177
PERCENTAGE TIME SPENT IN EACH ODOUR FIELD	36	20	21	23

A similar result was obtained with mated females (Table 5.2). It therefore appears that unparasitised scale insects produce some volatile chemical to which both mated and virgin females respond preferentially. Honeydew appears to be the source of this volatile chemical.

Many chemicals present in honeydew derive from the host plant sap (Srivastava & Varshney, 1966; Sidhu & Patton, 1970). Does the chemical to which C. atratus is attracted derive from the plant or is it

metabolised within the host? To answer this question, experiments were designed to test whether different plants influenced the attractiveness of F. gemina honeydew to C. atratus females.

TABLE 5.2 : Response of mated Coccophagus atratus females when provided with a choice between odours of parasitised and unparasitised Filippia gemina in an olfactometer (N = 25).

MATED <u>C. ATRATUS</u> FEMALES	ODOUR FIELDS			
	<u>FILIPPIA GEMINA</u>		CONTROL	CONTROL
	UNPARASITISED	PARASITISED	BLANK	BLANK
SCORE FOR EACH ODOUR FIELD	264	187	169	155
PERCENTAGE TIME SPENT IN EACH ODOUR FIELD	34	24	22	20

5.4. Influence of plants on the attractiveness of honeydew odour.

C. atratus never seems to parasitise Filippia gemina scale insects that feed on certain species of plants (Chapter 4). Ten to fifteen collections of scale insects were made from C. monolifera, C. strobilifera and O. europaea during the period of this study. Scale insects collected from O. europaea never yielded C. atratus parasitoids, whereas those collected on Chrysanthemoides monolifera and Cliffortia strobilifera were parasitised by C. atratus. Honeydew from F. gemina is attractive to the C. atratus females so why is F.gemina not parasitised on all three plant species?

In accordance with these field observations, C. monolifera and C. strobilifera were found to be attractive to C. atratus females (Fig. 4.2), but O. europaea was repellant (Fig. 4.3). The repellant nature of O. europaea odour in the field may possibly mask the honeydew odour, and effectively exclude C. atratus from this plant. This effect would be similar to the masking effect that odour from neighbouring non-host

plants may have on odours from otherwise attractive plants (Monteith, 1960). Alternatively, the volatile chemical present in the plant sap may be taken up by the scale insect, excreted in its honeydew, and thus render it repellent. Both possibilities may operate simultaneously but the latter, if correct, would minimise the importance of the former.

The effect of honeydew from F. gemina reared on C. monolifera has already been determined (Fig. 5.2). F. gemina ovisacs were collected from the culture on C. monolifera and were placed on O. europaea plants. When a colony had established, honeydew was collected using the same methods described earlier.

From the results of this test (Fig. 5.4) it is clear that females are repelled by honeydew from F. gemina scale insects feeding on O. europaea. For comparative purposes, the response of C. atratus to honeydew from F. gemina feeding on C. monolifera are also included. The volatile chemical repellent in the plant therefore seems to be taken up by the scale insects and excreted in the honeydew.

If the volatile chemical attracting the parasitoids is derived from the plant and not from the host insect, then honeydew excreted by any insect species feeding on the sap of a plant that produces these chemicals should be attractive to C. atratus females. An insect chosen to test this theory was whitefly nymphs (unidentified), which are commonly found on C. monolifera in Grahamstown. They were reared in the laboratory under similar conditions to the scale insect culture. Honeydew was collected as it fell from the insects.

C. atratus females were not attracted to whitefly honeydew and neither were they repelled by it. The volatile chemical attractant in the food plant may be taken up by F. gemina in the plant sap and metabolised before being excreted. If whiteflies do not metabolise the plant sap in the same way as F. gemina, this might explain why whitefly honeydew was neutral to C. atratus.

The parasitoid Diaeretiella rapae M'Intosh (Braconidae) has been shown by Read et al., (1970) to prefer aphids on collards to aphids on beet leaves. They also showed that aphids removed from collards and kept for

24h lost their attractiveness when tested in an olfactometer. Read et al., (1970) did not test aphid honeydew. If their results are interpreted in the light of the results obtained here, it would seem that the aphids gained their attractiveness from a chemical taken up from the plant sap and which was lost during the following 24h when the aphids had excreted all their honeydew.

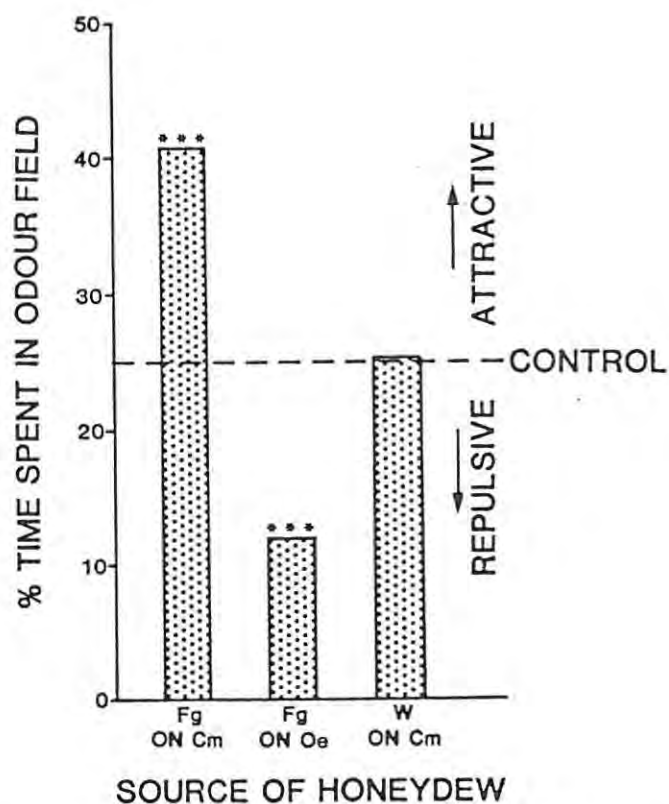


Fig. 5.4. Response of virgin Coccophagus atratus females to honeydew odour. The honeydew was obtained from Filippia gemina (Fg) reared on Chrysanthemoides monolifera (Cm) and on Oleae europaea (Oe), and from whiteflies (W) reared on C. monolifera (Cm). N=25 in each case. Levels of significance are represented by asterisks on the top of each histogram bar; ***: $P < 0.001$.

Inayatullah (1983) showed that the attractiveness of frass, produced by certain lepidopterous borer species, to the parasitoid Apanteles flavipes (Cameron) (Braconidae) varied, depending on which plant species the host had eaten. Frass from different host species feeding on the same plant species also differed in attractiveness to A.

flavipes. These results indicate that both the host species and its food plant species influence host location. However Inayatullah's (1983) use of the word attractiveness is misleading because what was actually measured was the amount of 'interest' displayed by the parasitoid (tapped frass with antennae or probed frass) when in CONTACT with the frass. An attractant is a chemical to which an insect can orientate in order to locate the source of the odour (Kennedy, 1978).

Lewis & Jones (1971) demonstrated that a host-seeking response was elicited in Microplitis croceipes (Cresson) (Braconidae) females by a chemical present in the frass, saliva, haemolymph and in the cuticle of its noctuid host Heliothis zea (Boddie). The chemical present in the frass, which elicits a response in the parasitoid, appears to be derived from the plant species on which the host feeds (Sauls et al., 1979; Nordlund & Sauls, 1981).

These examples given above all illustrate interactions between three trophic levels (Price et al., 1980). Properties of the plant affect the behaviour of the parasitoids via their herbivorous hosts. This will be dealt with in more detail in the general discussion.

Thus far, there appears to be no difference in the behaviour of virgin and mated females when presented with odours of parasitised and unparasitised scale insects. Observations of the females in the exposure chamber of the olfactometer did, however, indicated that mated females were more active than virgin females, and this was checked more thoroughly in the following section.

5.5. Activity index of virgin and mated females.

The activity of females in the olfactometer was examined to check whether mated females were more active than virgin females. To test this, an activity index was calculated for females exposed to different odours.

$$\text{Activity index} = \frac{\text{number of moves across odour fields}}{\text{number of parasitoids}}$$

Although the olfactometer is not specifically designed to measure activity, this equation does provide a means by which relative activities can be compared.

The activity indices of virgin and mated females are presented in Fig. 5.5. Mated females were found to be more active than virgin females in all cases examined ($G=12,92$; $P<0,05$; RxC test of independence). Virgin females probably produce a sex pheromone (Chapter 2). Excessive activity by virgin females is likely to disrupt the pheromone plume, and confuse attempts of males to locate females. This may explain why virgin females move less than mated females.

Females (virgin and mated) were more active in C. monolifera odour than in the controls (Fig. 5.5). C. monolifera odour also elicited greater activity than did scale insect odour (parasitised and unparasitised) ($P<0,001$) (Fig. 5.5). The leaves of this 'acceptable' plant may stimulate the females to search for hosts in the vicinity of the plant hence the increased activity levels observed in the olfactometer. Alternatively, odour from the hosts may cause activity to be concentrated in the region of the scale insects, and this might not be revealed in the methods used to measure activity here. The latter suggestion, is probably less likely because if females are active in one odour field, they may be expected to leave and return to that odour field often, because the boundary of the odour field would not be detected until it had been crossed.

Females activity was similar in odour from parasitised F. gemina and in the controls ($P<0,05$). Odour from unparasitised F. gemina scale insects did affect the activity levels of C. atratus females when compared with the activity in the control. Virgin females responded differently to mated females. Virgins were more active but mated females were inhibited ($P<0,001$) (Fig. 5.5). Mated females possibly concentrate their search effort within the F. gemina odour field while virgin females, because they cannot oviposit in unparasitised F. gemina, search the surrounding areas for hosts suitable for male eggs.

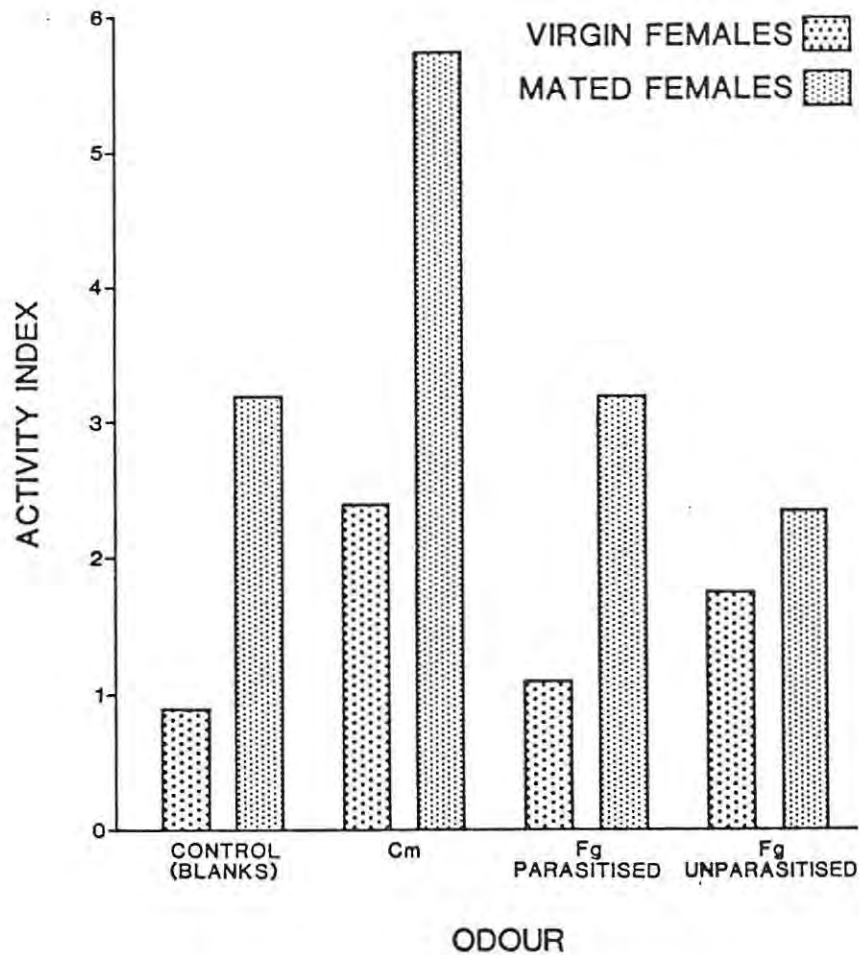


Fig. 5.5. Activity indices of virgin and mated *Coccophagus atratus* females when exposed to different odours. (Cm: *Chrysanthemoides monolifera*; Fg: *Filippia gemina*). Activity was measured in the olfactometer. N=25 in each case.

5.6. Modified hypothesis for host location.

The results presented in this chapter demonstrate that the section of the original hypothesis (Fig. 1.1) dealing with host location behaviour is incorrect and should be modified.

Originally it was believed that virgin females would be attracted only by hosts suitable for the oviposition of male eggs. Mated females were expected to be attracted by both types of hosts. However, results obtained have shown that both virgin and mated females are attracted to hosts suitable for female offspring (unparasitised scale insects). Presumably hosts for males (parasitised scale insects) may be found in the same area, even on the same leaf, as unparasitised scale insects. Once virgin females have been attracted to unparasitised scale insects,

they may then be stimulated to search randomly in that particular area for parasitised scale insects. This may be similar to Nasonia vitripennis which is attracted to carrion but locates its host puparia by searching the carrion randomly (Wylie, 1958). The results obtained in this chapter also indicate that the plant species upon which the host insect feeds may influence the attractiveness of the host to the parasitoid. The modified version of the host-searching hypothesis is presented in Fig. 5.6.

The interpretation of host-searching behaviour presented in this chapter is based only on results obtained from olfactometer experiments. To test the validity of these conclusions, observations on C. atratus in their normal habitat were undertaken and the results are presented in the following chapter.

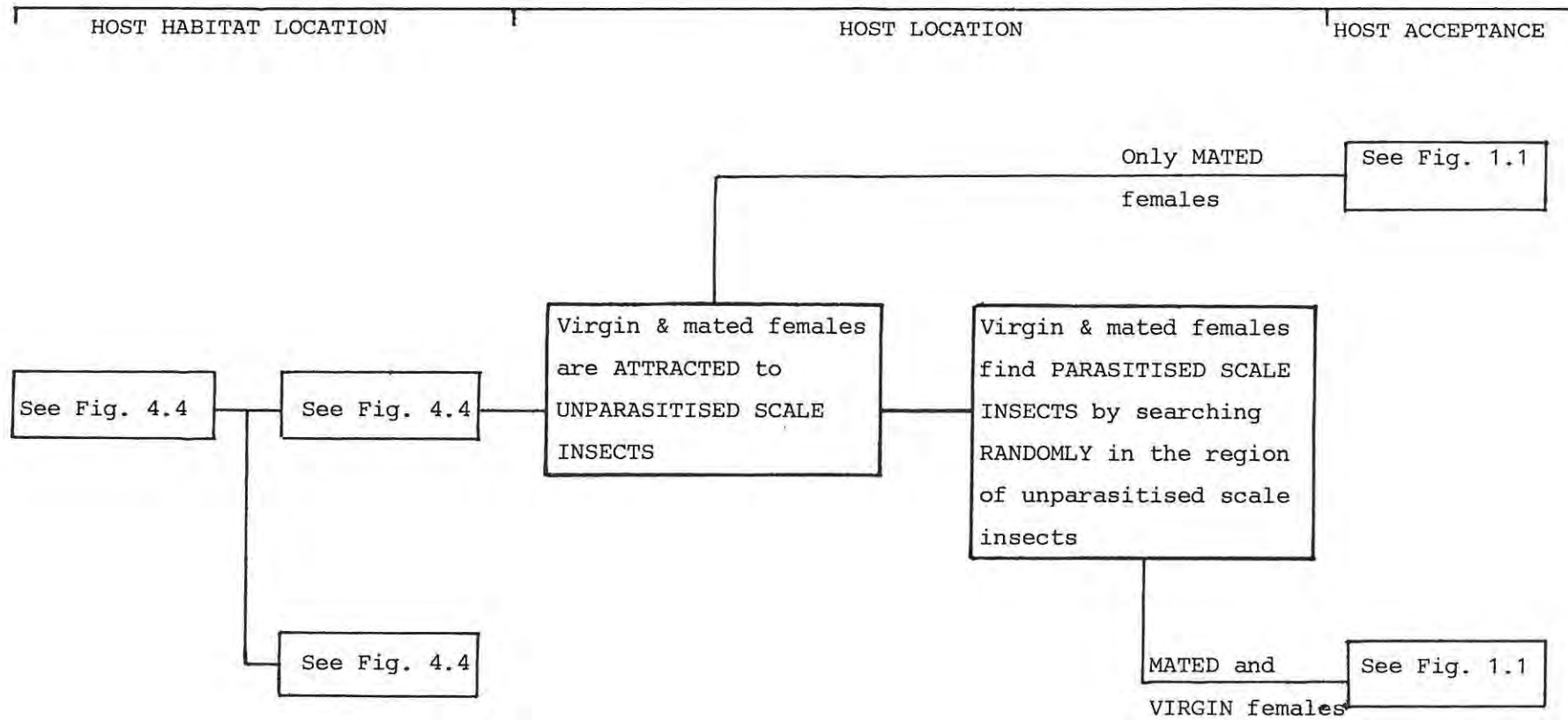


Fig. 5.6 Modified hypothesis to explain host-location by *Coccophagus atratus* females. The hypothesis has been modified from that proposed in Fig. 1.1. Host- habitat location and host acceptance remains as postulated in Figs 4.4 and 1.1 respectively.

CHAPTER 6: BEHAVIOUR OF C. ATRATUS IN THE FIELD

The results of laboratory experiments may be misleading because the conditions to which the parasitoids were exposed are artificial and probably do not resemble natural conditions at all. For example, the olfactometer used in chapters 4 and 5 prevented the parasitoids from flying and forced them to walk. The possibility exists, therefore, that parasitoids may respond differently to a stimulus when walking and when flying (Kennedy, 1977b).

To establish how realistic the laboratory results are, C. atratus females were observed in their natural habitat. In this way field observations act as a control for laboratory experiments.

There are two principal conclusions drawn from laboratory experiments that need to be verified in the field. Firstly, C. atratus may be attracted by odours from certain plant species and are therefore expected to be found searching only these plants whether they were infested with scale insects or not (Chapter 4). Secondly, it needs to be determined whether both virgin and mated females initially seek unparasitised scale insects before trying to locate parasitised scale insects (Chapter 5).

6.1. Effect of plants on searching behaviour in the field.

The plant C. monolifera is common around Grahamstown and has been identified as a host habitat of C. atratus parasitoids. Infestations of F. gemina on C. monolifera are usually small and isolated. Consequently there are many plants that are not infested with F. gemina. If the hypothesis, that females first locate the plant on which hosts might be present and only then search for hosts, is correct, then C. atratus females should be found on C. monolifera plants that are free of scales, as well as on C. monolifera plants that have scale insect infestations.

Preliminary observations of C. atratus parasitoids on F. gemina-infested plants indicated that the best observation periods were in the late morning and early afternoon on warm, windless days. Chalcidoidea

generally appear to be unaffected by wind (Juillet, 1960), but C. atratus is very small and difficult to observe on windy days because they cannot be seen on the moving foliage of the plants.

Scale insect-free C. monolifera plants were observed for an hour a day on 11 days when suitable conditions prevailed. Parasitoids had been seen on those same days on scale insect-infested plants within 50m of the observation site, so they were known to be in the area. C. atratus parasitoids were never observed on scale insect-free plants. Other parasitoids, for example braconids and ichneumonids, were, however, observed searching the plants.

C. atratus females were found on scale insect-infested plants. There may possibly be an accumulation of parasitoids on scale insect-infested plants because they had already searched those plants without hosts. However, at least some individuals would have been expected to be on host-free plants. To examine the conclusion that C. atratus parasitoids are not attracted by the plant alone, plants with infested and uninfested regions were examined to observe and compare the behaviour of parasitoids in the two areas. An even distribution of parasitoids around the plants was expected if the females were attracted to the plants themselves. Plants with small F. gemina infestations were chosen so that a large section of the plant was free of scale insects. The number of parasitoid females on scale insect-free areas was compared with the number on scale insect-infested areas. Once observed, each parasitoid was caught in a glass vial and taken back to the laboratory to have its identity confirmed.

The results presented in Table 6.1 show that the majority of parasitoids were found on parts of the plant that contained scale insects ($P < 0,001$). However this interpretation remains somewhat ambiguous because females already on the scale insect-infested parts may first have searched the scale insect-free areas. To test whether parasitoids are attracted to the plants before attempting to locate hosts, parasitoids were observed flying and landing on specific plants or parts of plants. The small size of the parasitoids (about 2mm in length) made such observations difficult, but a total of 16 observations are recorded in Table 6.1. Only parasitoids that were seen approaching the plant from about one metre away were recorded so that

their flight pattern could be observed. Two categories of flight were observed. Firstly, a directed flight towards the region of the plant that was infested with scale insects, and secondly a zig-zag flight that usually occurred when the parasitoid was within 5cm of the scale insect-infested leaves. The zig-zag flight always occurred prior to parasitoids landing near hosts. Parasitoids were seldom seen flying around scale insect-free areas of the plant, and when they were seen in these areas they exhibited the directed flight pattern. Only 4 parasitoids were observed landing on the uninfested areas.

TABLE 6.1 : Results of observations of Coccophagus atratus females on Chrysanthamoides monolifera plants in the field.

NO. <u>C. ATRATUS</u> FEMALES	<u>C. MONOLIFERA</u> PLANTS		
	<u>F. GEMINA</u>		<u>F. GEMINA</u>
	PRESENT		ABSENT
	INFESTED LEAVES	UNINFESTED LEAVES	
NO. OBSERVED			
LANDING	12	4	0
NO. PRESENT	56	13	0
TOTAL	68	17	0
NO. OBSERVATION HOURS	14	14	11

At close range (less than 5mm) it is probably difficult for small parasitoids to orientate to an odour source; the odour plume would be scattered by wind turbulence caused by the plant. Vision may therefore be important in close-range searching. The parasitoids appear to see quite accurately up to about 10cm because several were seen jumping from one leaf to another over this distance. Adult simuliids, which are

only slightly larger than adult C. atratus, are apparently able to distinguish between targets at distances as great as 180cm from them, on the basis of colour and in the absence of odour (Bradbury & Bennet, 1974).

From results obtained in the field it seems that the parasitoids search directly for the hosts. They are probably attracted by means of plant-derived chemicals, which are taken up by the host insect and modified by them before being excreted in the honeydew (Chapter 5). The parasitoids do not first locate the hosts' habitat. Again the original hypothesis (Fig. 1.1) has to be modified and this is done at the end of the chapter.

Parasitoids found on the scale insect-free parts of the plant were usually inactive and did not search for hosts. The reason for this inactivity was investigated. Firstly, Donaldson (1984) has shown that C. atratus females are inactive after they have laid their batch of 6 to 9 eggs each day, which, in the laboratory, took less than 30 minutes. Secondly, the females may be virgins, which are less active than mated females (Chapter 5) and may be producing a sex pheromone to attract males. To determine which of these alternatives is the more likely, or whether they act together, female parasitoids that were found on infested and uninfested parts of the plant were dissected to check the condition of their ovaries and to determine whether they had mated or not.

6.2. Effect of reproductive status on host-searching behaviour.

To determine whether females caught in the field had been mated or not, and to count the number of eggs in their ovaries, they had to be dissected. Only females whose immediate history was definitely known were dissected. For example, records were kept of whether they were found on infested or uninfested parts of the plant, and if on infested parts, whether they were on or near parasitised or unparasitised scale insects.

Female parasitoids caught in the field were kept separately in glass vials and taken immediately to the laboratory to be dissected. The dissection techniques were first practised on laboratory-reared females that had been observed mating. Dissection was performed in a drop of

saline on a watch-glass. Firstly, the female's abdomen was cut open and the ovaries and accessory glands removed. The number of mature eggs in the ovarioles were then counted to provide an indication of the female's physiological condition, and particularly to assess whether she was in a state to oviposit. A female with no mature eggs, for example, would not be expected to search for hosts as no eggs could be oviposited. The spermatheca was removed from the rest of the reproductive system. It could easily be identified by its light yellow colour; all other reproductive organs were white. The spermatheca was transferred to a drop of haematoxylin stain on a microscope slide. After 3 minutes, excess stain was removed, a drop of Canada Balsam added, and a coverslip placed over the the spermatheca. The presence of sperm was then sought under an oil emersion objective, and, if present, could be clearly seen (Fig. 6.1).



Fig. 6.1. Photograph of the spermatheca from a mated Coccophagus atratus female. The sperm can be clearly seen inside the spermatheca (X 1850).

All females that were caught appeared to be in the correct reproductive state to search for hosts and to oviposit because they had an average of 12.3 eggs (range, 3-18) in their ovaries. Most mated females were found on unparasitised scale insects ($P < 0.001$) (Table 6.2), but

similar numbers of virgin females were found on unparasitised and parasitised scale insects. Most of the females caught on the uninfested areas of the plant had been mated, so both reasons predicted earlier, explaining their presence on uninfested areas of the plant, do not hold. In a further attempt to establish the behaviour of females on scale insect-free C. monilifera plants, 25 mated females which were at least 48 hours old and therefore in a state ready to oviposit, were released singly onto a plant. The females were placed onto the plant by allowing them to walk from the holding vial up a glass tube, and onto a leaf.

TABLE 6.2 : Reproductive status of Coccophagus atratus females caught on parasitised Filippia gemina and unparasitised F. gemina infesting Chrysanthamoides monilifera plants and the number of C. atratus females caught on scale insect-free regions of C. monilifera plants.

NO. OF <u>C. ATRATUS</u> FEMALES	CONDITION OF <u>C. ATRATUS</u> FEMALES		
	VIRGIN	MATED	TOTAL
ON PARASITISED SCALE INSECTS	6	8	14
ON UNPARASITISED SCALE INSECTS	6	28	34
ON UNINFESTED PARTS OF <u>C. MONILIFERA</u> PLANTS	4	8	12
TOTAL	16	44	60

Of the 25 females released, 8 flew away immediately. The remaining females remained on the plant until they were chased by ants (11 females), or flew away for no apparent reason after preening themselves

(6 females). When on the plant, none of the females exhibited any searching behaviour. Most of the females moved very little and did not depart from the leaf on which they were initially placed. Their behaviour differed greatly from females on scale-infested plants. The latter moved around continually in search of hosts. The mated females placed on the uninfested plants were in the correct reproductive condition to search for hosts, so their inactivity may be due to some other reason(s). The females could possibly perceive, by the absence of host-derived odours, that there were no hosts present. This is further evidence that plants do not play a direct role in the parasitoids' host-searching behaviour. The hypothesis for host-searching behaviour must be further modified to account for the results obtained in this section.

6.3. Modified hypothesis for host location.

The original hypothesis on host-searching behaviour (Fig. 1.1) predicted that C. atratus females search initially for their hosts' habitat before searching for hosts. This hypothesis was modified (Fig. 4.4) to describe the hosts' habitat, for which the females search, as plant species that are acceptable to the females. The hypothesis also predicted that females would locate 'acceptable' plant species even when hosts were not present. Results obtained in this chapter show that C. atratus females, do not, in fact, search for the host insects' food plant in the field. Plants appear to have an indirect effect on host-searching behaviour by affecting the attractiveness of the host insects' honeydew (Chapter 5). A modified hypothesis for host location, incorporating these results, is presented in Fig. 6.2.

Theoretically, it is more advantageous for the parasitoids to search directly for their hosts rather than to waste time searching plants that have no hosts on them. This behaviour can be compared with that found in Cardiochiles nigriceps Viereck (Ichneumonidae) which does search host-free plants (Vinson, 1975). However the distribution of C. nigriceps' hosts is probably not as clumped as that of the scale insects of this study, there being only a few per plant. Therefore, it might be more advantageous for C. nigriceps to search randomly for hosts, while C. atratus would therefore benefit more from a host-directed searching behaviour.

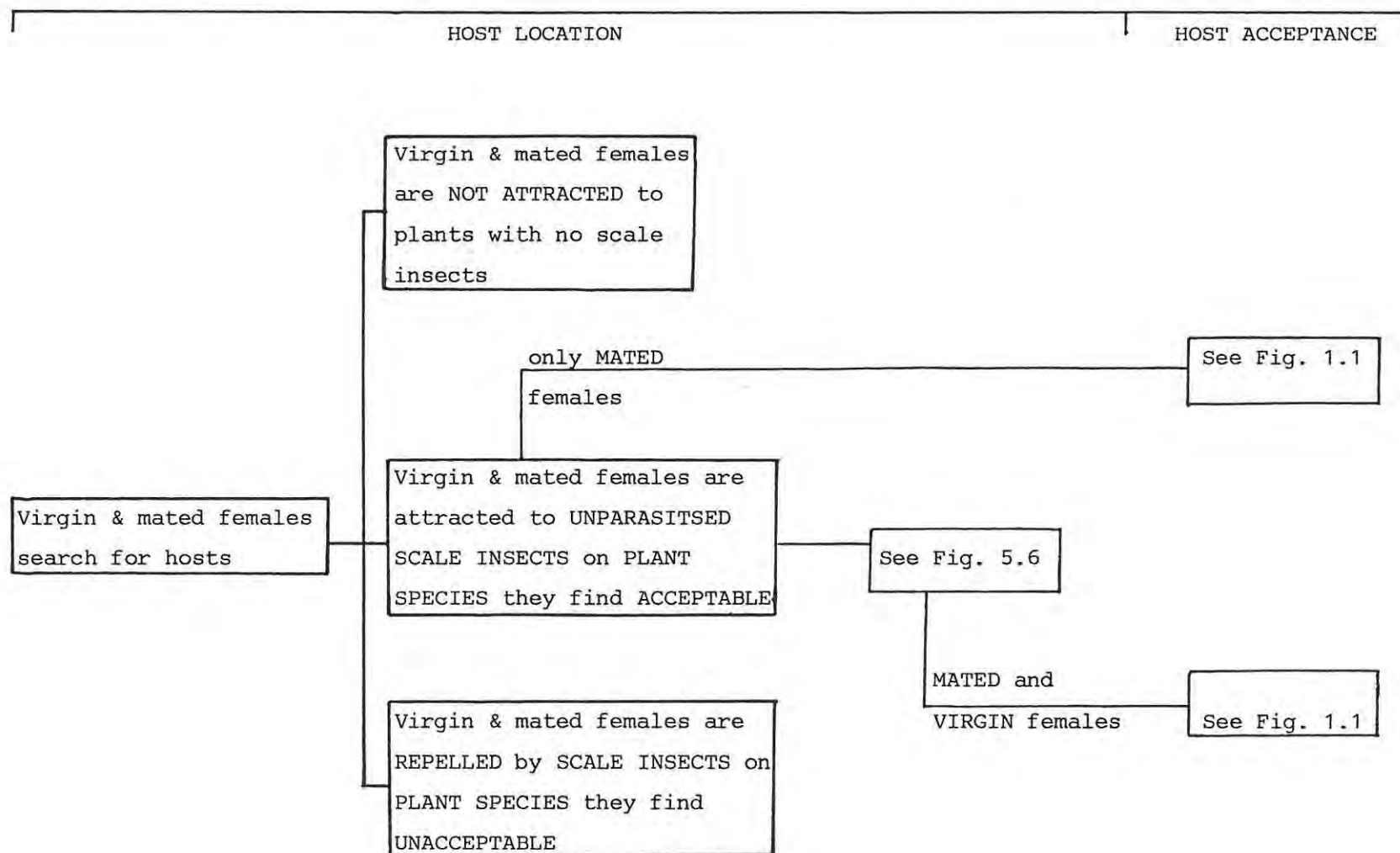


Fig. 6.2 Modified hypothesis to explain host location by Coccophagus atratus females. The hypothesis has been modified from that proposed in Fig. 5.6. Host habitat location has been dropped because C. atratus females do not locate plants without scale insects (see text). Host acceptance remains as postulated in Fig. 1.1.

CHAPTER 7: HOST RECOGNITION

Host acceptance is the final stage in the generalised sequence of host-searching behaviour (Vinson, 1976). Within host-acceptance behaviour there are two sequential behaviours. Firstly there is recognition (or identification) of the host as being of a species suitable for parasitisation. Secondly, the female may accept and parasitise a host or reject a host (Flanders, 1953). Hosts may be rejected for a number of reasons. For example, many primary parasitoid species can detect whether a host has been previously parasitised or not (host discrimination; Salt, 1961; van Lenteren, 1976; van Lenteren *et al.*, 1978), and refrain from parasitising it. The cues used by parasitoids to recognise hosts suitable for female eggs and hosts suitable for male eggs were examined in this chapter. An investigation was not done on the condition that the hosts must be in before they will be accepted by C. atratus.

The emphasis in this section is on how C. atratus females recognise their hosts and distinguish between parasitised and unparasitised scale insects. Initially, the point in the females searching behaviour at which the host is recognised had to be identified. The first question to answer is the following. At what point in the females searching behaviour is the host recognised? The female may recognise the host as suitable for parasitisation (1) before coming into contact with the host, (2) during an external inspection of the host or (3) during an internal inspection made by probing the host with the ovipositor. Once the moment of host recognition was identified, experiments could be designed to interpret what cues the females use in the recognition of the two types of hosts, and this is dealt with in the second part of the chapter.

7.1. Stage in searching behaviour at which hosts are recognised.

Female wasps investigate hosts to determine whether they are potentially suitable for oviposition. That point in the females' behaviour at which host recognition occurs, was sought. The method used simply entailed presentation of female wasps with suitable hosts, either parasitised or unparasitised, and observation of the parasitoids' behaviour. Virgin and mated females were tested separately to compare their behaviour.

In each experiment 5 hosts were placed within a 10mm diameter circle drawn in the center of a piece of filter paper placed on the bottom of a glass petri-dish (Fig. 7.1). Hosts are encountered on leaves in the field, so the females' behaviour may be affected by the unnatural experimental situation described. To test whether the absence of leaves affected parasitoid behaviour, females were also presented with 5 hosts on a leaf and the leaf was placed in the center of the petri-dish.



Fig. 7.1. Five parasitised Filippia gemina scale insects placed on filter paper in the center of a glass petri-dish.

Parasitoids were introduced into the petri-dish in groups of three and were observed for 15 minutes. Host recognition may occur before the host is located (Carton, 1971; Calvert, 1973), so the number of females that located hosts was recorded in addition to the number of females that probed them. At the end of each trial, the hosts were replaced with fresh specimens because individuals of some parasitoid species place a marking pheromone into (Wylie, 1965; 1970; 1971), or onto their hosts (Wilson, 1961; Vinson & Guillot, 1972; van Lenteren & DeBach, 1981). Parasitoids may also place a marking pheromone nearby their hosts (Salt, 1937; DeBach, 1944; Price, 1970; Greany & Oatman, 1972;

van Lenteren & Bakker, 1978; Waage, 1979; Galis & van Alphen, 1981). Therefore the petri-dish was cleaned and the filter paper replaced after each trial.

Virgin C. atratus females were presented with hosts in the following condition;

- 1) parasitised F. gemina on a C. monolifera leaf,
- 2) unparasitised F. gemina on a C. monolifera leaf,
- 3) parasitised F. gemina alone, and
- 4) unparasitised F. gemina alone.

Two controls are required for these different situations.

- 1) Females were presented with a fresh , clean, C. monolifera leaf. If the leaf helped the parasitoids to locate hosts, equal numbers of females would be expected on the control leaf and on leaves with hosts.
- 2) In place of hosts, female parasitoids were presented with just the 10mm diameter circle marked with pencil in the center of the petri-dish. This control would indicate the number of females that would locate the hosts if hosts are located by random search.

The results of these experiments are presented in Fig. 7.2. Fewer virgin females climbed onto scale insect-free leaves (Fig. 7.2a) than climbed onto scale insect-infested leaves and located hosts (Fig. 7.2b,c). Females readily climbed onto and searched the scale-infested leaves for hosts but they seemed reluctant to walk onto the scale-free leaves. Usually they landed on the scale-free leaf as they jumped around inside the petri-dish, so their arrival on the leaf appeared to be by chance. Little time was spent on the scale-free leaves ($\bar{x} \pm 1$ S.E. = $12,0 \pm 1,2s$), and females on these leaves did not appear to search the leaf for hosts. Apparently the females can detect whether there are hosts on a leaf before searching it. Vision and/or olfaction may be used although it seems doubtful that vision is important at this stage because females often walked past hosts without appearing to notice them.

When virgin females were confined in a petri-dish with only 5 hosts in the center, they located parasitised scale insects (Fig. 7.2.e) more times than they located the blank spot by chance (Fig. 7.2d). More

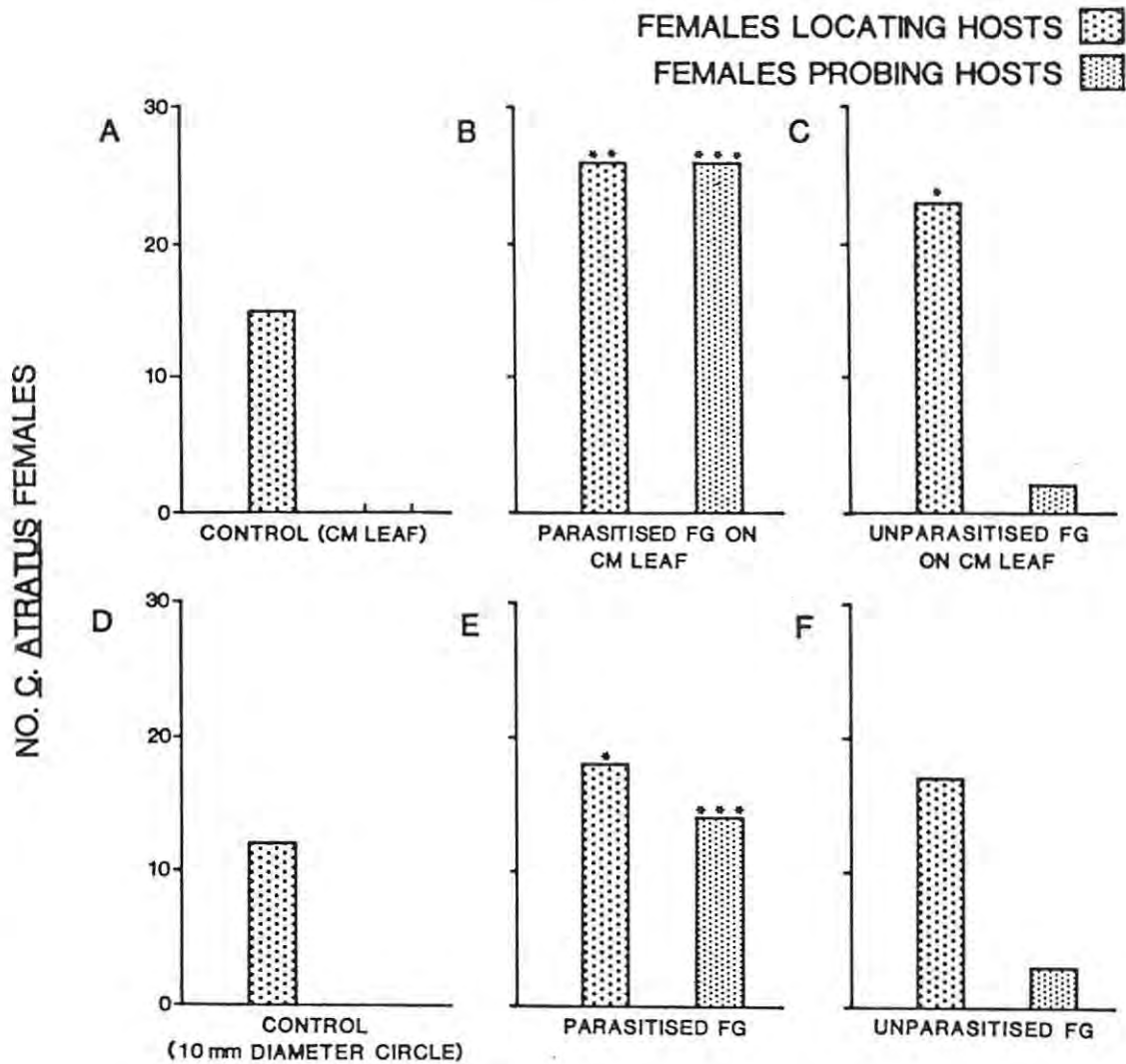


Fig. 7.2. Number of virgin *Coccophagus atratus* females locating hosts, either on a *Chrysanthemoides monolifera* leaf (B,C) or on filter paper (E,F), and the number of females that probed hosts. There are two controls for host location: (A) is the control for the number of females that locate hosts on leaves, and (D) is the number of females that locate hosts on filter paper. If the number of females that locate hosts (either on a leaf or filter paper) is significantly greater than in the relevant controls, then the females are influenced by the hosts and searching is not at random. The null hypothesis for the number of females probing hosts is that none of the hosts will be probed. If a significant number of females do probe a particular type of scale insect (parasitised or unparasitised), then that type of scale insect is acceptable to the females. $N=30$ in all cases. Levels of significance are represented by asterisks on the top of each histogram bar: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

unparasitised scale insects were also located than the control, but this was not statistically significant.

Similar numbers of virgin females located both parasitised and unparasitised scale insects (Fig. 7.2e,f). This supports conclusions from earlier results (Fig. 5.2) that virgin females search for unparasitised scale insects, even though they cannot oviposit in them. Therefore virgin females do not appear to differentiate between host types before they have found them. Quednau & Hübisch (1964) found that the Aphytis species they examined must also be in contact with its diaspidid host Aonidiella aurantii (Maskell) to recognise it as being potentially suitable for oviposition.

Once hosts are located, virgin females can identify whether a scale insect is parasitised or not before the host is probed. This was shown by the number of females probing the parasitised and unparasitised scale insects. A significant number of virgin females probed parasitised scale insects (Fig. 7.2b,e) but very few probed unparasitised scale insects (Fig. 7.2c,f). In the few examples where virgin females did probe unparasitised scale insects, the probe lasted less than 1s, too short a time to penetrate the cuticle. The virgin females appeared only to touch the unparasitised hosts' cuticle with the ovipositor before they stopped probing, then they walked off. Sensory structures present at the tip of the ovipositor (Gutierrez, 1970; King & Rafai, 1970; Weseloh, 1971b) may supply sensory information about the host.

To compare the behaviour of mated females with that of virgin females, mated females were tested in an identical manner using hosts on leaves (Fig. 7.3). Again, more females located parasitised and unparasitised scale insects than located the control (Fig. 7.3a). This result suggests that the females respond to a short-range cue from the hosts and do not search randomly for them. The main difference between virgin and mated females is apparent when the number of females that probe hosts is examined. Mated females probed both parasitised and unparasitised scale insects, as was expected.

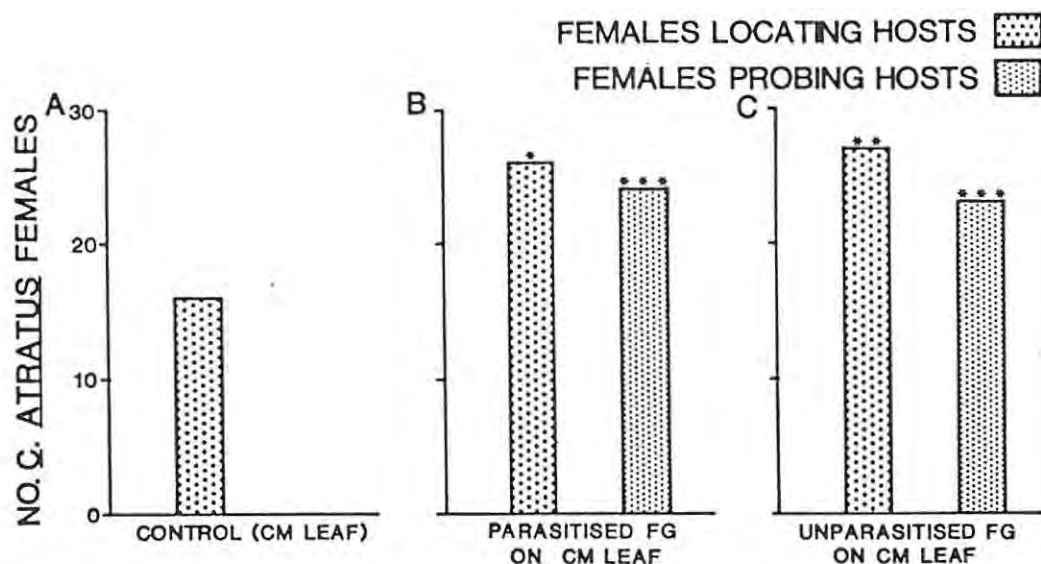


Fig. 7.3. Number of mated *Coccophagous atratus* females that locate hosts on leaves and that probe hosts. The control is for the number of females locating hosts. See Fig. 7.2 for more details. $N=30$ in each case. Levels of significance are presented by asterisks on the top of each histogram bar: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

In summary, the searching behaviour of virgin and mated females is identical up to the point where the parasitoid climbs onto a host. It is only when the females are in contact with a host and examine it with their antennae that they can distinguish between parasitised and unparasitised scale insects. Therefore, in all subsequent observations, probing of a host was taken as the criterion that the host has been identified as being potentially suitable for oviposition. The behaviour of *C. atratus* differs from that described in the hyperparasitoid *Cheiloneuris noxius* Compere (Encyrtidae). *C. noxius* probed both parasitised and unparasitised scale insects, even though, like virgin *C. atratus* females, they oviposit only in parasitised scale insects (Le Pelley, 1937; Weseloh, 1969; 1971a). *C. atratus* females appear to have evolved a more specialised behaviour that allows them to recognise their host earlier during oviposition behaviour. The oviposition behaviour of *C. noxius*, however, appears to be closer to that of a conventional primary parasitoid.

Several difficulties had to be overcome when designing experiments to investigate the cues used in host recognition by C. atratus females. Mated females respond to cues from both types of host and therefore cannot be used in experiments designed to isolate cues used by the wasps for identification of the two types of hosts. Virgin females respond, by ovipositing, only to cues from hosts suitable for male eggs and for this reason they were used in experiments to isolate the host recognition cues. In these experiments, hosts suitable for male egg deposition will be manipulated to isolate the host recognition cues. Cues identified in hosts suitable for male eggs that are not present in hosts suitable for female eggs are the most likely ones used by the females to distinguish between the two types of hosts. Cues common to both hosts are probably not important.

7.2. Cues used to identify hosts suitable for male eggs.

Physical cues like shape and size (Salt, 1940; 1958; Price, 1970; Schmidt, 1974; Wilson et al., 1974), movement (Salt, 1938; van den Assem & Kuenen, 1958; Jackson, 1968; Glas & Vet, 1983) and chemical cues (Vinson & Lewis, 1965; Wilson et al., 1974; Tucker & Leonard, 1977) are used by parasitoids to identify their hosts. Odour appears to be important in the process of host location by C. atratus, but, at even a short distance from the host, odour is probably not involved directly in the process of differentiating between hosts suitable for male eggs and hosts suitable for female eggs (ie. scale insects that are parasitised or unparasitised). This is because virgin females locate both parasitised and unparasitised scale insects, which are present together on the same leaf in equal numbers, with equal frequency.

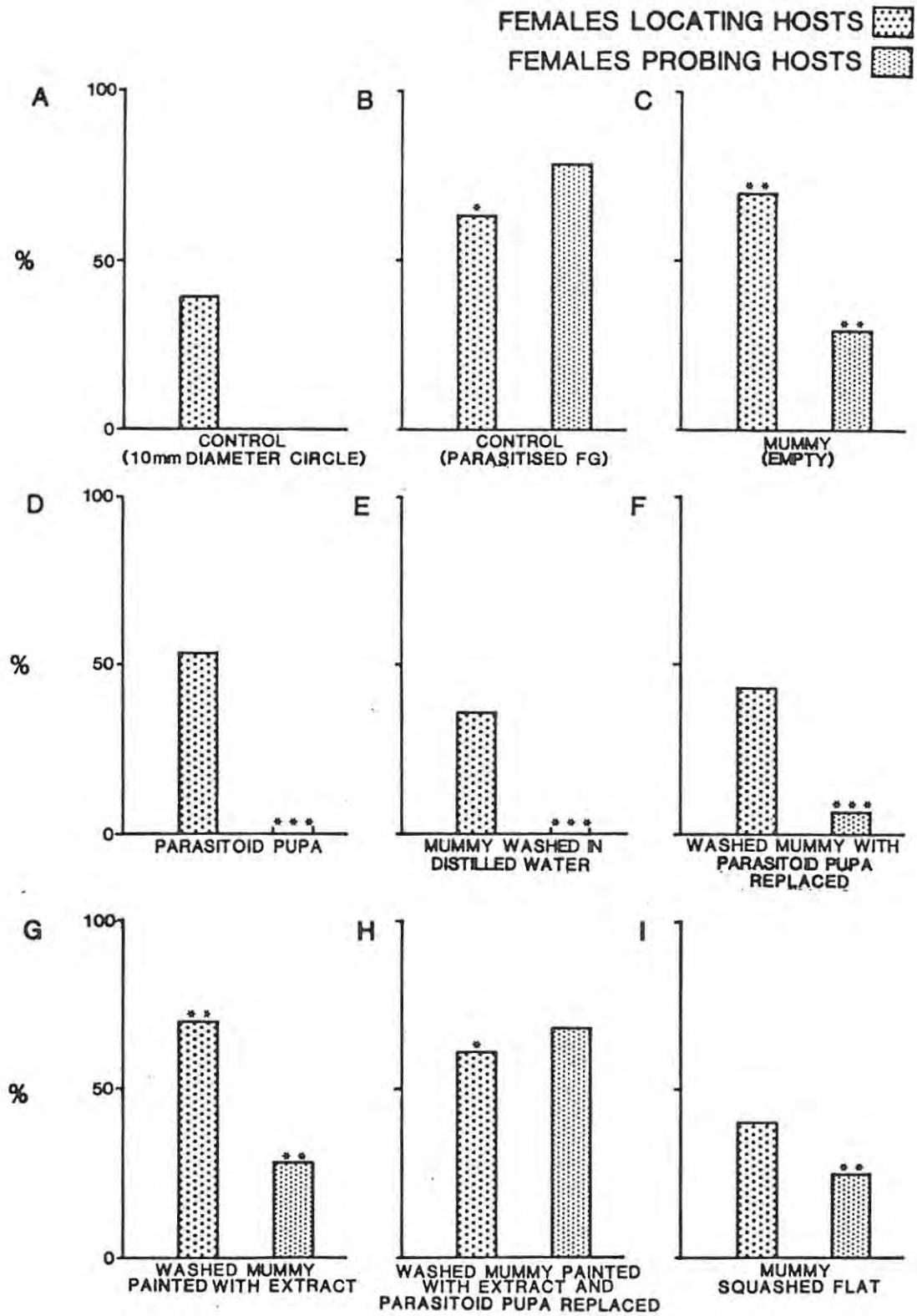
The basic experimental method used to determine how C. atratus differentiates between parasitised and unparasitised scale insects was similar to that used in the previous section. Virgin females were introduced into a glass petri-dish in groups of three and confined with the hosts for 15 minutes. To isolate the cues that C. atratus females might use to identify their hosts, the parasitised scale insects were altered in one way or another, before being presented to the female wasps. For example, the parasitoid pupae were removed from the

parasitised scale insects and the hollow shell or mummy was presented to the females. The number of females that located hosts and the number that probed them was recorded.

Two controls were used for these experiments, one for the number of females that located hosts and one for the number that probed hosts. The first control was identical to that used in the previous experiment (Fig. 7.2d): a 10mm diameter circle was drawn on filter paper in the center of the petri-dish and the number of females that encountered it was recorded (Fig. 7.4a). If significantly more females located hosts than encountered the control circle, then some factor, either olfactory or visual, may have influenced the females' behaviour. The second control (Fig. 7.4b) was a parasitised scale insect suitable for the deposition of male eggs: this is a control for the number of females that probed hosts. A significantly lower percentage of probing behaviour in any experiment would mean, the cue that usually elicits probing behaviour, and thereby host recognition, has been altered or removed.

Hosts suitable for the development of males differ in several ways from hosts suitable for female development. In 'male' hosts, a parasitoid replaces the scale insect, which is humped in shape and black in colour. Hosts for females, unparasitised scale insects, are flat and yellow. Colour was not investigated because C. atratus females also lay male eggs on developing parasitoids of other species that do not turn the scale insect mummy black in colour. Also, Weseloh (1971a) has shown that the hyperparasitoid Cheiloneurus noxius had no preference for black or transparent mummies.

In 'male' hosts, either the parasitoid within the mummy, the scale insect mummy itself, or both may be important sources of cues used in host recognition. Initially, parasitoid pupae were carefully removed from the mummy through a hole cut in the ventral surface of the scale insect. When such a mummy was placed on filter paper the hole could not be seen by a parasitoid and the scale insect looked like a normal host suitable for male egg deposition. More females located empty mummies than located the control spot in the centre of the filter paper. However, a significant decrease in the percentage of females that probed empty mummies was found (Fig. 7.4c). This suggests, firstly,



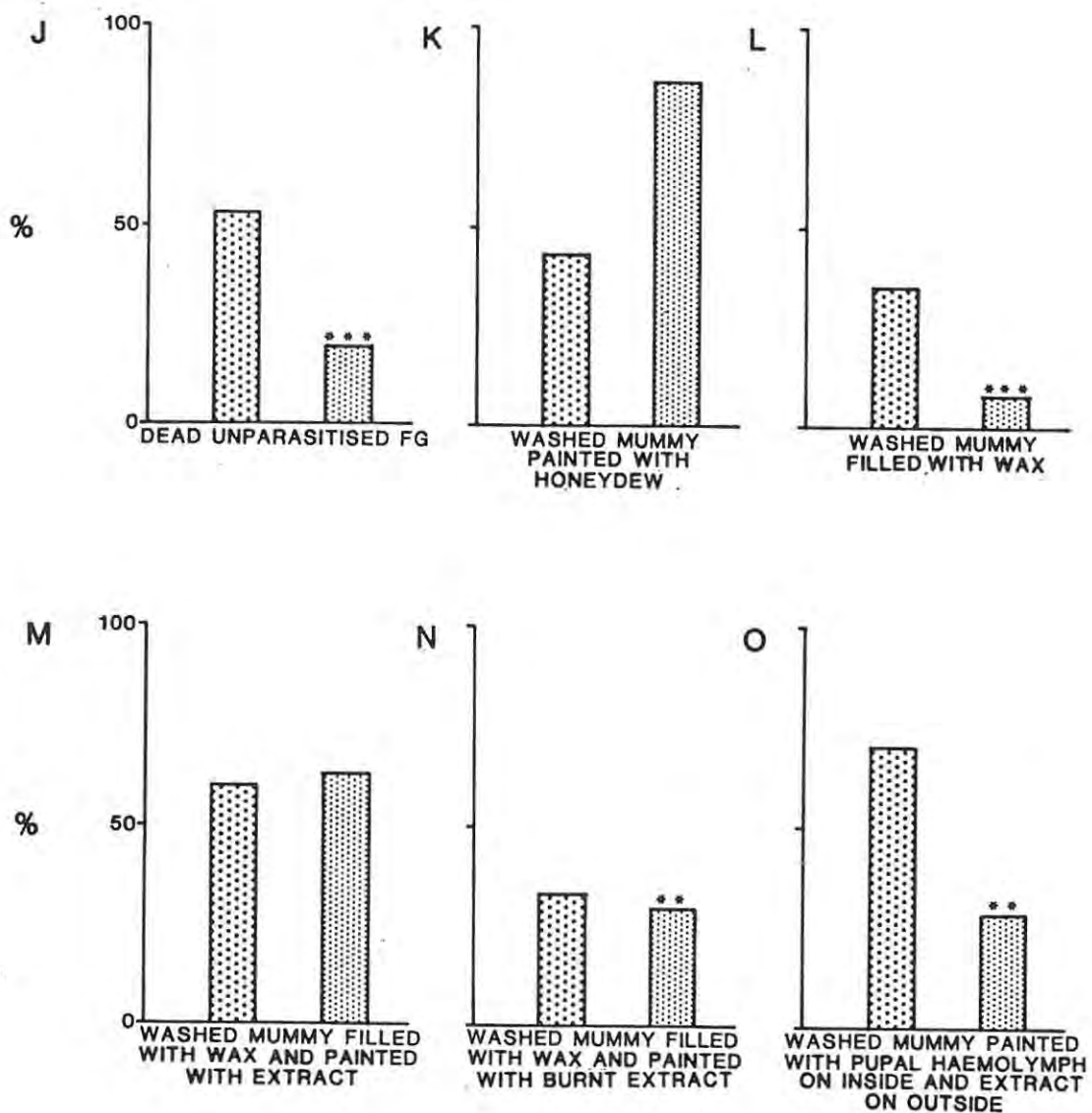


Fig. 7.4. The percentage of *Coccophagous atratus* females locating and probing hosts from a series of experiments designed to isolate host-recognition cues. There are two controls; A is the control for the percentage of females locating hosts and B is the control for the percentage of females probing hosts. Percentage probing is calculated from the number of females that located hosts and not from the total number of females used in the experiment. To test if hosts are located more frequently than at random, the percentage of females locating hosts is compared to A. Similarly, to determine the effect of an experiment on host probing, results are compared to the number of females probing normal hosts in B. $N=30$ in all cases. Levels of significance are represented by asterisks on the top of each histogram bar; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

that the host shell is important in host location and secondly that the parasitoid pupa elicits probing in virgin females. The effect of the parasitoid pupa may be due to (a) the physical presence of the pupa in the host shell, (b) to production by the pupa of a volatile chemical or (c) a combination of (a) and (b).

Isolated parasitoid pupae, removed from parasitised scale insects, did not attract virgin C. atratus females, and they were not probed by the wasps (Fig. 7.4d). However this is an unnatural situation with which the females would not normally be faced.

Empty mummies were washed in distilled water for 24h and presented to the virgin female wasps. The same number located the mummies as located the control circle (Fig. 7.4e). Therefore, washed mummies seem to contain a water-soluble chemical that affects female behaviour by attracting them to the mummy. Washed mummies found by the wasps were not probed, but this was similar to the result obtained for unwashed mummies. Quednau & Hübsch (1964) also discovered a water-soluble chemical present in scale covers that made them attractive to Aphytis coheni DeBach females. To test whether the attractant chemical is produced by the wasp pupa, parasitoid pupae were replaced into the washed mummies and presented to the virgin females (Fig. 7.4f). The results obtained did not differ markedly from those in the previous experiment (Fig. 7.4g) which indicates that the attractant is not derived from the parasitoid pupa.

To confirm that the attractant chemical was water soluble, an attempt was made to replace it on the mummy. Some of the distilled water in which the shells of the mummies were washed was evaporated off to obtain a higher concentration of extract. This extract was then painted onto the outer dorsal surface of the washed mummies which were then presented to the virgin females. The results (Fig. 7.4g) indicate that the females responded in the same way as they did to the empty mummies (Fig. 7.4c). A similar result was obtained by Quednau & Hübsch (1964) for Aphytis coheni on red scales. The hyperparasitoid Cheiloneuris noxius also responds to chemicals in the host integument (Weseloh & Bartlett, 1971). Virgin C. atratus females should accept and probe washed mummies painted with extract, and in which parasitoid pupae have been replaced, as normal hosts suitable for the deposition of male

eggs. Virgin females exposed to these hosts (Fig. 7.4h) did exhibit the same behaviour as those females presented with normal parasitised hosts (Fig. 7.4b). The presence of the parasitoid pupa inside the mummy TOGETHER with the water soluble chemical painted on the scale elicits the expected (Fig. 7.4h) amount of probing behaviour in the virgin female wasps.

Parasitised scale insects are humped in shape because of the parasitoid inside them, but unparasitised scale insects are flat in shape. This is a clear morphological difference between parasitised and unparasitised hosts that could possibly be used by females to differentiate between the two types of hosts. To determine the role of host shape, empty scale insect mummies were squashed flat and presented to the C. atratus females. The number of females that located 'squashed' mummies (Fig. 7.4i) was the same as that in the control (Fig. 7.4a) and therefore the shape of the host may be important in the identification of parasitised scale insects. If shape had no effect, the number of females that located squashed mummies should have been similar to the number that located normal hosts (Fig. 7.4c). The hyperparasitoid Cheiloneuris noxius was also found, by Weseloh (1971b), to be influenced by host shape; they preferred a convex shape, like that of their normal hosts, to a flat shape. Quednau & Hübsch (1964), however, found that the shape of the scale cover of Aonidiella aurantii, whether convex or flat, did not affect the behaviour of Aphytis females. A. aurantii are diaspidids, which are relatively flat in shape, even when parasitised, so they are not really comparable with F. gemina scale insects, which are flat in shape when unparasitised but become humped when parasitised.

Another difference between hosts suitable for male offspring and hosts for females is the condition of the scale insect's cuticle. In unparasitised scale insects, the cuticle forms part of a living scale insect, but in parasitised scale insects it forms a dead dry shell, or mummy, around the parasitoid pupa. If one of the cues used by females to identify parasitised scale insects is the dead cuticle of the scale insect then females should respond in the same way to dead, unparasitised, scale insects. The results obtained when virgin females were presented with such dead unparasitised scale insects (Fig. 7.4j), that had been killed by starvation, were similar to those obtained for

flattened mummies (Fig. 7.4i) and the females showed little interest in them. The water soluble chemical present in scale insect mummies, which attracted C. atratus females, was not present in the dead unparasitised scale insect.

Vinson et al. (1978) found that honeydew from Coccus hesperidum L. was a search stimulant and a recognition cue used by the parasitoid Microterys flavus (Howard). Honeydew is soluble in water so it may be the water soluble chemical present in the mummy that seems to attract C. atratus females to hosts. To test this idea, honeydew was painted onto washed, empty, mummies, which were then presented to the female parasitoids (Fig. 7.4k). The number of females that located hosts was the same as the control (Fig. 7.4a), but the number of females probing increased and did not differ significantly from that obtained for females exposed to normal hosts (Fig. 7.4b). Honeydew is therefore not the attractant but it may elicit probing behaviour by the parasitoids. However, the high percentage of females that probed may have been caused by the unrealistically high concentrations of honeydew. Honeydew was found to stimulate Microterys flavus females to search for their scale insect hosts, Coccus hesperidum, but the honeydew did not elicit probing in M. flavus females.

To determine whether the physical presence of the parasitoid pupa in the mummy elicited probing, or whether probing behaviour occurs in response to a volatile chemical produced by the parasitoid pupa, the parasitoid was removed from the mummy and replaced with paraffin wax. A small amount of molten wax was drawn up into a fine glass capillary tube. The capillary tube had to be heated to prevent the wax cooling and solidifying as it entered the tube. To place the wax inside an empty scale insect mummy, the end of the capillary tube was held against the hole made in the ventral side of the mummy. The molten wax was drawn into the empty shell by capillary action and it solidified as it came into contact with the cold cuticle.

When wax was placed into washed mummies and presented to virgin females, the females did not probe the mummies (Fig. 7.4l). When the water soluble extract, obtained from empty mummies, was painted onto

wax-filled mummies (Fig. 7.4m), the percentage probing increased and was not significantly different from that obtained for normal hosts (Fig. 7.4b).

It therefore appears that the physical presence of the parasitoid is important in eliciting probing behaviour by the virgin females. C. atratus females therefore differ from some hyperparasitoids (Gutierrez, 1970; Weseloh, 1971a; 1971b; Weseloh & Bartlett, 1971) and other heteronomous hyperparasitoids (Gerling, 1966; Williams, 1972; Flanders, 1969; Jarraya, 1975). The females of these species could detect the presence of the primary parasitoid only after they had probed the parasitised scale insect. If the water soluble extract was painted onto the mummy before the mummy was filled with wax, the hot wax appeared to destroy the active component of the extract, because there was no increase in the percentage of females that probed the wax-filled mummies (Fig. 7.4n). Microterys flavus females also did not probe scale insects that had been killed by heat (Vinson et al., 1978). C. atratus females therefore appear to detect the presence of the parasitoid pupa as they tap the surface of the mummy with their antennae.

To confirm that a volatile chemical given off by the parasitoid pupa is not important in eliciting probing, female wasps were presented with washed mummies painted on the outside with extract and on the inside with haemolymph removed from parasitoid pupae. The results (Fig. 7.4o) show that there was an increase (compared with the control experiment (Fig. 7.4a)) in the percentage of females that located the hosts. This was probably due to the extract painted onto the mummy. Percentage probing was significantly lower than that obtained for normal hosts (Fig. 7.4b). Female parasitoids seem able, therefore, to detect with their antennae, the physical presence of a parasitoid pupa inside the scale insect shell. Females may be able to detect the presence of a parasitoid pupa from the resonance of the parasitised scale insect as they tap it with their antennae. Therefore an auditory signal may be used by C. atratus females and this has also been reported for Encarsia formosa Gahan (Nell et al., 1976). To summarise, physical and chemical cues appear to be the cause of host-probing behaviour by C. atratus.

Further support for the conclusions drawn above may be gained from the sequence of behavioural events that occur prior to a female probing her ovipositor into a host. In Chapter 2 it was reported that the sequence of behavioural events that occurred before the host was probed was, (1) antennal inspection of the host, followed by (2) a more detailed inspection when the female climbed onto the scale insect, still tapping it with her antennae, and then (3) performed a series of rapid 180° turns while tapping either end of the scale insect with its antennae. Nell *et al.* (1976) suggested that the antennal-tapping and turning behaviour of *E. formosa* on their hosts is done to measure the host size.

Fig. 7.5 shows at which point during the inspection of the scale insects, the virgin females rejected them. The observations reported in Fig. 7.5 are from the previous experiment (Fig. 7.4). Virgin females may require specific cues to continue from one behaviour in the sequence to the next. If these cues were absent in the hosts presented to the females then they would reject the host at that point in their investigation.

When presented with normal parasitised scale insects suitable for the deposition of a male egg, most *C. atratus* females complete a full examination of the host (Fig. 7.5a). Empty mummies were more often rejected earlier on in the oviposition sequence (Fig. 7.5b) and parasitoid pupae were rejected mainly at the beginning of the sequence. After the mummies were washed in distilled water, host rejection occurred early (Fig. 7.5d,e), but when the extract was painted on, most females completed a full inspection of the host (Fig. 7.5f). Results obtained for females presented with washed mummies that had been painted with extract and had the parasitoid pupa replaced (Fig. 7.5g) were similar to results obtained with normal parasitised scale insects (Fig. 7.5a). Squashed mummies and dead unparasitised scale insects were usually rejected before completion of the oviposition sequence (Fig. 7.5h,i). Washed mummies painted with honeydew were examined completely by most of the females (Fig. 7.5j), indicating that honeydew may be an important stimulus. Washed mummies filled with wax did not elicit much attention from the females (Fig. 7.5k), but when they were painted with extract, most females examined them completely (Fig. 7.5l) and this result was similar to that obtained for normal parasitised scale

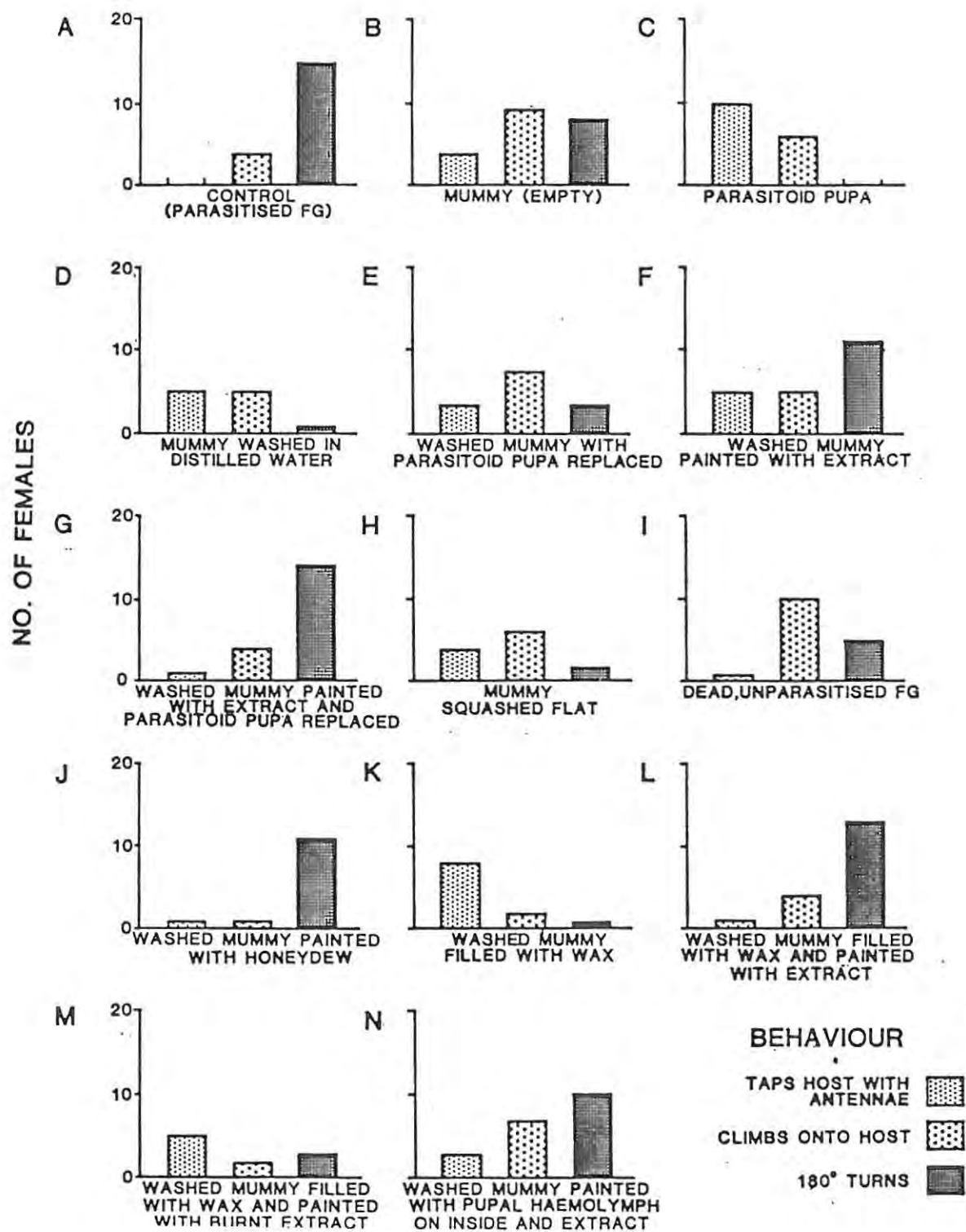


Fig. 7.5. Ovipositional sequence of *Coccophagous atratus* females when presented with parasitised *Filippia gemina* that have been manipulated to isolate host recognition cues. N=30 in each case.

insects (Fig. 7.5a). Mummies filled with wax and painted with extract that got burnt by the hot wax, did not elicit much response from the virgin females (Fig. 7.5m). Mummies painted on the inside with pupal haemolymph were examined completely by most females (Fig. 7.5n) but this response was probably due to the extract painted on the outside. In summary, it appears that females can be stimulated to examine a 'host' if it is painted on the outside with extract. The physical presence of the parasitoid pupa is probably the major cue eliciting probing by the females.

7.3 Hypothesis for host-acceptance behaviour.

The hypothesis advanced in Fig. 7.6 to explain host-acceptance behaviour has not been altered from the original hypothesis (Fig. 1.1). The latter correctly predicted that virgin females recognise and oviposit male eggs only in parasitised scale insects, while mated females recognise and oviposit female eggs in unparasitised scale insects as well. Therefore host acceptance behaviour is the point, in the host-searching sequence, at which the behaviour of virgin and mated females differ.

A comparison between the oviposition behaviour of virgin and mated females confined with parasitised or unparasitised scale insects showed that the females can identify their hosts with only an external inspection and do not require to probe the host like other hyperparasitoid species (Gutierrez, 1970; Weseloh & Bartlett, 1971).

The cues used by virgin females to identify hosts suitable for male eggs were both chemical and physical and these are summarised in Fig. 7.7. A water soluble chemical present in the scale insect mummy appeared to be responsible for more females locating the parasitised scale insects than if searching was done at random. This chemical may be a short-range cue. The humped shape of the parasitised scale insect also appeared to help the females locate the hosts. Because these two cues are not present in unparasitised scale insects, they may possibly be used for host recognition. When a female locates a parasitised scale insect, two cues, one chemical and one physical, must be present TOGETHER to elicit an exploratory probe. These cues are the water-soluble chemical present in the scale insect mummy, and the physical presence of the parasitoid pupa inside the mummy.

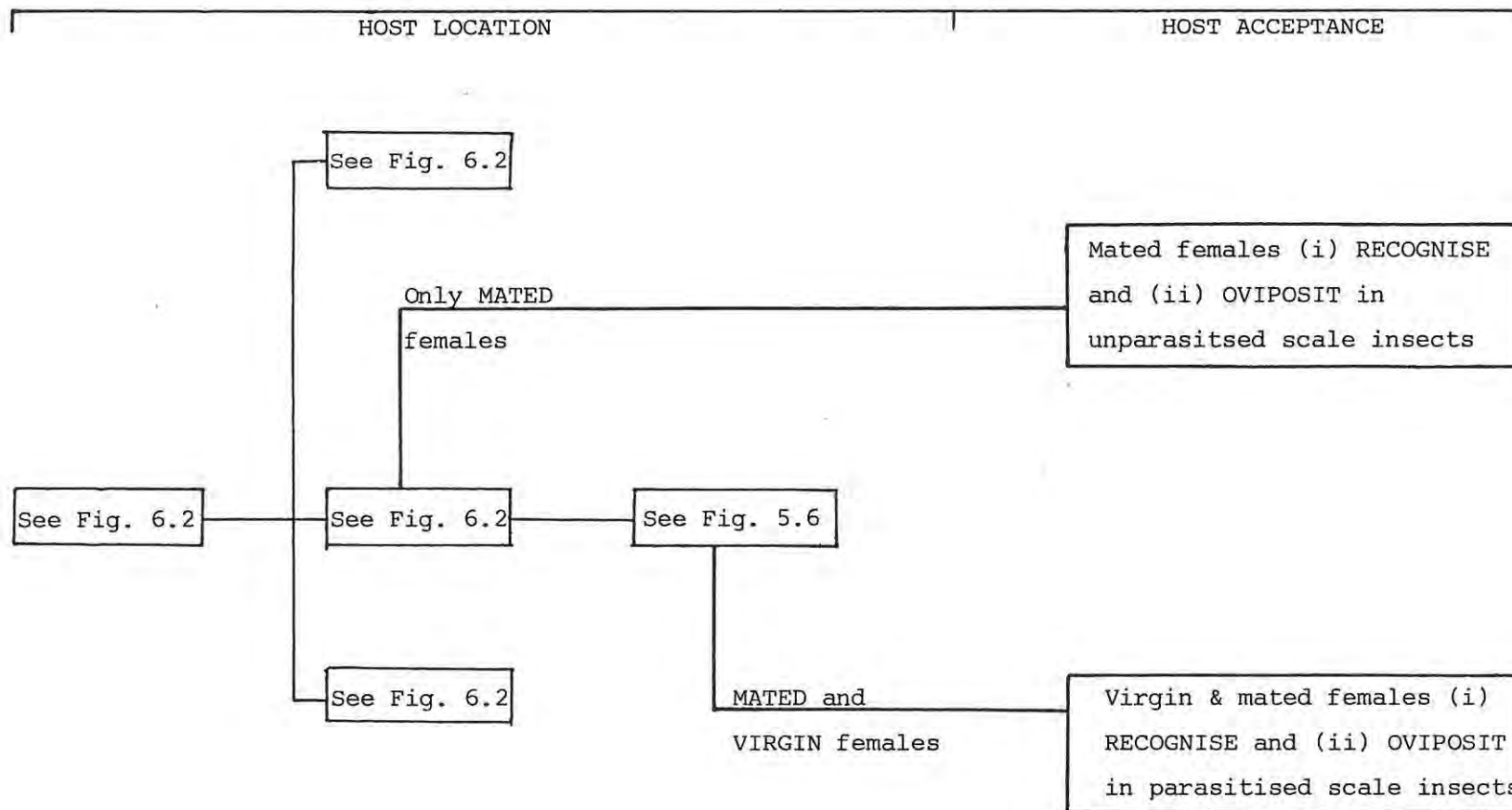


Fig. 7.6. Modified hypothesis to explain host-acceptance behaviour by Coccophagus atratus females. The hypothesis has been modified from that proposed in Fig. 1.1. host location remains as postulated in Fig. 6.2.

CUE	PARASITOID BEHAVIOUR	
SHAPE		HOST LOCATION
EXTRACT		HOST LOCATION
WAX		
PARASITOID PUPA		
EXTRACT AND WAX		PROBING
EXTRACT AND PUPA		PROBING

Fig. 7.7. A summary of the main cues which elicited a behavioural response in Coccophagous atratus females

Because these cues are not present in unparasitised scale insects, they may be used by the females to differentiate between hosts suitable for male eggs and hosts suitable for female eggs. Alternatively, females may not differentiate between hosts, but may merely have two sets of behaviours, one for each type of host. When a female contacts a 'male' host, a cue(s) from this host may set off a train of investigations leading to oviposition of a male egg. Different cues on unparasitised scale insects may elicit a different set of behaviours leading to the oviposition of a female egg.

CHAPTER 8: DISCUSSION

Results obtained in this study are discussed initially with respect to parasitoid host-searching behaviour. Firstly, the behaviour of C. atratus is discussed in detail, then these observations are extended to a general discussion of the theory of host-searching behaviour in parasitoids. Next the implications of this study for the interpretation of both individual and population sex ratios of C. atratus are discussed. Following on this, the interpretation of Walter's (1983a) proposed evolutionary sequence for heteronomous parasitoids is dealt with. Finally, the host-searching behaviour of C. atratus females will be related to the use of parasitoids in biological control.

8.1. Host-searching behaviour.

8.1.1. C. atratus.

The original hypothesis postulated to explain the host-searching behaviour of C. atratus females (Fig. 1.1), was based on the current theory of parasitoid host-searching behaviour (Doutt, 1964; Vinson, 1975; 1976; 1981; Weseloh, 1981). The hypothesis (Fig. 1.1) predicted that virgin and mated females would search initially for the hosts' habitat. Mated females would then search for parasitised OR unparasitised scale insects. On the other hand, virgin females were expected to search only for parasitised scale insects. Finally, mated females were expected to recognise and oviposit in hosts suitable for male development and in hosts suitable for female development. Virgin females were expected to oviposit only in hosts suitable for the development of males.

Results obtained in experimental tests of the predictions generated by this hypothesis have necessitated several modifications to the original theory. The modified hypothesis for host-searching behaviour in C. atratus females is presented in Fig. 8.1.

C. atratus females appear to search directly for their hosts and do not search initially for the hosts' habitat as predicted by the original hypothesis. Females find hosts by responding to cues in honeydew excreted by the scale insects (Chapter 5). Plants did affect parasitoid host-location behaviour, but only indirectly. Certain chemicals, presumably present in the sap of plants, seem to be modified by the

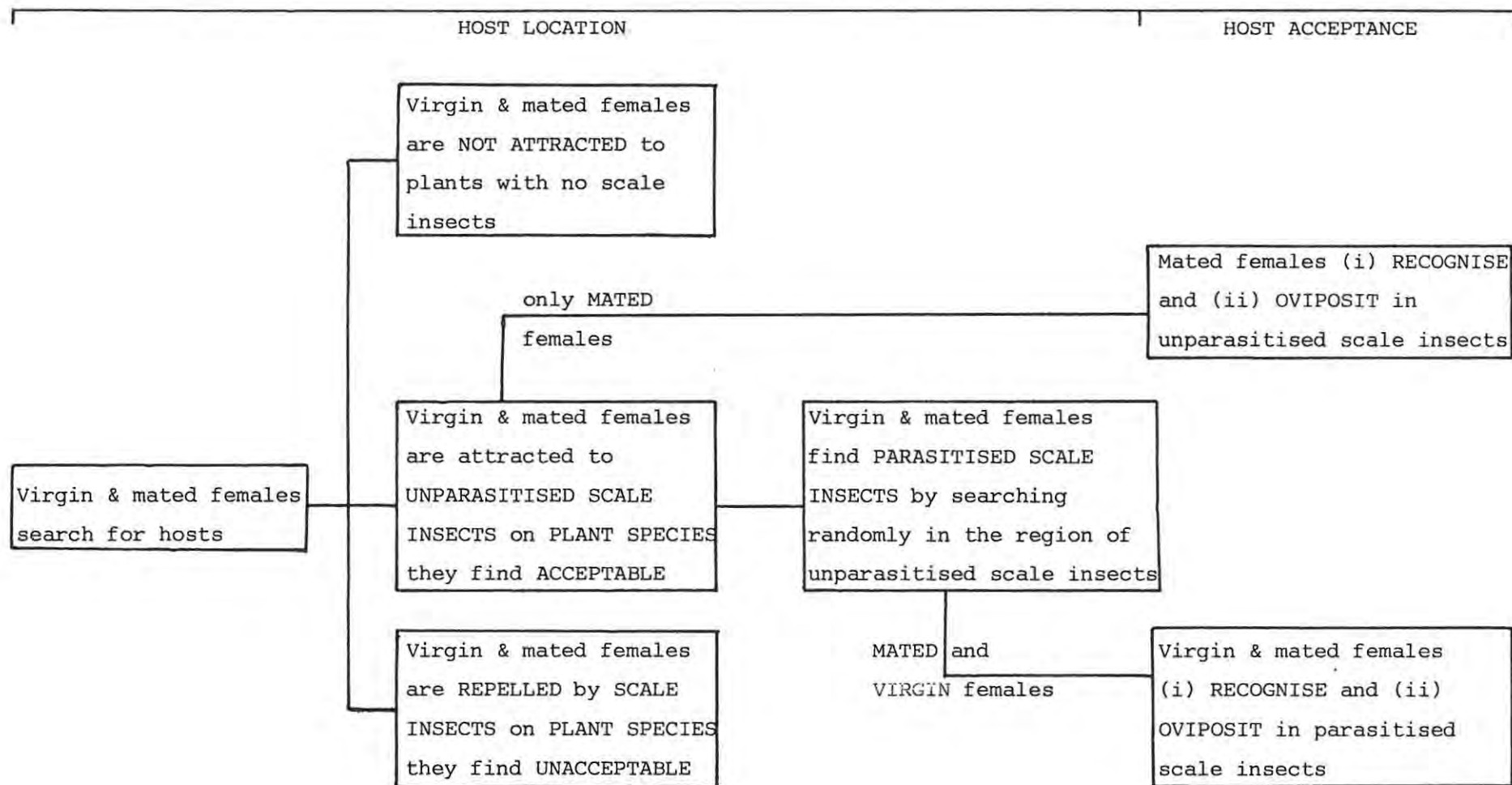


Fig. 8.1. Modified hypothesis to explain host-searching behaviour by *Coccophagus atratus* females. Modified from the original hypothesis proposed in Fig. 1.1 using results obtained in this study (see Figs 4.4, 5.6, 6.2 and 7.6)

coccoid hosts of C. atratus. These plant-derived chemicals are then excreted in the honeydew of the host and thus affect the attractiveness of honeydew to the females. Only hosts feeding on plant species acceptable to C. atratus females are located and attacked by them (Fig. 5.5).

Future research, at the biochemical level, should be directed at determining whether the plant-derived chemical is indeed modified by the hosts, and the chemicals involved should be identified. This may provide useful information for the manipulation of parasitoids in biological control programmes.

Both virgin and mated females first locate unparasitised scale insects and this was not predicted by the original hypothesis (Fig. 1.1). It appears that virgin females use the more 'primitive' cues and locate unparasitised hosts, which they cannot parasitise, before searching randomly in the vicinity (on the same leaf or branch) for parasitised scale insects. C. atratus females seem not to have a separate searching behaviour that allows them to locate parasitised scale insects independently of the presence of unparasitised scale insects.

Only when hosts had been physically located, did the host-searching and ovipositional behaviour of virgin females differ from that of mated females. Mated females probed and oviposited into unparasitised scale insects, but virgin females did not even probe these scale insects (Figs 7.2 and 7.3). Virgin females probed only suitably parasitised scale insects and oviposited male eggs onto parasitoid larvae or prepupae. Flanders (1967) suggested that the cause of the change in behaviour is "psychological" in nature and is activated by mating or may be indirectly caused by the availability of hosts.

8.1.2. General host-searching behaviour.

The concept of host-habitat location caused several problems in this study, and there are probably two reasons for this. Firstly, there is the difficulty of identifying the hosts' habitat and secondly, there are problems in defining host-habitat location and these two points are discussed below.

The problem of identifying the hosts' habitat appears to be due to the terminology used. Salt's (1935; 1938) original description of this phase of host-searching behaviour, as the attraction of a parasitoid to a particular environment, avoids any ambiguity. It does not imply, as current theory does, that the parasitoid can identify the habitat of its host. According to Salt's (1935; 1938) description, the parasitoid simply responds preferentially to an environmentally derived stimulus.

The definition of host-habitat location may cause difficulties, both in the designing of experiments and the interpretation of results. To test whether a parasitoid does search for its hosts' habitat, it should be observed to do this in the absence of hosts. However, Vinson (1975; 1981) has stated that cues used by females to locate the host habitat may derive directly or indirectly from the host itself. Host-derived cues should, strictly, be included in host location and not host-habitat location. Even if host-derived cues only attract the parasitoid to the general area of the host, these cues may be part of a hierarchy of stimuli that, in combination, combine to attract the parasitoid to the host.

Because of the difficulties encountered with the term "host-habitat location", it should be dropped in favour of Salt's (1935; 1938) original description that parasitoids are simply attracted to a particular environment.

8.2. Interpretation of *C. atratus* sex ratios.

Females of 'conventional' parasitoids have only one type of host into which they can lay either male or female eggs. Heteronomous hyperparasitoid females have a 'choice' between two types of hosts, one for the deposition of male eggs and the other type for the deposition of female eggs. Therefore, if heteronomous hyperparasitoid females could 'decide' the sex of egg they wish to lay and then seek out the appropriate host, the sex ratio of the female could be predetermined by the female.

Alternatively, if females do not make a 'choice' between the two host types, but instead, respond to host-searching cues as they are perceived, the sex ratio of the female would, theoretically, be influenced by the availability of each type of host.

This study has shown that C. atratus females cannot choose which type of host to locate. Females first locate the 'primitive' host (unparasitised scale insect) and may then locate hosts for males by searching randomly in the vicinity of unparasitised scale insects. Therefore no decision can be made in advance, by the female, of which type of host to locate. Once a female has located a scale insect infestation, the location of both types of hosts is likely to be random and dependent on their availability. Donaldson (1984) found that mated C. atratus females, presented with both types of hosts on leaf discs, located parasitised and unparasitised scale insects randomly.

Heteronomous hyperparasitoid females have less choice than 'conventional' parasitoid females as to which sex of egg to lay. The only 'choice' faced by heteronomous parasitoid females is whether or not to oviposit, because the type of host encountered determines the sex of egg to be oviposited.

My colleague Donaldson (1984), in a detailed study of C. atratus sex ratios, has, as predicted by the results of this study, found that host availability does govern sex ratios in this species, both in the field and in the laboratory. Other authors have also noted that sex ratios of heteronomous parasitoids is influenced by the availability of hosts (Flanders, 1967; Williams, 1977).

8.3. Proposed sequence for the evolution of heteronomous parasitoids.

Three principal types of heteronomous parasitoids are recognised (Walter, 1983a), diphagous parasitoids, heteronomous hyperparasitoids (eg. C. atratus) and heterotrophic hyperparasitoids. Diphagous parasitoids are characterised by having males that are primary ectoparasitoids of scale insects, while heterotrophic parasitoids have males that are primary endoparasitoids of lepidopterous eggs. Females of ALL heteronomous parasitoids develop as endoparasitoids of Coccoidea or Aleyrodoidea. The sequence of evolution in heteronomous parasitoids may have evolved in three different ways (Walter, 1984), and each will now be discussed.

(1) Flanders (1967) suggested that the most primitive form of heteronomous parasitism are indirect hyperparasitoids (Flanders, 1943; 1963; Walter, 1983a; 1983b) which lay male eggs into coccoid hosts

regardless of whether they are parasitised or not. Heteronomous hyperparasitoids are believed to have evolved from indirect heteronomous parasitoids, which in turn gave rise to diphagous and heterotrophic parasitoids (Flanders, 1964). However, according to Walter (1984), indirect heteronomous parasitoids were unlikely to have evolved from 'conventional' parasitoids without intermediate steps.

(2) Walter (1984) suggested that heteronomous hyperparasitoids may have evolved from facultative hyperparasitoids, but he rejected this idea for three reasons. Firstly, facultative hyperparasitism is more advantageous than heteronomous hyperparasitism because they suffer no constraints as to the sex of egg they oviposit, in either parasitised or unparasitised hosts. Secondly, most facultative hyperparasitoids recorded in the literature are ectoparasitic (Muesebeck & Dohanian, 1927; Gahan, 1933; Force, 1974; Askew, 1975; Dahms, 1984). This means that, in addition to the male becoming an obligate hyperparasitoid, the female would simultaneously have to become endoparasitic. Lastly, very few facultative hyperparasitoids are parasitic on scale insects (Walter, 1984).

(3) Walter (1983a) based the evolutionary sequence for heteronomous parasitoids on host-searching behaviour. Diphagous parasitoids require the simplest set of behaviours to locate and oviposit in their host (identical hosts, but different oviposition sites). This system is believed to have led to the evolution of heteronomous hyperparasitoids, which have more complex host-searching behaviours (similar hosts, ie. coccoid and coccoid + parasitoid). Heterotrophic parasitoids may represent the extreme development of the sequence, because they probably have the most complex host-searching behaviour to locate two different hosts (coccoids and lepidopterous eggs). A similar sequence was proposed by Zinna (1962), but no reasons were given for his theory.

Results obtained in the present study provide additional reasons for accepting Walter's (1983a) sequence (number 3 above). C. atratus females are able to locate, recognise and oviposit in two types of hosts. This is more complex than the host-searching behaviour expected of diphagous parasitoids.

Although C. atratus females do not have a separate set of behaviours to locate hosts for the deposition of male eggs, other heteronomous hyperparasitoids may have evolved the necessary behaviour to do this and may be a link to heterotrophic parasitoids. Males of Coccophagous malthusi Girault develop on immature parasitoids in scale insects, but the species of scale insect is different from that in which C. malthusi females develop. Furthermore, these scale insect species are usually found on different plant species (Compere, 1926; Flanders, 1937; Annecke, 1964; Annecke & Insley, 1974). A more complex host-searching behaviour to that found in C. atratus females is necessary for C. malthusi females to locate host species suitable for male development on one species of plant, and host species suitable for female development on another plant species.

In summary, this study provides additional support for Walter (1983a), by showing that the host-searching behaviour of heteronomous parasitoids does, in fact, fit the postulated evolutionary sequence for heteronomous parasitoids, which is based on the parasitoids' host-searching behaviour.

8.4. Use of heteronomous hyperparasitoids in biological control.

Many species of heteronomous hyperparasitoids have been used in biological control programmes. Examples include Coccophagous capensis Compere introduced against Saissetia oleae, Coccophagus lycimnia (Walker) against Coccus hesperidum (Clausen, 1956), and Coccophagous gurneyi Compere against Pseudococcus fragilis Brain (Clausen, 1958).

An important attribute of parasitoids used in biological control is the parasitoid's ability to locate hosts, especially when the host population is at a very low density (Viggiani, 1984). There are two aspects of host-searching behaviour, discovered during this study, which may possibly be manipulated in biological control programmes to increase the effectiveness of parasitoids.

The first aspect is the attraction of parasitoids to Coccoidea, Aleyrodoidea and Aphidoidea. Honeydew from the scale insect hosts of C. atratus was identified as a possible long-range cue that these

females could use to locate their hosts (Chapter 5). Other parasitoids of honeydew-producing insects may also be attracted by honeydew (see Quednau & Hübsch, 1964; Vinson et al., 1978).

It may be possible to attract more parasitoids to plants that are heavily infested with pests, with a formulation of artificial honeydew. Artificial honeydews have already been used in field trials, but so far with limited success (Hagen et al., 1976; Saad & Bishop, 1976). Use of honeydew in this way appears to be part of a general trend towards using chemicals to manipulate natural enemies in biological control programmes (see Shorey & McKelvey, 1977; Nordlund et al., 1981).

Secondly, different plant species influence the host-searching behaviour of parasitoid females in different ways. The effect of plants on the searching behaviour of C. atratus was indirect and acted through the host insect species (Chapter 5). Additionally, different plant species had different effects on the attractiveness or repulsiveness of scale insect honeydew to the females (Fig. 5.5). The attractiveness of insect frass to parasitoids may also be influenced by the plant species on which the host is feeding (Sauls et al., 1979; Nordlund & Sauls, 1981; Inayatullah, 1983). Even different strains of plants may have different effects on parasitoid-searching behaviour. For example, the tachinid Lydella grisescens Robineau-Desvoidy is more effective against the European corn borer on one corn hybrid than another (Franklin & Holdaway, 1966).

It is therefore essential to ensure that host plants of the pest species, against which the parasitoids are released, do not negatively influence the female's host-searching ability. More effective use of parasitoids can be made in biological control programmes of insect pests, where interactions across three trophic levels are involved (Price et al., 1980; Price, 1981). It may be possible to increase the effectiveness of parasitoids of pests in crops or orchards, by cultivating varieties of plants that are more acceptable to the parasitoid species. In other words, the parasitoids (third trophic level) can be manipulated by altering the plants (first trophic level) on which the hosts feed.

SUMMARY

1. The host-searching behaviour of Coccophagous atratus, a heteronomous hyperparasitoid, was investigated to determine how the females are able to locate and parasitise two different types of hosts. Mated females oviposit both male and female eggs into the appropriate type of hosts, while virgin females oviposit only male eggs and are therefore not expected to locate hosts suitable for the deposition of male eggs.
2. C. atratus females were found to parasitise at least 21 species of scale insects which are found on at least 33 species of plants. The distribution of C. atratus is restricted to the Western and Eastern Cape Province, and did not coincide with the distribution of either their hosts or with the hosts' food plants.
3. Mated and virgin females were attracted to the odours from Chrysanthemoides monolifera, Cliffortia strobilifera and Trichilia emetica leaves in an olfactometer. However they were repelled by odours from Carpobrotus edulis, Dodonaea viscosa and Olea europaea. This response was found to be correlated with the presence of C. atratus-infested scale insects on the first three plant species in the field. None were present on the latter three plant species.
4. Odours from both parasitised and unparasitised scale insects were attractive to virgin and mated females but parasitised scale insect odour was only slightly attractive. When offered a choice between the two odours, females preferred odours from unparasitised scale insects and ignored odours from parasitised scale insects. The main host finding cue was identified as honeydew, so virgin females are able to locate unparasitised scale insects, even though they cannot oviposit in them. Virgin and mated females both appear to locate unparasitised scale insects initially and then locate parasitised scale insects by searching in the vicinity of unparasitised scale insects.
5. Field observations showed that C. atratus females do not locate C. monolifera plants without scale insect infestations. Mated females

- were placed on scale-free C. monolifera plants exhibited no searching behaviour and were clearly able to detect the absence of hosts without searching.
6. The behaviour of mated and virgin females differed once the hosts were located. Virgin females oviposited only in parasitised scale insects. Mated females oviposited in both parasitised and unparasitised scale insects. Females were able to identify their hosts with an external inspection before they probed.
 7. Cues used by virgin females to identify parasitised scale insects were; the humped shape of the scale insect mummy, a water soluble chemical present on the mummy and the physical presence of a parasitoid inside the mummy. The latter two had to be present TOGETHER before females would probe. As these cues are not present in unparasitised scale insects, they may possibly be used by both virgin and mated females to distinguish between parasitised and unparasitised scale insects.
 8. The results obtained in this study suggest that C. atratus cannot decide what sex of egg to lay and locate the required type of host. Sex ratios in this species are therefore probably influenced by the availability of hosts.
 9. The searching-behaviour of C. atratus, a heteronomous hyperparasitoid, is more complex than that of diphagous parasitoids, yet less complex than that postulated for heterotrophic parasitoids. This study, therefore, supports the postulated evolutionary sequence for heteronomous parasitoids, which is based on the complexity of their host searching behaviour.
 10. The efficiency of parasitoids in biological control programmes could be improved if (1) honeydew was sprayed on plants heavily infested with coccoid pests and (2) if varieties of plants, acceptable to the parasitoids, are cultivated.

REFERENCES

- ACOCKS, J.P.H. 1975. Veld types of South Africa. Memoirs of the Botanical Survey of South Africa 40 (second edition).
- ALLEN, D.C. 1972. Insect parasites of the saddled prominent, Heterocampa guttivitta (Lepidoptera: Notodontidae) in the northeastern United States. Canadian Entomologist 104: 1609-1622.
- ANDREWARTHA, H.G. & BIRCH, L.C. 1954. The Distribution and Abundance of Animals. University of Chicago Press, Chicago.
- ANNECKE, D.P. 1964. The encyrtid and aphelinid parasites (Hymenoptera: Chalcidoidea) of soft brown scale, Coccus hesperidum Linnaeus (Hemiptera: Coccidae) in South Africa. Entomology Memoirs. Department of Agricultural Technical Services. Republic of South Africa 7: 1-74.
- ANNECKE, D.P. & INSLEY, H.P. 1974. The species of Coccophagus Westwood, 1833 from the Ethiopian region (Hymenoptera: Aphelinidae). Entomology Memoirs. Department of Agricultural Technical Services. Republic of South Africa 37: 1-62.
- ARTHUR, A.P. 1962. Influence of host tree on abundance of Itoplectis conquisitor (Say), a polyphagous parasite of the European shoot moth Rhyacionia buoliana (Schiff.). Canadian Entomologist 99: 337-347.
- ARTHUR, A.P. 1966. Associative learning in Itoplectis conquisitor (Say) (Hymenoptera: Ichneumonidae). Canadian Entomologist 98: 213-223.
- ASKEW, R.R. 1971. Parasitic Insects. pp. 316. Heinemann Educational Books, London.
- ASKEW, R.R. 1975. The organisation of chalcid-dominated parasitoid communities centred upon endophytic hosts. In Evolutionary Strategies of Parasitic Insects and Mites pp. 130-153 Ed. P.W. Price. Plenum Press, New York.

- BRADBURY, W.C. & BENNETT, G.F. 1974. Behaviour of adult Simuliidae (Diptera).II. Vision and olfaction in near orientation and landing. Canadian Journal of Zoology 52: 1355-1364.
- BROODRYK, S.W. & DOUTT, R.L. 1966. The biology of Coccophagoides utilis Douth (Hymenoptera: Aphelinidae). Hilgardia 37: 233-254.
- BROWN, W.L., Jr., EISNER, T. & WHITTAKER, R.H. 1970. Allomones and kairomones: transpecific chemical messengers. Biological Science 20: 21-22.
- CADE, W. 1975. Acoustically orientating parasitoids: fly phonotaxis to cricket song. Science 190: 1312-1313.
- CALVERT, D.J. 1973. Experimental host preferences of Monoctonus paulensis (Hymenoptera: Braconidae), including a hypothetical scheme of host selection. Annals of the Entomological Society of America 66: 28-33.
- CAMORS, F.B. & PAYNE, T.L. 1972. Response of Hydenia unica (Hymenoptera: Pteromalidae) to Dendroctonus frontalis (Coleoptera: Scolytidae) pheromones and a host-tree terpene. Annals of the Entomological Society of America 65: 31-33.
- CAMORS, F.B. & PAYNE, T.L. 1973. Sequence of arrival of entomophagous insects to trees infected with the southern pine beetle. Environmental Entomology 2: 267-270.
- CARTON, Y. 1971. Biologie de Pimpla instigator F. 1793 (Ichneumonidae, Pimplinae) 1. Mode de perception de l'hôte. Entomophaga 16: 285-296
- CENDAÑA, S.M. 1937. Studies on the biology of Coccophagus (Hymenoptera), a genus parasitic on nondiaspidine Coccidae. University of California Publications in Entomology 6: 337-399.

- CILLIERS, C.J. 1967. A comparative biological study of three Ceroplastes species (Hem. Coccoidea) and their natural enemies. Entomology Memoirs. Department of Agricultural Technical Services. Republic of South Africa 13: 1-59.
- CLAUSEN, C.P. 1956. Biological control of insect pests in the continental United States. Technical Bulletin. United States Department of Agriculture No. 1139, pp. 151.
- CLAUSEN, C.P. 1958. Biological control of insect pests. Annual Review of Entomology 3: 291-310.
- CLAUSEN, C.P. (Ed.) 1977. Introduced parasites and predators of arthropod pests and weeds: a world review. Agricultural Research Service. United States Department of Agriculture. Agriculture Handbook 480: 1-545.
- COMPERE, H. 1926. Description of new coccid-inhabiting chalcidoid parasites (Hymenoptera). University of California Publications 4: 1-31.
- DAHMS, E.C. 1984. A review of the biology of species in the genus Melittobia (Hymenoptera: Eulophidae) with interpretations and additions using observations on Melittobia australica. Memoir of the Queensland Museum 21: 337-360.
- DEBACH, P. 1944. Environmental contamination by an insect parasite and the effect on host selection Annals of the Entomological Society of America 37: 70-74.
- DEBACH, P. (Ed.) 1964. Biological Control of Insect Pests and Weeds. pp 844. Chapman and Hall, London.
- DELEON, D. 1935. The biology of Coeloides dendroctoni Cushman (Hymenoptera: Braconidae) an important parasite of the mountain pine beetle (Dendroctonus monticolae Hpk). Annals of the Entomological Society of America 28: 411-424.

- DE LOTTO, G. 1974. New species of Filippia Targioni Tozzetti, 1868 (Homoptera: Coccoidea: Coccidae) from South Africa. Journal of the Entomological Society of Southern Africa 37: 207-214.
- DONALDSON, J.S. 1984. Sex ratios of Coccophagus atratus Compere (Hymenoptera : Aphelinidae) in relation to host abundance. Unpublished MSc Thesis, Rhodes University, Grahamstown.
- DOUTT, R.L. 1959. The biology of parasitic Hymenoptera. Annual Review of Entomology 4: 161-182.
- DOUTT, R.L. 1964. Biological characteristics of entomophagous adults. In: Biological Control of Insect Pests and Weeds. pp. 145-167. Ed. P. DeBach. Chapman & Hall, London.
- DOWELL, R.V., PUCKETT, D. & JOHNSON, M. 1981. Searching and ovipositional behaviour of Amitus hesperidum (Hym.: Platygasteridae) and Encarsia opulenta (Hym.: Aphelinidae) parasitoids of the citrus blackfly (Hom.: Aleyrodidae). Entomophaga 26: 233-239.
- ELZEN, G.W., WILLIAMS, H.J. & VINSON, S.B. 1983. Response by the parasitoid Campoletis sonorensis (Hymenoptera : Ichneumonidae) to chemicals (synomones) in plants: implications for host habitat location. Environmental Entomology 12: 1873-1877.
- FARKAS, S.R., SHOREY, H.H. & GASTON, L.K. 1974. Sex pheromones of Lepidoptera. Influence of pheromone concentration and visual cues on aerial odor-trail following by males of Pectinophora gossypiella. Annals of the Entomological Society of America 67: 633-638.
- FISHER, T.W. 1961. Biology of Physcus sp. (Eulophidae, Aphelinidae) from Burma. Journal of Economic Entomology 54: 444-446.
- FLANDERS, S.E. 1936. Sexual dimorphism of hymenopterous eggs and larvae. Science 84: 85.

- FLANDERS, S.E. 1937. Ovipositional instincts and developmental sex differences in the genus Coccophagus. University of California Publications in Entomology 6: 403-422.
- FLANDERS, S.E. 1953. Variations in susceptibility of citrus-infesting coccids to parasitization. Journal of Economic Entomology 46: 266-269.
- FLANDERS, S.E. 1959. Differential host relations of the sexes in parasitic Hymenoptera. Entomologia Experimentalis et Applicata 2: 125-142.
- FLANDERS, S.E. 1963. Hyperparasitism, a mutualistic phenomenon. Canadian Entomologist 95: 716-720.
- FLANDERS, S.E. 1964. Dual ontogeny of the male Coccophagus gurneyi Comp. (Hymenoptera: Aphelinidae): a phenotypic phenomenon. Nature 204: 944-946.
- FLANDERS, S.E. 1967. Deviate-ontogenies in the aphelinid male (Hymenoptera) associated with the ovipositional behavior of the parental female. Entomophaga 12: 415-427.
- FLANDERS, S.E. 1969. Alternative differential mortality of the sexes concomitant to sex differentiation in host relations. Entomophaga 14: 335-346.
- FLANDERS, S.E. 1973. Particularities of diverse egg deposition phenomena characterising carnivoroid Hymenoptera (with morphological and physiological correlations). Canadian Entomologist 105: 1175-1187.
- FLANDERS, S.E., BARTLETT, B.R. & FISHER, T.W. 1961. Coccophagus basalis (Hymenoptera: Aphelinidae): its introduction into California with studies of its biology. Annals of the Entomological Society of America 54: 227-236.
- FORCE, D.C. 1974. Ecology of insect host-parasitoid communities. Science 184: 624-632.

- FRANKLIN, R.T. & HOLDAWAY, F.G. 1966. A relationship of the plant to parasitism of the European corn borer by the tachinid parasite Lydella griscens. Journal of Economic Entomology 59: 440-441.
- GAHAN, A.B. 1933. The serphoid and chalcidoid parasites of the hessian fly. United States Department of Agriculture. Miscellaneous Publications 174: 1-147.
- GALIS, F. & VAN ALPHEN, J.J.M. 1981. Patch time allocation and search intensity of Asobara tabida Nees (Braconidae), a larval parasitoid of Drosophila. Netherlands Journal of Zoology 31: 595-611.
- GERLING, D. 1966. Studies with whitefly parasites of southern California. I. Encarsia pergandiella Howard (Hymenoptera: Aphelinidae). Canadian Entomologist 98: 707-724.
- GLASS, P.C.G. & VET, L.E.M. 1983. Host-habitat location and host location by Diachasma alloeum Muesebeck (Hym., Braconidae), a parasitoid of Rhagoletis pomonella Walsh (Dipt., Tephritidae). Netherlands Journal of Zoology 33: 41-54.
- GREANY, P.D. & OATMAN, E.R. 1972. Demonstration of host discrimination in the parasite Orgilus lepidus (Hymenoptera: Braconidae). Annals of the Entomological Society of America 65: 375-376.
- GREANY, P.D., TUMLINSON, J.H., CHAMBERS, D.L. & BOUSH, G.M. 1977. Chemically mediated host finding by Biosteres (Opus) longicaudatus, a parasitoid of Tephritid fruit fly larvae. Journal of Chemical Ecology 3: 189-195.
- GUTIERREZ, A.P. 1970. Studies on host selection and host specificity of the aphid hyperparasite Charips victrix (Hymenoptera: Cynipidae). 6. Description of sensory structures and a synopsis of host selection and host specificity. Annals of the Entomological Society of America 63: 1705-1709.

- HAGEN, K.S., GREANY, P., SAWALL Jr, E.F. & TASSAN, R.L. 1976. Tryptophan in artificial honeydews as a source of an attractant for adult Chrysopa carnea. Environmental Entomology 5: 458-468.
- HAYAT, M. 1983. The genera of Aphelinidae (Hymenoptera) of the world. Systematic Entomology 8: 63-102.
- HERREBOUT, W.M. & VAN DEN VEER, J. 1969. Habitat selection in Eucarelia rutilla Vill. III. Preliminary results of olfactometer experiments with females of known age. Zeitschrift für Angewandte Entomologie 64: 55-61.
- INAYATULLAH, C. 1983. Host selection by Apanteles flavipes (Cameron) (Hymenoptera: Braconidae): influence of host and host plant. Journal of Economic Entomology 76: 1086-1087.
- JACKSON, D.J. 1968. Observations on the female reproductive organs and the poison apparatus of Caraphractus cinctus Walker (Hymenoptera: Mymaridae). Zoological Journal of the Linnean Society 48: 59-81.
- JACOBI, E.F. 1939. Über lebensweise, auffinden des Wirtes und regulierung des individuenzahl von Mormoniella vitripennis Walker. Archives neerlandaises de zoologie 3: 197-282.
- JARRAYA, A. 1975. Contribution à l'étude des interactions hôte-parasite chez Coccus hesperidum L. (hom. Coccidae) et son parasite Coccophagus scutellaris Dalmon (hym. Aphelinidae). I. Etude expérimentale du comportement de ponte du parasite. Archives de l'Institut Pasteur de Tunis 52: 415-456.
- JUILLET, J.A. 1960. Some factors influencing the flight activity of hymenopterous parasites. Canadian Journal of Zoology 38: 1057-1061.
- KENNEDY, J.S. 1977a. Behaviourally discriminating assays of attractants and repellents. In: Chemical Control of Insect Behavior : Theory and Application. pp. 215-229. Eds. H.H. Shorey & J.J. McKelvey Jr. John Wiley & Sons, New York.

- KENNEDY, J.S. 1977b. Olfactory responses to distant plants and other odors. In: Chemical Control of Insect Behavior: Theory and Application. pp. 67-91. Eds H.H. Shorey & J.J. McKelvey Jr. John Wiley & Sons, New York.
- KENNEDY, J.S. 1978. The concepts of olfactory 'arrestment' and 'attraction'. Physiological Entomology 3: 91-98.
- KING, P.E. & RAFAI, J. 1970. Host discrimination in a gregarious parasitoid Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae). Journal of Experimental Biology 53: 245-254.
- KRUGER, F.J. 1979. Introduction. In: Fynbos Ecology : a Preliminary Synthesis. Eds J. Day, W.R. Siegfried, G.N. Louw & M.L. Jarman. National Scientific Programmes Report No 40: 1-6. CSIR, Pretoria.
- LAING, J. 1937. Host-finding by insect parasites. I. Observations on the finding of hosts by Alysia manductor, Mormoniella vitripennis and Trichogramma evanescens. Journal of Animal Ecology 6: 298-317.
- LECOMTE, C. & THIBOUT, E. 1984. Etude olfactométrique de l'action de diverses substances allélochimiques végétales dans la recherche de l'hôte par Diadromus pulchellus (Hymenoptera, Ichneumonidae). Entomologia Experimentalis et Applicata 35: 295-303.
- LEGNER, E.F. & THOMPSON, S.N. 1977. Effects of the parental host on host selection, reproductive potential, survival and fecundity of the egg-larval parasitoid, Chelonus sp. near curvimaculatus, reared on Pectinophora gossypiella and Phthorimaea operculella. Entomophaga 22: 75-84.
- LE PELLEY, R.H. 1937. Notes on the life-history of Cheiloneuris noxius Compere (Hym.). Bulletin of Entomological Research 28: 181-183.
- LEWIS, W.J. & JONES, R.L. 1971. Substance that stimulates host-seeking by Microplitis croceipes (Hymenoptera: Braconidae) a parasite of Heliothis species. Annals of the Entomological Society of America 64: 471-473.

- MALTAISE, J.B. & AUCLAIR, J.L. 1952. Occurrence of amino acids in honeydew of the crescent-marked lily aphid Myzus circumflexus (Buck.). Canadian Journal of Zoology 30: 191-193.
- MCDANIEL, J.R. & MORAN, V.C. 1972. The parasitoid complex of the citrus psylla Trioza erytreae (Del Guercio) (Homoptera: Psyllidae). Entomophaga 17: 297-317.
- MITTLER, T.E. 1958. Studies on feeding and nutrition of Tuberolachnus salignus (Gmelin) (Homoptera, Aphididae). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. Journal of Experimental Biology 35: 74-84.
- MOERICKE, V., PROKOPY, R.J., BERLOCHER, S. & BUSH, G.L. 1975. Visual stimuli eliciting attraction of Rhagoletis pomonella flies to trees. Entomologia Experimentalis et Applicata 18: 497-507.
- MOLL, E.J. & JARMAN, M.L. 1984. A clarification of the term fynbos. South African Journal of Science 80: 351-352.
- MONTEITH, L.G. 1955. Host preferences of Drino bohemica Mesn. with particular reference to olfactory responses. Canadian Entomologist 87: 509-530.
- MONTEITH, L.G. 1956. Influence of host movement on selection of hosts by Drino bohemica Mesn. as determined in an olfactometer. Canadian Entomologist 88: 583-586.
- MONTEITH, L.G. 1958. Influence of food plant of host on attractiveness of the host to tachinid parasites with notes on preimaginal conditioning. Canadian Entomologist 90: 478-482.
- MONTEITH, L.G. 1960. Influence of plants other than the food plants of their host on host-finding by tachinid parasites. Canadian Entomologist 92: 641-652.

- MUESEBECK, C.F.W. & DOHANIAN, S.M. 1927. A study in hyperparasitism, with particular reference to the parasites of Apanteles melanoscelus (Ratzeburg). United States Department of Agriculture. Department Bulletin 1487: 1-35.
- NEL, H.W., SEVENSTER-VAN DER LELIE, L.A., WOETS, J. & VAN LENTEREN, J.C. 1976. The parasite-host relationship between Encarsia formosa (Hymenoptera: Aphelinidae) and Trialeurodes vaporariorum (Homoptera: Aleyrodidae). II. Selection of host stages for oviposition and feeding by the parasite. Zeitschrift für Angewandte Entomologie 81: 372-376.
- NISHIDA, T. 1956. An experimental study of the ovipositional behaviour of Opius fletcheri Silvestri, a parasite of the melon fly. Proceedings of the Hawaii Entomological Society 16: 126-134.
- NORDLUND, D.A., JONES, R.L. & LEWIS, W.J. 1981. Eds Semiochemicals: Their role in pest control. pp. 306. John Wiley & Sons, New York.
- NORDLUND, D.A. & SAULS, C.E. 1981. Kairomones and their use for management of entomophagous insect. XI. Effects of host plants on kairomonal activity of frass from Heliothis zea larvae for the parasitoid Microplitis croceipes. Journal of Chemical Ecology 7: 1057-1061.
- NOYES, J.S. 1982. Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). Journal of Natural History 16: 315-334.
- PASTEELS, J.M. 1982. Is kairomone a valid and useful term? Journal of Chemical Ecology 8: 1079-1081.
- PIMM, S.L. & LAWTON, J.H. 1980. Are food webs divided into compartments? Journal of Animal Ecology 49: 879-898.
- PRICE, P.W. 1970. Trail odours: recognition by insects parasitic on cocoons. Science 170: 546-547.

- PRICE, P.W. 1981. Semiochemicals in evolutionary time. In Semiochemicals: Their role in pest control. Eds D.A. Nordlund, R.L. Jones & W.J. Lewis pp. 251-279. John Wiley & Sons, New York.
- PRICE, P.W., BOUTON, C.E., GROSS, P., MCPHERON, B.A., THOMPSON, J.N. & WEISS, A.E. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology and Systematics 11: 41-65.
- PRINSLOO, G.L. 1980. An illustrated guide to the families of African Chalcidoidea (Insecta: Hymenoptera). Department of Agriculture and Fisheries Science. Republic of South Africa. Science Bulletin 395: 1-66.
- QUEDNAU, F.W. & HÜBSCH, H.M. 1964. Factors influencing the host-finding and host-acceptance pattern in some Aphytis species (Hymenoptera: Aphelinidae). South African Journal of Agricultural Science 7: 543-554.
- READ, D.P., FEENY, P.P. & ROOT, R.B. 1970. Habitat selection by the aphid parasite Diaeretiella rapae (Hymenoptera: Braconidae) and hyperparasite Charips brassicae (Hymenoptera: Cynipidae) Canadian Entomologist 102: 1567-1578.
- RICHERSON, J.V. & BORDEN, J.H. 1972. Host finding behaviour of Coeloides brunneri (Hymenoptera: Braconidae). Canadian Entomologist 104: 1235-1250.
- ROELOFS, W.L. & CARDÉ, R.T. 1977. Responses of Lepidoptera to synthetic sex pheromone chemicals and their analogues. Annual Review of Entomology 22: 377-405.
- ROSEN, D. & DEBACH, P. 1979. Species of Aphytis of the World (Hymenoptera: Aphelinidae). In: Series Entomologica 17. Dr W. Junk, The Hague.

- ROTHERAY, G.E. 1981. Host searching and oviposition behaviour of some parasitoids of aphidophagous Syrphidae. Ecological Entomology 6: 79-87.
- RYAN, R.B. & RUDINSKY, J.A. 1962. Biology and habits of the douglas fir beetle parasite, Coeloides brunneri Viereck (Hymenoptera: Braconidae) in western Oregon. Canadian Entomologist 94: 748-763.
- SAAD, A.A.B. & BISHOP, G.W. 1976. Attraction of insects to potato plants through use of artificial honeydews and aphid juice. Entomophaga 21: 49-57.
- SALT, G. 1935. Experimental studies in insect parasitism. III. Host selection. Proceedings of the Royal Society of London. Series B 117: 413-435.
- SALT, G. 1937. Experimental studies in insect parasitism. V. The sense used by Trichogramma to distinguish between parasitized and unparasitized hosts. Proceedings of the Royal Society of London. Series B 122: 57-75.
- SALT, G. 1938. Experimental studies in insect parasitism. VI. Host suitability. Bulletin of Entomological Research 29: 223-246.
- SALT, G. 1940. Experimental studies in insect parasitism. VII. The effects of different hosts on the parasite Trichogramma evanescens Westw. (Hym., Chalcidoidea). Proceedings of the Royal Entomological Society of London. Series A 15: 81-124.
- SALT, G. 1958. Parasite behaviour and the control of insect pests. Endeavour 17: 145-148.
- SALT, G. 1961. Competition among insect parasitoids. Symposia of the Society for Experimental Biology. 15:96-119.
- SALT, G. 1975. The fate of an internal parasitoid, Nemeritis canescens, in a variety of insects. Transactions of the Royal Entomological Society of London 127: 141-161.

- SALT, G. 1976. The hosts of Nemeritis canescens, a problem in the host specificity of insect parasitoids. Ecological Entomology 1: 63-67.
- SAULS, C.E., NORDLUND, D.A. & LEWIS, W.J. 1979. Kairomones and their use for management of entomophagous insects. VIII. Effect of diet on the kairomonal activity of frass from Heliothis zea (Boddie) larvae for Microplitis croceipes (Cresson). Journal of Chemical Ecology 5: 363-369.
- SCHMIDT, G.T. 1974. Host-acceptance behaviour of Campoletis sonorensis towards Heliothis zea. Annals of the Entomological Society of America 67: 835-844.
- SHAHJAHAN, M. 1974. Erigeron flowers as a food and attractive odor source for Peristenus pseudopallipes, a braconid parasitoid of the tarnished plant bug. Environmental Entomology 3: 69-72.
- SHAHJAHAN, M. & STREAMS, F. 1973. Plant effects on host-finding by Leiophron pseudopallipes (Hymenoptera: Braconidae), a parasitoid of the tarnished plant bug. Environmental Entomology 2: 921-926.
- SHOREY, H.H. 1977. Interaction of insects with their chemical environment. In: Chemical Control of Insect Behaviour : Theory and Application. pp. 1-5. Eds H.H. Shorey & J.J. McKelvey Jr. Wiley-Interscience, New York.
- SHOREY, H.H. & MCKELVEY, J.J., Jr. 1977. Eds Chemical Control of Insect Behaviour: Theory and Application. pp. 414. John Wiley & Sons, New York.
- SIDHU, H.S. & PATON, R.L. 1970. Carbohydrates and nitrogenous compounds in the honeydew of the mustard aphid, Lipaphis erysini. Journal of Insect Physiology 16: 1339-1348.
- SIEGEL, S. 1956. Nonparametric Statistics for the Behavioral Sciences. pp. 311. McGraw-Hill, New York.

- SNOW, J.W., HAMM, J.J. & BRAZZEL, J.R. 1966. Geranium carolinianum as an early host for Heliothis zea and H. virescens in the southeastern United States with notes on associated parasites. Annals of the Entomological Society of America 59: 506-509.
- SOKAL, R.R. & ROHLF, F.J. 1981. Biometry. pp. 859. Second Edition. Freeman, San Francisco.
- SOPER, R.S., SHEWELL, G.W. & TYRRELL, D. 1976. Colcondamyia auditrix nov. sp. (Diptera: Sarcophagidae), a parasite which is attracted by the mating song of its host, Okanagana rimosa (Homoptera: Cicadidae). Canadian Entomologist 108: 61-68.
- SRIVASTAVA, P.N. & VARSHNEY, R.K. 1966. Composition of honeydew excreted by the lac insect, Kerria lacca (Homoptera: Coccoidea). I. Free amino acids. Entomologia Experimentalis et Applicata 9: 209-212.
- STARKS, K.J. & SCHUSTER, D.J. 1974. Response of Lysiphlebus testaceipes in an olfactometer to a host and a non-host insect and to plants. Environmental Entomology 3: 1034-1035.
- STEINWEDEN, J.B. 1929. Bases for the generic classification of the coccoid family Coccidae. Annals of the Entomological Society of America 22: 197-243.
- STERNLICHT, M. 1973. Parasitic wasps attracted by the sex pheromone of their coccid host. Entomophaga 18: 339-342.
- STREAMS, F.A., SHAHJAHAN, M. & LE MASURIER, H.G. 1968. Influence of plants on the parasitization of the tarnished plant bug by Leiophron pallipes. Journal of Economic Entomology 61: 996-999.
- SUBBA RAO, B.R. & RAI, P.S. 1969. Studies on the Indian species of the genus Coccophagus Westwood, 1883. Journal of Natural History 3: 381-392.

- TAMAKI, Y. 1977. Complexity, diversity, and specificity of behavior-modifying chemicals in Lepidoptera and Diptera. In: Chemical Control of Insect Behavior : Theory and Application. pp. 253-285. Eds H.H. Shorey & J.J. McKelvey Jr. John Wiley & Sons, New York.
- TAYLOR, J.S. 1932. Report on cotton insect and disease investigation. II. Notes on the American Bollworm (Heliothis obsoleta F.) on cotton and its parasite (Microbracon brevicornis Wesm.). Scientific Bulletin. Department of Agriculture. Union of South Africa 113.
- TAYLOR, T.A. & STERN, V.M. 1971. Host preference studies with the egg parasite Trichogramma semifumatum (Hymenoptera: Trichogrammatidae) Annals of the Entomological Society of America 64: 1381-1390.
- THORPE, W.H. & CAUDLE, H.B. 1938. A study of the olfactory responses of insect parasites to the food plant of their host. Parasitology 30: 523-528.
- THORPE, W.H. & JONES, F.G.W. 1937. Olfactory conditioning in a parasitic insect and its relation to the problem of host selection. Proceedings of the Royal Society. Series B 124: 56-81.
- TUCKER, J.E. & LEONARD, D.E. 1977. The role of kairomones in host recognition and host acceptance behavior of the parasite Brachymeria intermedia. Environmental Entomology 6: 527-531.
- UDVARDY, M.F.D. 1959. Notes on the ecological concepts of habitat, biotope and niche. Ecology 40: 725-728.
- VAN DEN ASSEM, J. & KUENEN, D.J. 1958. Host finding of Choetospila elegans Westw. (Hym., Chalcid.) a parasite of Sitophilus granarius L. (Coleopt., Curcul.). Entomologia Experimentalis et Applicata 1: 174-180.
- VAN LENTEREN, J.C. 1976. The development of host discrimination and the prevention of superparasitism in the parasite Pseudeucoila bochei (Hym.: Cynipidae). Netherlands Journal of Zoology 26: 1-83.

- VAN LENTEREN, J.C. & BAKKER, K. 1978. Behavioural aspects of the functional response of a parasite (Pseudocoila bochei Weld.) to its host (Drosophila melanogaster). Netherlands Journal of Zoology 28: 213-233.
- VAN LENTEREN, J.C., BAKKER, K. & VAN ALPHEN, J.J.M. 1978. How to analyse host discrimination? Ecological Entomology 3: 71-75.
- VAN LENTEREN, J.C. & DEBACH, P. 1981. Host discrimination in three ectoparasites (Aphytis coheni, A. lignanensis and A. melinus) of the oleander scale (Aspidiotus nerii). Netherlands Journal of Zoology 31: 504-532.
- VAN LENTEREN, J.C., NELL, H.W., SEVENSTER-VAN DER LELIE, L.A. & WOETS, J. 1976. The parasite-host relationship between Encarsia formosa (Hymenoptera: Aphelinidae) and Trialeurodes vaporariorum (Homoptera: Aleyrodidae). I. Host finding by the parasite. Zeitschrift für Angewandte Entomologie 81: 377-380.
- VET, L.E.M., VAN LENTEREN, J.C., HEYMANS, M. & MEELIS, E. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiological Entomology 8: 97-106.
- VIGGIANI, G. 1981. Hyperparasitism and sex differentiation in the Aphelinidae. In: The Role of Hyperparasitism in Biological Control: A Symposium. pp. 19-26. University of California, Division of Agricultural Sciences.
- VIGGIANI, G. 1984. Bionomics of the Aphelinidae. Annual Review of Entomology 29: 257-276.
- VINSON, S.B. 1975. Biochemical coevolution between parasitoids and their hosts. In: Evolutionary Strategies of Parasitic Insects and Mites. pp. 14-48. Ed. P.W. Price. Plenum Press, New York.
- VINSON, S.B. 1976. Host selection by insect parasitoids. Annual Review of Entomology 21: 109-133.

- VINSON, S.B. 1981. Habitat Location. In: Semiochemicals : Their Role in Pest Control. pp. 51-77. Eds D.A. Nordlund, R.L. Jones & W.J. Lewis. John Wiley & Sons, New York.
- VINSON, S.B. & GUILLOT, F.S. 1972. Host marking: source of a substance that results in host discrimination in insect parasitoids. Entomophaga 17: 241-245.
- VINSON, S.B., HARLAN, D.P. & HART, W.G. 1978. Response of the parasitoid Microterys flavus to the brown soft scale and its honeydew. Environmental Entomology 7: 874-878.
- VINSON, S.B. & IWANTSCH, G.F. 1980a. Host suitability for insect parasitoids. Annual Review of Entomology 25: 397-419.
- VINSON, S.B. & IWANTSCH, G.F. 1980b. Host regulation by insect parasitoids. Quarterly Review of Biology 55: 143-165.
- VINSON, S.B. & LEWIS, W.J. 1965. A method of host selection by Cardiochiles nigriceps. Journal of Economic Entomology 58: 869-871.
- WAAGE, J.K. 1979. Forging for patchily-distributed hosts by the parasitoid, Nemeritis canescens. Journal of Animal Ecology 48: 353-371.
- WALKER, M.G. 1940. Notes on the distribution of Cephus pygmaeus Linn. and of its parasite, Collyria calcitrator Grav. Bulletin of Entomological Research 30: 551-573.
- WALTER, G.H. 1983a. 'Divergent male ontogenies' in Aphelinidae (Hymenoptera: Chalcidoidea): a simplified classification and a suggested evolutionary sequence. Biological Journal of the Linnean Society 19: 63-92.

- WALTER, G.H. 1983b. Differences in host relationships between male and female heteronomous parasitoids (Aphelinidae: Chalcidoidea): a review of host location, oviposition and pre-imaginal physiology and morphology. Journal of the Entomological Society of Southern Africa 46: 261-282.
- WALTER, G.H. 1984. The evolution of heteronomous host relationships in Aphelinidae (Hymenoptera: Chalcidoidea), with special reference to the biology of Coccophagus bartletti Annecke & Insley. Unpublished PhD Thesis, Rhodes University, Grahamstown.
- WESELOH, R.M. 1969. Biology of Cheiloneurus noxius, with emphasis on host relationships and oviposition behaviour. Annals of the Entomological Society of America 62: 299-305.
- WESELOH, R.M. 1971a. Influence of primary (parasite) hosts on host selection of the hyperparasite Cheiloneurus noxius (Hymenoptera: Encyrtidae). Annals of the Entomological Society of America 64: 1233-1236.
- WESELOH, R.M. 1971b. Influence of host deprivation and physical host characteristics on host selection behaviour of the hyperparasite Cheiloneurus noxius (Hymenoptera: Encyrtidae). Annals of the Entomological Society of America 64: 580-589.
- WESELOH, R.M. 1981. Host location by parasitoids. In: Semiochemicals: Their Role in Pest Control. pp. 79-95. Eds D.A. Nordlund, R.L. Jones & W.J. Lewis. John Wiley & Sons, New York.
- WESELOH, R.M. & BARTLETT, B.R. 1971. Influence of chemical characteristics of the secondary scale host on host selection behavior of the hyper-parasite Cheiloneurus noxius (Hymenoptera: Encyrtidae). Annals of the Entomological Society of America 64: 1259-1264.
- WHITTAKER, R.H. LEVIN, S.A. & ROOT, R.B. 1973. Niche, habitat and ecotope. American Naturalist 107: 321-328.

- WILK, B.M. & KITAYAMA, C.Y. 1981. Host stage preference for deposition of male eggs by Coccophagus cowperi (Hym.: Aphelinidae). Entomophaga 26: 313-318.
- WILLIAMS, D.A. 1976. Improved likelihood ratio tests for complete contingency tables. Biometrika 63: 33-37.
- WILLIAMS, J.R. 1951. The factors which promote and influence the oviposition of Nemeritis canescens Grabs. Proceedings of the Royal Society of London. Series A 26: 49-58.
- WILLIAMS, J.R. 1972. The biology of Phycus seminotus Silv. and P. subflavus Annecke & Insley (Aphelinidae), parasites of the sugar-cane scale insect Aulacaspis tegalensis (Zhnt.) (Diaspididae). Bulletin of Entomological Research 61: 463-484.
- WILLIAMS, J.R. 1977. Some features of sex-linked hyperparasitism in Aphelinidae (Hymenoptera). Entomophaga 22: 345-350.
- WILSON, D.D., RIDGWAY, R.L. & VINSON, S.B. 1974. Host acceptance and oviposition behavior of the parasitoid Campoletis sonorensis. Annals of the Entomological Society of America 67: 271-274.
- WILSON, F. 1961. Adult reproductive behaviour in Asolcus basalis (Hymenoptera: Scelionidae). Australian Journal of Zoology 9: 739-751.
- WYLIE, H.G. 1958. Factors that affect host finding by Nasonia vitripennis (Walk.) (Hymenoptera: Pteromalidae). Canadian Entomologist 90: 597-608.
- WYLIE, H.G. 1965. Discrimination between parasitized and unparasitized housefly pupae by females of Nasonia vitripennis (Walk.) (Hymenoptera : Pteromalidae). Canadian Entomologist 97: 279-286.
- WYLIE, H.G. 1970. Oviposition restraint of Nasonia vitripennis on hosts parasitized by other hymenopterous species. Canadian Entomologist 103: 1537-1544.

- WYLIE, H.G. 1971. Oviposition restraint of Muscidifurax zaraptor on parasitized housefly pupae. Canadian Entomologist 103: 1537-1544.
- ZINNA, G. 1962. Ricerche sugli insetti entomofagi. III Specializzazione entomoparassitica negli Aphelinidae: Interdependenze biocenotiche tra due specie associate. Studio morfologico, etologico e fisiologico del Coccophagus similis (Masi) e Azotus matritensis Mercet. Bolletino del Laboratorio di Entomologia Agraria 'Filippo Silvestri' 20: 73-182.
- ZWÖLFER, H. & KRAUS, M. 1957. Bioclimatic studies on the parasites of two fir- and two oak tortricids. Entomophaga 2: 173-196.