THE EVOLUTION OF HETERONOMOUS HOST RELATIONSHIPS IN APHELINIDAE (HYMENOPTERA: CHALCIDOIDEA), WITH SPECIAL REFERENCE TO THE BIOLOGY OF *COCCOPHAGUS BARTLETTI* ANNECKE & INSLEY

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by

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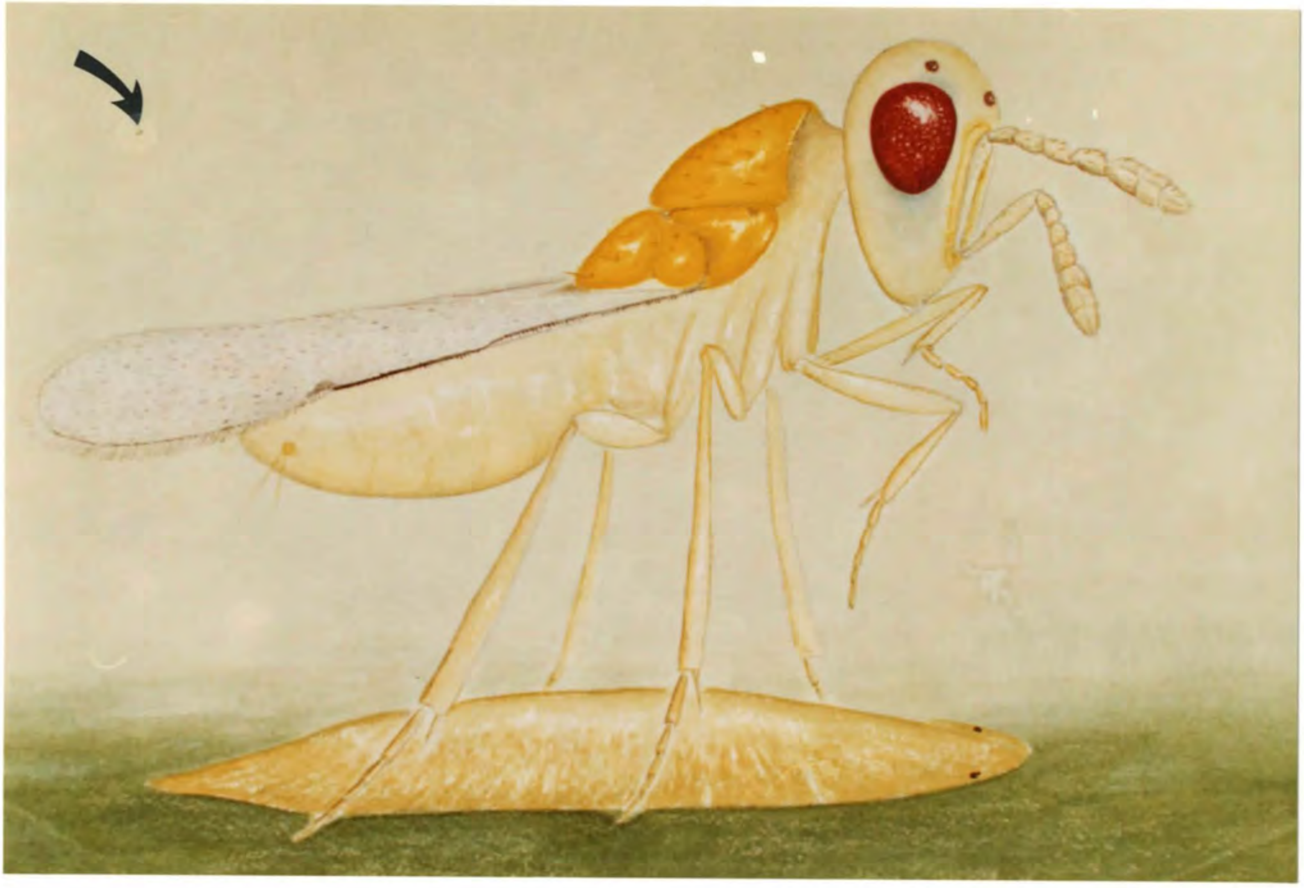
FRONTISPIECE



A female Coccophagus bartletti wasp preens before drinking haemolymph from a scale insect she has just drilled for this purpose. A specimen of C. bartletti (arrowed) indicates the actual size of this insect. Photograph of a painting by J.S. Donaldson.

CONTENTS

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RÉSUMÉ

Heteronomous host relationships are unique to parasitoids in about 8 aphelinid genera. Males have host relationships quite distinct from those of their females. Females of ALL species are primary endoparasitoids of homopterous hosts. Males, on the other hand, may be either primary ectoparasitoids of the same host species as their conspecific females, or they may be hyperparasitic upon parasitoids within Homoptera, and some are primary endoparasitoids of moth eggs. Species in these groups are termed DIPHAGOUS PARASITOIDS, HETERONOMOUS HYPERPARASITOIDS and HETEROTROPHIC PARASITOIDS, respectively.

The selective advantages proposed to explain the evolution of these unusual host relationships are examined in this thesis. The biology of a diphagous parasitoid was examined in detail because diphagous parasitism is considered the most primitive of heteronomous host relationships. Diphagous parasitism is thought to have evolved during a period when ovipositing females continuously encountered large proportions of parasitised hosts. Larval competition may have generated the selection pressures that favoured male ectoparasitism. Ectoparasitoids are known to be superior in competition against other larvae, even older ones.

A series of observations was carried out on the diphagous parasitoid, _Coccophagus bartletti_. Information was gathered on oviposition and host-feeding behaviour, daily activity patterns, and sex ratios in the laboratory and field. This enabled the design and interpretation of a series of observations on the responses of mated _C. bartletti_ females to already-parasitised hosts.

The results indicate that competition from other parasitoids probably played no role in the evolution of diphagous host relationships. Heteronomous hyperparasitoids, thought to have evolved from diphagous parasitoids, appear to be strong competitors because their males kill other parasitoids. However, an alternative hypothesis to the competition one, and based on the present study, is presented. Implications for the generally-held view, that competition is important in moulding species' characters, are discussed.

CHAPTER 1 : INTRODUCTION

Aphelinid host relationships. The aphelinids form a relatively small family of parasitic chalcidoid wasps, with more than 700 described species in over 40 genera (Yasnosh 1980, 1983; Hayat 1983). They are minute insects that rarely exceed 1 mm in length (Viggiani 1981; Hayat 1983), and which attack hosts from a number of insect orders. The most common and best-known hosts are aphids, scale insects and white flies (Yasnosh 1980; Viggiani 1981), and various aphelinid species have been used against these pest insects in numerous biological control programmes (Clausen 1940, 1977).

The host relationships of many aphelinid species in about 8 genera (Appendix 1) are unique among parasitic organisms: males have host relationships that are quite different from those of their conspecific females. Walter (1983a) referred to these as heteronomous host relationships. Although known for almost fifty years (Flanders 1936a, b, c, 1937), a convenient and generally-accepted terminology for the various categories of heteronomous parasitism never developed, and published records about the biology of the species involved are often anecdotal and difficult to locate. Pertinent evolutionary and ecological questions were difficult to frame because of the disordered nature of the literature. For these reasons I proposed a classification and evolutionary sequence for heteronomous parasitoids (Walter 1983a), and reviewed aspects of their biology (Walter 1983b). These two papers have been reproduced in this thesis as chapters 2 and 3 because they are of direct relevance to an understanding of aphelinid biologies discussed here, and they also provide background information about various aspects of behaviour and larval development.

Walter's (1983a) classification was based on the host relationships of MALES only, because females of all species are invariably endoparasitic in Coccoidea or Aleyrodoidea (Flanders 1936a, 1959; Ferrière 1965). Male larvae, depending on their species, may be (i) primary ectoparasitoids of Coccoidea or Aleyrodoidea, or (ii) secondary parasitoids (ecto- or endoparasitoids) of chalcidoid wasps within coccoids or aleyrodids, or (iii) primary endoparasitoids of lepidopterous eggs. Species in these groups are called DIPHAGOUS PARASITOIDS, HETERONOMOUS HYPERPARASITOIDS and HETEROTROPHIC PARASITOIDS respectively. This terminology was not used by earlier authors, but, for uniformity, I have incorporated these terms into their interpretations of aphelinid host relationships.

Evolution of heteronomous parasitism. The evolution and adaptive significance of heteronomous host relationships has been discussed to a limited extent only by Flanders (1937, 1959) and Zinna (1961, 1962), both of whom devoted most attention to heteronomous hyperparasitoids in discussions of the adaptive significance of heteronomous parasitism. Their dominant interest in heteronomous hyperparasitoids probably hinges on a number of factors: considerably more heteronomous hyperparasitoid species are known than diphagous or heterotrophic parasitoids; heteronomous hyperparasitoids have been used most often in biological control programmes; and, especially, the males of some of these species parasitise and kill conspecific females (autoparasitism, see chapter 2, p. 14). Autoparasitism is obligatory in some species.

Flanders (1937, 1959) argued that heteronomous hyperparasitism increases the bias in sex ratios toward females, because males inhabit a different, rarer, host than females. He also assumed (incorrectly) that all mated aphelinid females could produce only female offspring (Walter 1983b), and that this added to the bias in sex ratios. The selective advantage ('survival value' : Flanders 1959) proposed was that, for a given number of offspring, proportionately more females would be produced by heteronomous hyperparasitoids than by other parasitoids, and thus the host-searching capacity of these forms would be enhanced. However, parasitised hosts are not necessarily rarer than unparasitised ones, and the arrhenotokous parthenogenesis prevalent in almost all Hymenoptera (Crozier 1975), coupled with spermathecal control of fertilisation (Flanders 1939), theoretically permits the deposition of male eggs and female eggs in any ratio. So the female-biased sex ratios of heteronomous hyperparasitoids are probably not causally related to the sexes having host relationships different from each other.

Zinna (1961, 1962) thought that heteronomous hyperparasitism, particularly autoparasitism, evolved to stabilise population numbers of these parasitoids on the patches of hosts (*sensu* Hassell & Southwood 1978) they occupied. In this way resources would be conserved for future generations. This theory is generally favoured over that of Flanders' (Williams 1977; Yasnosh 1980; Viggiani 1981), but it is a group selectionist argument (see Maynard Smith 1964), and many have argued for group selection being ineffective in the evolution of adaptations, particularly where the postulated selection pressures are self-regulation of population numbers (eg Williams 1966; Lewontin 1970; Maynard Smith 1976; Alexander & Borgia 1978). Field data also contradict Zinna's ideas. The heteronomous hyperparasitoid, *Coccophagus atratus* Compere, usually parasitises all scale insects in a given patch (Donaldson 1984), and emergent females would have to fly off in search of more patches of hosts. If more than one female often colonises a patch of hosts, the effects of group selection would be diluted (Alexander & Borgia 1978). Zinna had assumed, too, that successive generations of parasitoids occupied a single patch: he even said that the majority of such species are practically incapable of flying, and therefore of leaving over-exploited resources.

I suggest an alternative hypothesis, that within a given patch of hosts heteronomous hyperparasitism allows an individual female to attack more hosts at the expense of competing parasitoids. Fig. 1.1 illustrates, in a hypothetical example, how more offspring can be produced (ten instead of five) in a given patch of hosts. Females develop within unparasitised hosts and males develop upon competitors. The sex ratio would then depend upon the ratio of parasitised to unparasitised scale insects present, and Donaldson (1984) has demonstrated that this is so in *C. atratus*.

The most likely sequence in the evolution of heteronomous parasitoids is perhaps the following (Walter 1983a), because it is the simplest. Diphagous parasitism probably evolved initially. Here males and females develop on the same host species, and only oviposition behaviour

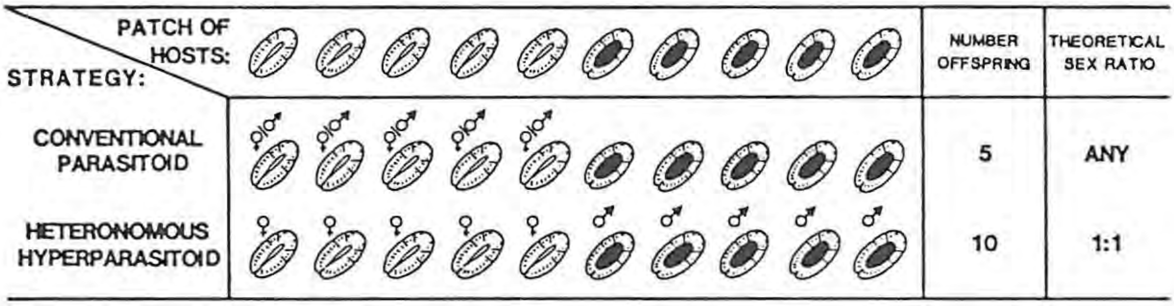

Fig. 1.1: Hypothetical example to illustrate how the unusual host relationships of heteronomous hyperparasitoids (females primary parasitoids within scale insects and males hyperparasitic) could increase their reproductive potential, relative to 'conventional' primary parasitoids, when mixed patches of parasitised and unparasitised hosts are encountered. Light models represent unparasitised scale insects, and dark ones contain parasitoids. A 'conventional' primary parasitoid could deposit male or female eggs within only 5 hosts in the patch illustrated, whereas a heteronomous hyperparasitoid is able to deposit female eggs in unparasitised (light) scale insects and male eggs in parasitised (dark) hosts to produce twice that number of offspring. Theoretically, the sex ratio of offspring produced by the 'conventional' parasitoid in this situation is variable, whereas that of the heteronomous hyperparasitoid is dependent upon the ratio of parasitised to unparasitised hosts.

need differ for eggs of either sex. Therefore host-searching behaviour and presumably host-acceptance criteria are similar whether the female is producing males or females. Heteronomous hyperparasitoids may have evolved from diphagous parasitoids and differ from them in having an additional complexity, in that males develop on different hosts (Hymenoptera) from females (Homoptera). Heterotrophic parasitism, which seems to involve an even greater modification of female behaviour, may then have evolved.

If heteronomous hyperparasitism evolved as a mechanism to widen the host range (Fig. 1.1), why did diphagous parasitism evolve first? If an individual of a primary parasitoid species lays an egg in or on a previously-parasitised host, generally it will overcome the older larva only if the incumbent is endo- or ectoparasitic and it itself is ectoparasitic (Flanders 1971; Askew 1975; Vinson & Iwantsch 1980). For example, the endoparasitic larvae of Coccophagoides utilis Doutt, Encarsia inquirenda (Silvestri) and Pteroptrix smithi (Compere) are easily destroyed by ectoparasitic Aphytis larvae that are younger than themselves (Kennett et al. 1966; Gerson 1968; Rivnay 1968 respectively). I propose that this superiority of ectoparasitic larvae in competition with older parasitoid larvae is the selective advantage for the evolution of ectoparasitism in male diphagous parasitoids. Presumably, ectoparasitism was limited to males because individuals that developed successfully in already-parasitised hosts may have been smaller than normal. In previously-parasitised scale insects there may be less food available because some of the nutrients within the host have already been consumed and formed into indigestible cuticle. It is often presumed (eg van den Assem 1976) that there is less penalty, in terms of fitness, to males being smaller than females (but see Charnov et al. 1981). In fact, females of many species tend to lay female eggs in larger hosts and male eggs in smaller ones.

The question I attempt to answer in this thesis is as follows, 'Is male ectoparasitism an adaptation that expands the range of hosts that can be parasitised by diphagous parasitoids?' If this is so, then diphagous parasitoids also have the hypothetical advantage proposed for heteronomous hyperparasitoids in Fig. 1.1, and presumably represent a step that resulted in the evolution of heteronomous hyperparasitism. The results obtained serve as a basis for discussing the evolution of heteronomous host relationships.

The species chosen for experimental work was Coccophagus bartletti Annecke & Insley, because Flanders (1959) reported it to be a diphagous parasitoid (under the name *C.* sp. nr. *flavidus* Compere; Flanders *in litt.* 2. *ix*. 1977). During a years' preliminary sampling of a large, persistent population of *Saissetia* species, I determined that *C. bartletti* is one of the commonest parasitoids of these scale insects in Grahamstown (33°19'S; 26°31'E). However a cryptic (sibling) species of *C. bartletti*, *C. lutescens* Compere, is sympatric, presumably over a wide area that includes Grahamstown (Fig. 1.2, compiled from published records, specimens in the National Collection of Insects in Pretoria (henceforth NCI), and personal collections). Therefore the morphology and coloration of both species was examined in detail (chapter 5) to find a rapid, reliable method to differentiate them. Observations on mating were also conducted on local populations to confirm the validity of the species, and whether any undescribed cryptic species existed locally. Once *C. bartletti* was isolated, behavioural observations were begun.

The first step in answering the question outlined above involved quantification of the behaviour associated with oviposition of male and female eggs and with host-feeding (chapter 6). This information, derived from observations on females exposed to unparasitised hosts, enabled subsequent observations of *C. bartletti* activity to be interpreted more realistically. Thereafter, daily activity patterns of *C. bartletti* were analysed and correlated with changes in the reproductive system (chapter 7). On exposure to hosts, *C. bartletti* females deposit their whole complement of eggs, the majority of them during the first three hours. This enabled these bouts of ovipositional activity to be examined for fixed patterns of male and female egg deposition (chapter 8). Sex ratios obtained in the laboratory were compared with those in the field to establish the reliability and realism of the experimental methods. It was then possible to determine whether mated *C. bartletti* respond to previously-parasitised hosts by depositing male eggs (chapter 9). Finally, the results obtained in chapter 9 are used to discuss the most likely pathway, and the possible selection pressures, in the evolution of heteronomous host relationships (chapter 10). Also in the final chapter, the implications of this study for competition theory in evolutionary biology are considered.

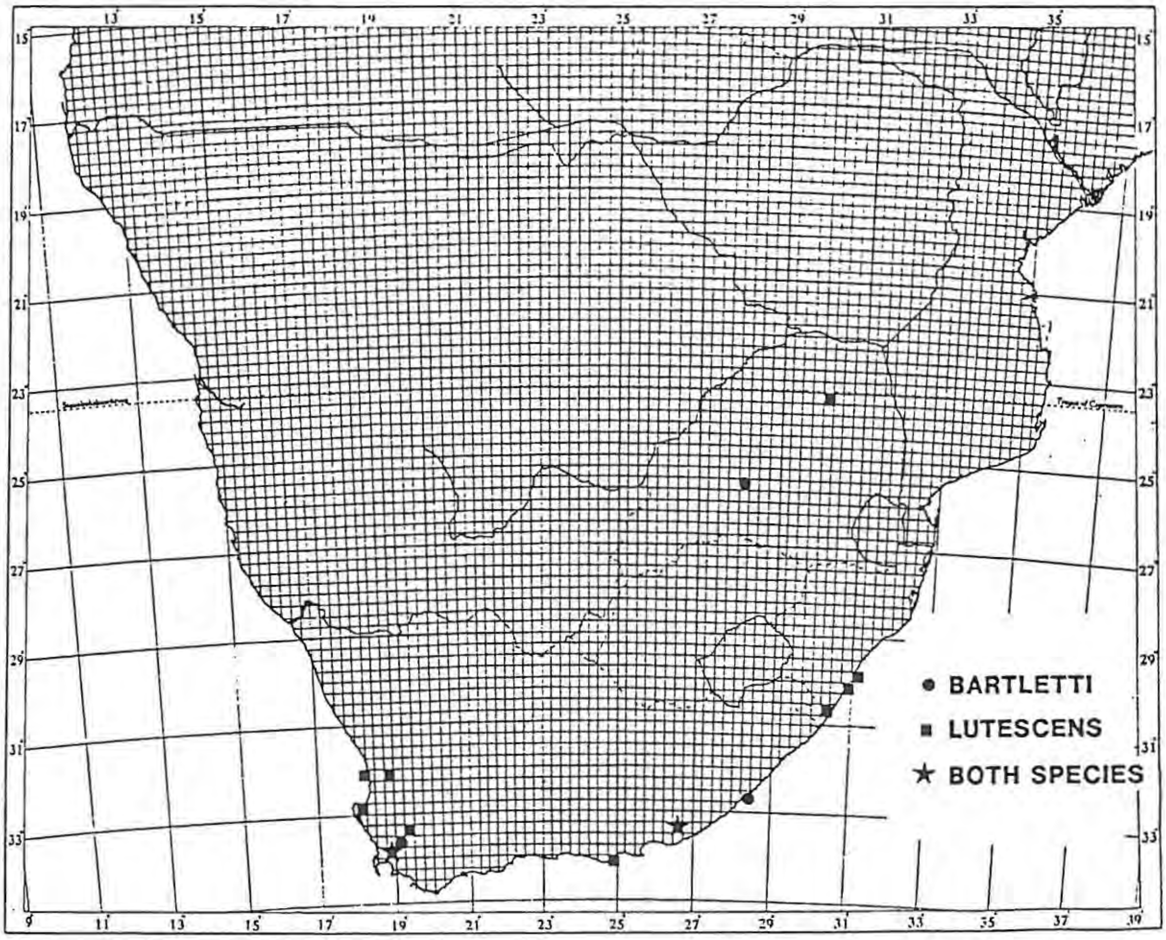

Fig. 1.2 : Distribution records for *Coccophagus bartletti* and *C. lutescens* in southern Africa. Although data are few, these species do occur together at widely-separated localities, which indicates a broad sympatric distribution.

CHAPTER 2 : TERMINOLOGY OF APHELINID HOST RELATIONSHIPS

Biological Journal of the Linnean Society (1983), *19*: 63–82. With 2 figures

'Divergent male ontogenies' in Aphelinidae (Hymenoptera: Chalcidoidea): a simplified classification and a suggested evolutionary sequence

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A classification of the different types of divergent male ontogeny in Aphelinidae is proposed to replace the little known and cumbersome systems previously published. All the Aphelinidae in which conspecific males and females have different host relationships, i.e. those that have divergent ontogenies, are here designated heteronomous parasitoids. Females of these species are primary endoparasitoids of Homoptera. The males of (i) diphagous parasitoids are primary ectoparasitoids of the same host species exploited by their females, (ii) heteronomous hyperparasitoids develop hyperparasitically, and (iii) heterotrophic species are endoparasitoids of lepidopterous eggs. Heteronomous hyperparasitoids can be classified further as obligate autoparasitoids, facultative autoparasitoids or alloparasitoids. All the species of heteronomous aphelinids whose biologies are known are listed according to the new classification, and the evolutionary sequence of these unusual host relationships is discussed.

KEY WORDS:—Divergent male ontogenies – Aphelinidae – heteronomous parasitoids – hyperparasitism – parthenogenesis.

CONTENTS

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INTRODUCTION

Unusual host relationships, unknown in any other group of insect parasitoids, have evolved in several related genera of the hymenopterous family Aphelinidae. These peculiarities were first described by Flanders (1936a, b, c, 1937). Females of these aphelinid species always develop as primary endoparasitoids of homopterous hosts. The males have host relationships that differ from those of conspecific females: they may be primary ectoparasitoids of Homoptera, hyperparasitoids of chalcidoid larvae or pupae within their homopterous hosts, or primary endoparasitoids of lepidopterous eggs. The hyperparasitic males of some species may even develop as facultative or obligate hyperparasitoids on females, and sometimes males, of their own species. Male eggs and first- and second-instar larvae may differ considerably in structure from the corresponding stages in their females (Flanders, 1936d, 1937, 1959a).

This phenomenon, where males of a parasitoid species have different host relationships from the females, has been referred to as 'differential development of the sexes' (Flanders, 1936b), 'differential host relations of the sexes' (Flanders, 1936a), 'divergent male ontogeny' (Flanders, 1967), and 'sexual ditrophicity' (Yasnosh, 1976). These phrases are cumbersome and cannot be used adjectivally. They are here replaced by a single word, heteronomy (subject to different laws or modes of growth). Heteronomous parasitoids can therefore be easily referred to.

Heteronomy is restricted to three of the seven aphelinid subfamilies recognized by Yasnosh (1976), the Coccophaginae, Physcinae and Prospaltellinae, and perhaps a fourth, the Azotinae. This unusual mode of development has been unequivocally demonstrated in species belonging to nine genera; *Aneristus, Coccophagus, Euxanthellus, Prococcophagus* and *Lounsburyia* (Coccophaginae); *Physcus* (Physcinae); and *Coccophagoides, Encarsia* and *Prospaltella* (Prospaltellinae). The biology of these genera has been studied because of their importance in biological control. Details of the biology of the other genera in these subfamilies are still unknown, although it has been suggested that certain species of *Archenomus* (Ferrière, 1965), *Pteroptrix* (formerly *Casca*) (Flanders *et al.*, 1958; Flanders, 1966; but see Bar & Gerling, 1971) (Prospaltellinae) and *Azotus* (Viggiani, 1973) (Azotinae) may be heteronomous.

The primary aim of this paper is to develop a simplified classification of the unusual host relationships of heteronomous aphelinids. This classification is then used as the basis for (i) a series of lists of species that exhibit the phenomenon in its various forms, and (ii) a postulated sequence for the evolution of different categories of heteronomous parasitism. A brief résumé of parthenogenesis prefaces the account because heteronomy is a consequence of, and is maintained by, the parthenogenetic characteristics of the Hymenoptera.

PARTHENOGENESIS IN HYMENOPTERA

To classify parthenogenesis of any hymenopteran species, two questions must be answered. (a) Are females produced sexually or by parthenogenesis? All females are diploid regardless of how they develop. (b) Are males produced or not? All males develop parthenogenetically and are haploid. Details of exceptional diploid males are available in White (1973) and Kerr (1974).

Based on these criteria, three categories of parthenogenesis exist in Hymenoptera.

(i) Arrhenotokous species have diploid females that develop sexually from fertilized eggs, and parthenogenetic males that are haploid and develop from unfertilized eggs. Most hymenopteran species have these characteristics, and mated females of many of them have been proved capable of controlling fertilization of the egg that is being deposited, thus determining the sex of each offspring (e.g. Brunson, 1937, 1938; Sandlan, 1979). This latter process has been termed selective fertilization (Flanders, 1969a).

(ii) In some Hymenoptera, there are no males and diploid females are produced parthenogenetically. This phenomenon, strictly, is thelytoky, a term that may be used for any organism producing only female offspring.

(iii) Other Hymenoptera produce *both* diploid females and haploid males by parthenogenesis. In these species most eggs yield females, but male production is dependent on a postulated variation of meiosis that gives rise to haploid eggs (Flanders, 1946, 1965). The mechanism is unknown, although haploidy may be induced if the developing parent is subjected to high temperatures (Wilson & Woolcock, 1960; Wilson, 1962; Flanders, 1965; Bowen & Stern, 1966). Nutrition may also be involved (Flanders, 1944a, b, 1945, 1946; Smith, 1957). Parthenogenetic development of both haploid males and diploid females is termed deuterotoky, amphitokous parthenogenesis or ampherotokous parthenogenesis (Flanders, 1945; Doutt, 1959, 1964). Although deuterotoky (deutero = second) is the more common term, amphitoky (amphi = both) is retained here because of its more correct etymology. These terms apply also to diploid organisms that produce both males and females parthenogenetically (White, 1973; Enghoff, 1976). The males of amphitokous Hymenoptera are not necessary for reproduction although they may be viable (Orphanides & Gonzalez, 1970; Rössler & DeBach, 1973).

CLASSIFICATION OF HETERONOMOUS PARASITISM

The remarkable biologies of heteronomous aphelinids have possibly remained little known and poorly understood because of the lack of an appropriate and generally accepted terminology. Heteronomous species have the following attributes.

(i) Females develop as primary endoparasitoids of either Aleyrodidae, Pseudococcidae, Diaspididae or Eriococcidae (Flanders, 1936a, 1952a, 1959a; Ferrière, 1965). Females of some species parasitize scale insects in other coccoid families, but the host relationships of their males have not been determined (see the list of host insects in Annecke & Insley, 1974).

(ii) Males, on the other hand, develop either:

(a) as primary ectoparasitoids of coccoid or aleyrodid hosts (Flanders, 1959a, 1967; Zinna, 1961; Ferrière, 1965);

or (b) as hyperparasitoids of other species only, conspecifics only, or both;

or (c) as primary endoparasitoids of lepidopterous eggs.

In addition, the hyperparasitic males referred to in category (iib) are either direct or indirect hyperparasitoids. Where the male is a direct hyperparasitoid the female deposits haploid eggs only in coccoids that are already parasitized. This is the predominant group. In contrast, females of species with indirect hyperparasitic males oviposit in the coccoid whether it is parasitized or not (see Smith, 1916; Flanders, 1943, 1963). The males of direct hyperparasitic species develop consistently either as endoparasitoids or as ectoparasitoids. Indirect hyperparasitoid males develop only ectoparasitically.

Hyperparasitic males usually develop on encyrtids, eulophids or other aphelinids (Chalcidoidea) (Flanders, 1937, 1959a; Zinna, 1961; McDaniel & Moran, 1972; Williams, 1972), although a platygasterid (Proctotrupoidea) host has also been recorded (Flanders, 1969b). In addition, they are hyperparasitic in the same homopterous host species normally parasitized by conspecific females. There are exceptions. For example, *Coccophagus malthusi* females parasitize wax scales on *Acacia karroo* trees, and males are reared only from other coccid genera, usually on different host plants (Compere, 1926; Flanders, 1937; Annecke, 1964; Annecke & Insley, 1974). *Euxanthellus* sp. males show a similar relationship, and may be reared from psyllids (McDaniel & Moran, 1972) and aleyrodids (Valentine, 1964), whereas *Euxanthellus* females parasitize coccids (Compere, 1936; Annecke & Prinsloo, 1976).

Flanders (1959a) proposed the first comprehensive classification of heteronomous parasitism (Appendix 1), which he based on ovipositional behaviour. Later Zinna (1961, 1962) introduced a classification (Appendix 2) very similar to the generalized scheme discussed in the introduction to this section, but it has two important differences: (i) no distinction was made between indirect and direct hyperparasitism, or between endo- and ectoparasitism in direct hyperparasitoids; and (ii) his terminology is complicated and cumbersome (Appendix 2). A third scheme was published by Ferrière (1965), who combined Flanders' (1959a) and Zinna's (1961, 1962) systems (Appendix 3). This compounded the disadvantages of Zinna's classification.

Flanders (1967) extended his 1959 classification in the form of a key, and this is represented diagrammatically in Appendix 4. The only major change to the older

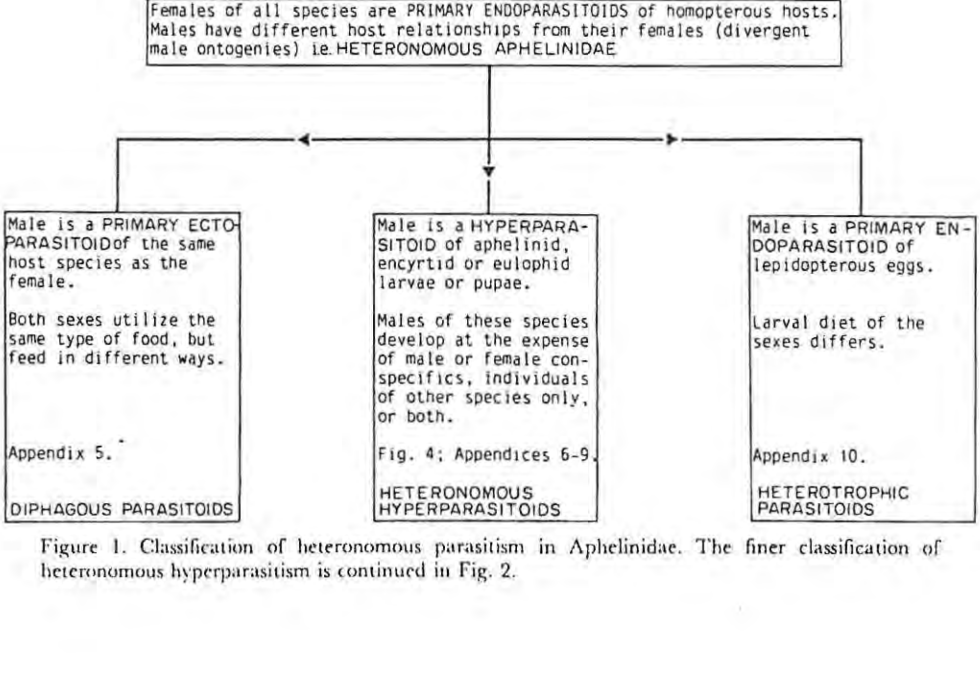

Figure 1. Classification of heteronomous parasitism in Aphelinidae. The finer classification of heteronomous hyperparasitism is continued in Fig. 2.

system is his consideration that ovipositing females have to alter their ovipositional behaviour to deposit male eggs ectoparasitically on unparasitized scale insects. Flanders introduced order into a complex subject, but his key has limited practical applicability.

The existence of a number of different classifications has hindered communication about the biology of these aphelinids. A simpler and more manageable classification is proposed in Fig. 1. The terms chosen are adapted from Zinna's (1961, 1962) terminology. The three principal types of heteronomous parasitoids are:

(i) Diphagous parasitoids, which have males that are primary ectoparasitoids of scale insects.
(ii) Heteronomous hyperparasitoids with hyperparasitic males.
(iii) Heterotrophic parasitoids, whose males are primary endoparasitoids of lepidopterous eggs.

Zinna's (1961, 1962) term, adelphoparasitism, becomes superfluous since heteronomous hyperparasitoids are more simply described as such without the need for another term. Heteronomous hyperparasitism may be subdivided as shown in Fig. 2. Those species whose males always develop on conspecifics of either sex are obligate autoparasitoids, those whose males are parasitic on individuals of other species or their own species are facultative autoparasitoids, and the males of alloparasitoids always develop on other species. Species from any of these categories may be either direct or indirect heteronomous hyperparasitoids (Fig. 2).

It is important to distinguish true heteronomous parasitoids from those in which individuals of the same species may develop with different host relationships, but without these bearing an obligatory relationship to the sex of the individual, as in the aphelinids. An example is the non-heteronomous aphelinid *Marietta* which has hyperparasitic males and females (Compere in Clausen, 1940; Flanders, 1953a;

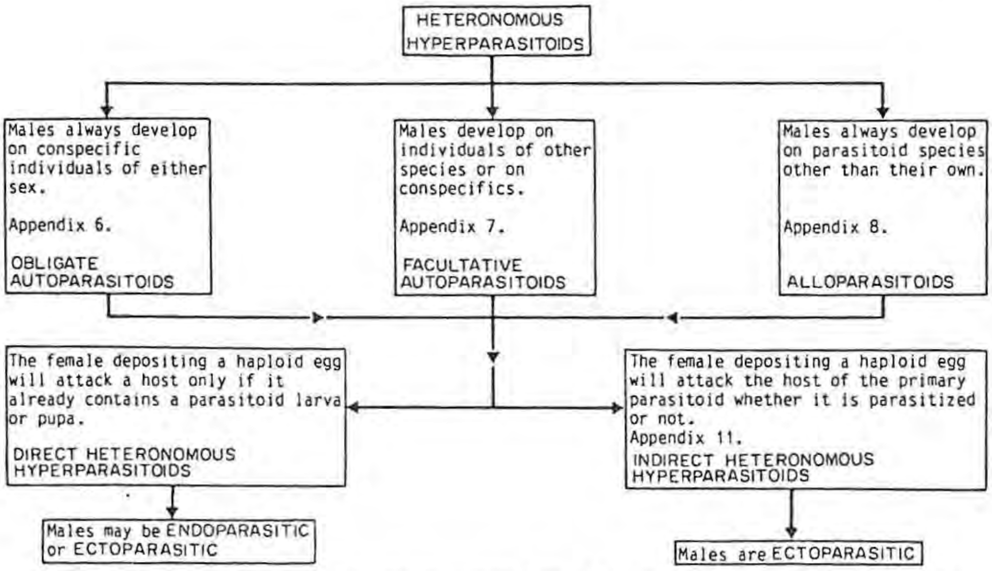

Figure 2. Classification of heteronomous hyperparasitism. This classification is a continuation from Fig. 1.

Zinna, 1962). However, *Marietta* males may develop upon occasion as 'incidental autoparasitoids' of *Marietta* females (Zinna, 1962), but this is not an obligate relationship. Such autoparasitism usually occurs in culture or in the field when population densities are high relative to those of the host (Spencer, 1926; Narayanan, 1939; Chumakova & Goryunova, 1963).

HOST RELATIONSHIPS OF HETERONOMOUS APHELINIDS

The classification proposed in Figs 1 & 2 is applicable to all those species of heteronomous aphelinids whose host relationships have been reported in the literature (see Nikol'skaya & Yasnosh (1966) for a review of aphelinid biology in general). Appendix 5 lists those species that are diphagous, Appendix 6 lists obligate autoparasitoids, Appendix 7 facultative autoparasitoids, Appendix 8 alloparasitoids and Appendix 9 lists those heteronomous hyperparasitoids whose exact host relationships are unknown. Heterotrophic parasitoids are included in Appendix 10. The references cited in these appendices are not all definitive works; many of them contain information that is anecdotal or circumstantial.

There are two points of relevance in the interpretation of Appendices 5–10. Firstly, the assumption has been made that if a female heteronomous hyperparasitoid deposits a male egg endo- or ectoparasitically, the male will develop accordingly. This is based on Hagen's (1964) assertion that in the parasitic Hymenoptera, development is endoparasitic where the egg is deposited in the host, but where the egg is deposited externally to the host, endoparasitic development is rare. Hagen draws on Flanders (1937) in maintaining that the few examples of the latter are found in the genus *Coccophagus*; the species Hagen referred to is presumably *C. gurneyi*, but the biology of this species is not correctly reported in Flanders (1937), and is not necessarily exceptional in this respect (Flanders, 1964).

Secondly, when one species in a genus is heteronomous, all species generally are. The exception to this pattern occurs in heteronomous genera that also contain thelytokous or amphitokous species (Appendix 12). The thelytokous species seldom, if ever, produce males. Male production in amphitokous species is more common. Ovipositing amphitokous females have no control over the sex of the eggs they lay: all are diploid except for those altered to the haploid state by environmental conditions. They therefore cannot deposit eggs of either sex differentially. These factors exclude amphitokous species from being diphagous or heterotrophic parasitoids: larvae that hatch from male eggs deposited into their females' hosts would die because they are not adapted to these conditions. Therefore only heteronomous hyperparasitism is consistent with amphitoky because the host of the male is found within the female's homopterous host. The haploid eggs of these amphitokous heteronomous hyperparasitoids are deposited indirectly within the host of the female, and the male larva must locate a developing primary parasitoid. A list of the heteronomous parasitoids that deposit male eggs indirectly is contained in Appendix 11. All the amphitokous species listed in Appendix 12 also appear in Appendix 11.

POSTULATED EVOLUTIONARY SEQUENCE

The most primitive category of heteronomous parasitism is probably diphagous parasitism. In these species, as in non-heteronomous parasitoids, male and female larvae attack the same host species, but the sexes do feed in different ways, males externally and females internally. There is also a possible link with non-heteronomous parasitoids, found in recent observations on *Aphytis melinus* and *A. lingnanensis*. Although both sexes of these species are primary ectoparasitoids, adult females usually deposit male and female eggs on different parts of the hosts' bodies, males below and females above (R. Kfir, pers. commn, 26 March 1982). If the ancestral heteronomous parasitoid practised differential oviposition like this, the evolution of sexually dimorphic larvae adapted to live under different conditions would be a relatively small step.

Clearly related to diphagous parasitism is heteronomous hyperparasitism, particularly as found in those species that affix male eggs directly onto parasitoid larvae and pupae. Ovipositing heteronomous hyperparasitoid females select different sites *and* different hosts for eggs of either sex, but this latter trait is not as drastic a deviation from the behaviour of ovipositing diphagous parasitoid females as initially seems. The same type of host is still sought for deposition of both male and female eggs (coccoids and parasitoids within coccoids), but now only discrimination of hosts for eggs of either sex has changed. Species with other forms of heteronomous hyperparasitism radiated from this primitive stock. Species evolved in which male eggs are deposited (i) directly and endoparasitically into parasitoid larvae or pupae, and (ii) indirectly into unparasitized or parasitized coccoids or aleyrodids. In addition, species such as *Coccophagus malthusi* and *Euxanthellus* sp. evolved. Their males develop on parasitoids within coccoids and other homopterous insects not parasitized by conspecific female larvae.

Heterotrophic parasitism may represent an extreme development of this trend to select different host species for male and female eggs. In order for these host relationships to evolve, it seems most likely that females possessed the ability to orientate to two distinct host types, hence the clear link with heteronomous hyperparasitism.

Zinna (1962) proposed a similar evolutionary sequence, but he gave no reasons for believing why evolution proceeded along this particular pathway. The sequence proposed here is based simply on host-searching behaviour. It leads from the simplest set of behaviours (same host species; different oviposition sites) through intermediate situations (similar hosts, i.e. coccoid & coccoid + parasitoid; different oviposition sites) to the most complex set of behaviours (different hosts).

No statement is made on the selective pressures that led to the evolution of heteronomous parasitism. Previous speculation has concentrated almost entirely on the selective advantages of heteronomous hyperparasitism, and particularly on autoparasitism because this poses the interesting problem of female offspring being sacrificed for the production of males. The most popular theory suggests that autoparasitism is a group-advantageous trait that confers stability on isolated populations of coccid hosts and parasitoids (Zinna, 1962; Williams, 1977; Viggiani, 1981).

Before this or other theories become generally accepted, however, the biology and ecology of heteronomous hyperparasitoids requires close scrutiny. A more detailed examination of the role of natural selection in the evolution of autoparasitism and other forms of heteronomy will be made in a further publication.

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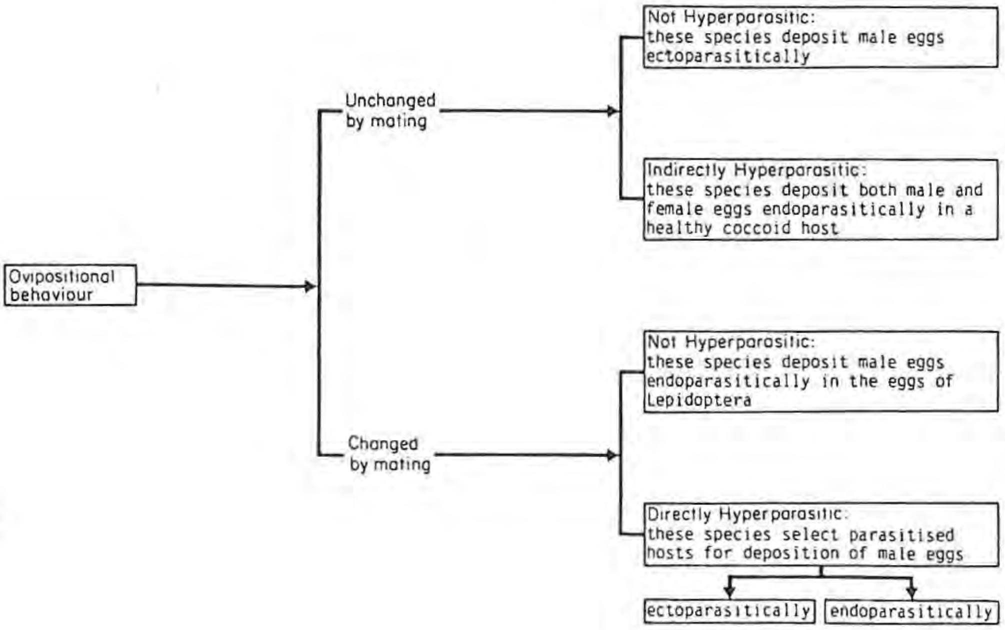
ZINNA, G., 1962. Ricerche sugli insetti entomofagi. III. Specializzazione entomoparassitica negli Aphelinidae: Interdipendenze biocenotiche tra due specie associate. Studio morfologico, etologico e fisiologico del *Coccophagoides similis* (Masi) e *Azotus matritensis* Mercet. *Bollettino del Laboratorio di entomologia agraria 'Filippo Silvestri', 20:* 73–182.

APPENDIX I

Flanders' (1959a) classification of heteronomous parasitism in the Aphelinidae, modified from his original table



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*Also quoted as A. O. Novoa Zañartu.

APPENDIX 2

Zinna's (1961, 1962) classification of heteronomous parasitism

Male development	Zinna's terminology
Primary ectoparasitoid	Primary ditrophic arrhenoparasitism
Secondary parasitoid of other primary parasitoid species	Obligatory secondary heterotrophic arrhenoparasitism
Secondary parasitoid *either* (i) of individuals of its own species *or* (ii) on individuals of other primary parasitoid species	(i) Facultative male adelphoparasitism* (ii) Facultative secondary heterotrophic arrhenoparasitism
Secondary parasitoid of individuals of its own species	Obligatory male adelphoparasitism*
Primary endoparasitoid of lepidopterous eggs	Primary heterotrophic arrhenoparasitism

*These two categories can be grouped, termed either adelphoparasitic male arrhenoparasitism or autotrophic arrhenoparasitism.

APPENDIX 3

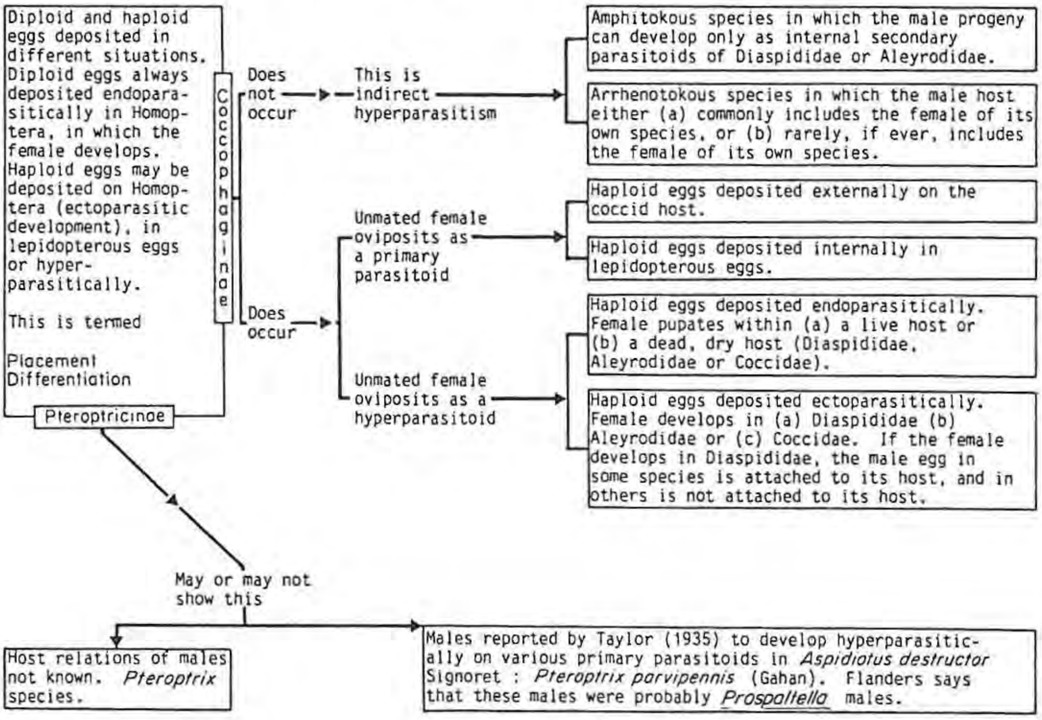
Ferrière's (1965) classification of heteronomous parasitism in the Aphelinidae. This, with the introduction of new terms is a combination of Flanders' (1959a) (appendix 1) and Zinna's (1961, 1962) (appendix 2) classification

Male development	Ferrière's terminology
Ectoparasitoid of the same scale insect species as the female	Primary ditrophic arrhenoparasitism
Secondary parasitoid of a female of its own species	Secondary autotrophic arrhenoparasitism *or* obligatory adelphoparasitism*
Hyperparasitoid of individuals of other primary parasitoid species, often in other scale insect species;	Secondary heterotrophic arrhenoparasitism*
and when a male can develop on either a larvae of other parasitoid species or on a larva of its own species	Facultative adelphoparasitism*
Endoparasitic in lepidopterous eggs	(No term)

*Males in these sections may be endo- or ectoparasitic.

APPENDIX 4

A diagrammatic representation of Flanders' (1967) classification of heteronomous parasitism in aphelinids. The subfamilies of Aphelinidae accepted by Flanders are different from those of Yasnosh (1976)



Diploid and haploid eggs deposited in different situations. Diploid eggs always deposited endoparasitically in Homoptera, in which the female develops. Haploid eggs may be deposited on Homoptera (ectoparasitic development), in lepidopterous eggs or hyperparasitically.

This is termed

Placement Differentiation

Amphitokous species in which the male progeny can develop only as internal secondary parasitoids of Diaspididae or Aleyrodidae.

Arrhenotokous species in which the male host either (a) commonly includes the female of its own species, or (b) rarely, if ever, includes the female of its own species.

Haploid eggs deposited externally on the coccid host.

Haploid eggs deposited internally in lepidopterous eggs.

Haploid eggs deposited endoparasitically. Female pupates within (a) a live host or (b) a dead, dry host (Diaspididae, Aleyrodidae or Coccidae).

Haploid eggs deposited ectoparasitically. Female develops in (a) Diaspididae (b) Aleyrodidae or (c) Coccidae. If the female develops in Diaspididae, the male egg in some species is attached to its host, and in others is not attached to its host.

Host relations of males not known. *Pteroptrix* species.

Males reported by Taylor (1935) to develop hyperparasitically on various primary parasitoids in *Aspidiotus destructor* Signoret : *Pteroptrix parvipennis* (Gahan). Flanders says that these males were probably *Prospaltella* males.

APPENDIX 5

Diphagous parasitoids: these are heteronomous species in which both sexes develop as primary parasitoids of homopterous hosts. Females develop endoparasitically and males ectoparasitically (Fig. 1)

Species	Author
Coccophagus bartletti Annecke & Insley	Flanders, 1959a
Coccophagus hemera (Walker)	Zinna, 1961
Coccophagus longifasciatus Howard	Flanders, 1959a, 1967
Coccophagus ochraceus Howard	Flanders, 1942a, 1953a, 1967, 1969a
Coccophagus scutatus Howard*	Flanders, 1937
Encarsia conjugata (Masi)	Ferrière, 1965
Encarsia partenopea Masi	Ferrière, 1965
Prococcophagus saissetiae Annecke & Mynhardt	Y. Rössler (pers. commn. 25 Jan. 1979)
Prospaltella ectophaga Silvestri†	Silvestri, 1935

*Flanders (1937, p. 408) listed the male of this species as a primary endoparasitoid. This was, however, based on incomplete life-history studies, so it probably develops as a primary ectoparasitoid or as a heteronomous hyperparasitoid (and is therefore also listed in Appendix 9).

†Silvestri (1935) recorded both sexes as ectoparasitoids, but Flanders (1959a) considered this questionable, and hinted at the species being a diphagous parasitoid.

APPENDIX 6

Obligate autoparasitoids: females of these heteronomous species are primary endoparasitoids of homopterous hosts, and males develop hyperparasitically only through individuals of their own species (Figs 1 & 2)

Species	Male development (ecto/endoparasitic)	Author(s)
Coccophagoides abnormicornis Girault	?	Doutt in Zinna, 1962
Coccophagoides kuwanai Silvestri*	Ecto	Flanders, 1960
Coccophagoides utilis Doutt	Ecto	Broodryk & Doutt, 1966; Kennett *et al.*, 1966
Coccophagus gossypariae Gahan	?	Sumaroka, 1965; Ceianu, 1968
Coccophagus semicircularis (Foerster)†	Endo	Flanders, 1936b, c, 1937, 1953a, 1959a, 1967; Jarraya, 1975, 1977, 1978; Zinna, 1961
Encarsia formosa Gahan	? Egg laid in or on female larva	Gerling, 1966a
Physcus fulvus Compere & Annecke	Endo	Flanders, 1952b, 1953b; Clausen, 1956
Physcus intermedius Gahan	Endo but completes feeding as an ecto	Taylor, 1935; Flanders, 1936c, 1967
Physcus sp. nr. *varicornis* (Howard)‡	Endo	Viggiani, 1970
Prospaltella lahorensis Howard	Endo but completes feeding as an ecto	Viggiani & Mazzone, 1978
Prospaltella perniciosi Tower, San José scale race, bisexual form	Endo	Rice, 1937; Flanders, 1939a, 1960; Chumakova & Goryunova, 1963

*Flanders (1960) says that this species is supposedly an obligate autoparasitoid because R. L. Doutt (unpub. notes) found this to be the case with another species of the genus (presumably *Coccophagoides utilis* described in Doutt, 1966). This reasoning does not necessarily hold: *Coccophagoides similis* is a facultative autoparasitoid (Zinna, 1962: see appendix 7).

†Flanders (1937), however, found this species ovipositing in the prepupae and pupae of *Lounsburyia trifasciata* (Compere) and *Coccophagus caridei* (Brèthes) females.

‡This species is possibly a facultative autoparasitoid. This point is not clear from the review of Viggiani's (1970) paper.

APPENDIX 7

Facultative autoparasitoids: females are primary endoparasitoids of homopterous hosts, and males are hyperparasitoids of conspecifics or individuals of other species (Figs 1 & 2)

Species	Male development (ecto/endoparasitic)	Author(s)
Coccophagoides similis (Masi)	Ecto*	Zinna, 1962
Coccophagus capensis Compere	Endo	Smith & Compere, 1926; Flanders, 1936b, c, 1937, 1953a, 1959a, 1967
Coccophagus caridei (Brèthes)	Ecto	Flanders, 1939a, 1959a, 1967
Coccophagus cowperi Girault	Ecto	Flanders in Compere, 1940; Flanders, 1953a, 1959a, b, 1967; Wilk & Kitayama, 1981
Coccophagus eleaphilus Silvestri	Endo Ecto	van den Bosch *et al.*, 1955 Flanders, 1959a, 1967
Coccophagus eritreaensis Compere	Endo	van den Bosch *et al.*, 1955
Coccophagus gurneyi Compere	Ecto or Endo†	Compere & Smith, 1932; Bess, 1939; Flanders, 1964
Coccophagus insidiator (Dalman)‡	Ecto	Flanders, 1952a, 1967
Coccophagus lycimnia (Walker)	Ecto	Flanders, 1936b, c, 1937, 1942b, 1953a, 1959a, 1967; Timberlake, 1913; Rubin & Beirne, 1975
Coccophagus nigritus Compere	Endo	van den Bosch *et al.*, 1955; Flanders, 1959a, 1967
Coccophagus pseudococci Compere	?Ecto	Flanders, 1959c, 1967
Coccophagus rusti Compere	Endo	Flanders, 1939b, 1953a, 1959a, 1967
Encarsia luteola Howard	?	Zinna in Poinar, 1964
Encarsia pergandiella Howard	Endo	Gerling, 1966b; Flanders, 1967
Encarsia tricolor (Foerster)	?	Mazzone, 1976
Encarsia sp.	?	Beingolea, 1959
Physcus debachi Compere & Annecke	Ecto*	Fisher, 1961; Flanders, 1967
Physcus seminotus Silvestri	Ecto	Williams, 1972, 1977
Physcus subflavus Annecke & Insley	Ecto	Williams, 1972
Physcus testaceus Masi	Ecto	Flanders, 1942c, 1957, 1959a, 1967
Prospaltella clypealis Silvestri	Ecto	Flanders, 1959a, 1969b; Smith *et al.*, 1964; Smith, 1951, cited by Flanders, 1967

APPENDIX 7 (*contd*)

Prospaltella opulenta Silvestri	Endo	Flanders, 1959a, 1969b, Smith *et al.*, 1964; Smith, 1951, cited by Flanders, 1967
Prospaltella smithi Silvestri	Endo	Clausen & Berry, 1932; Flanders, 1969b

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*The male eggs of *Coccophagoides similis* and *Physcus* spp. are often fastened to the inner body wall of a mummified coccoid, not actually touching the male's host (Flanders, 1959a, Fisher, 1961; Zinna, 1962; Williams, 1972).

†*Coccophagus gurneyi:* endoparasitic when oviposition is direct and ectoparasitic when indirect.

‡Flanders (1952a) says it is a parasitoid of its sister female. This however, is probably just an incomplete statement of the male's host relationship.

APPENDIX 8

Alloparasitoids: females are primary endoparasitoids of homopterous hosts, and males develop hyperparasitically on individuals of other species only (Figs 1 & 2)

Species	Male development (ecto/endoparasitic)	Author(s)
Aneristus ceroplastae Howard	Endo	Flanders, 1959a, b, 1967; Doutt *et al.*, 1976
Coccophagus basalis Compere	Ecto	Flanders *et al.*, 1961; Flanders, 1967
Coccophagus malthusi Girault	Ecto	Flanders, 1937, 1967; Annecke, 1964; Annecke & Insley, 1974
Coccophagus pulvinariae Compere	Ecto	Flanders in Compere, 1940; Flanders, 1953a, 1959a, b, 1967
Lounsburyia trifasciata (Compere)	Ecto	Flanders, 1936c, 1937, 1967

APPENDIX 9

Heteronomous hyperparasitoids: females are primary endoparasitoids of homopterous hosts, and males develop hyperparasitically (Fig. 1). These species are not included in Appendices 7–9 because the exact host relationships of the males are not known

Species	Male development (ecto/endoparasitic)	Author(s)
Coccophagus baldassarii Compere	Endo	Flanders, 1959a, 1967; van den Bosch *et al.*, 1955
Coccophagus hawaiiensis Timberlake	Endo Ecto	Flanders, 1953a Flanders, 1959a, 1967
Coccophagus japonicus Compere	Ecto	Flanders, 1959a, 1967
Coccophagus scutatus Howard*	Endo	Flanders, 1937
Coccophagus yoshidai Nakayama	Endo	Flanders, 1967
Coccophagus sp. nr. *yoshidai* Nakayama	Endo	Flanders, 1959a
Coccophagus sp.	Endo	McDaniel & Moran, 1972
Euxanthellus philippiae Silvestri	Ecto	Smith & Compere, 1928; Compere, 1931; McDaniel & Moran, 1972
Euxanthellus sp.†	Ecto	van den Bosch *et al.*, 1955; Flanders, 1959a, 1967
Physcus flaviceps Girault & Dodd	Endo	Fisher, 1961
Physcus howardi Compere‡	?	Luck & Dahlsten, 1974
Physcus reticulatus Compere & Annecke	?	Flanders, 1953b
Physcus sp. nr. *flaviceps* Girault & Dodd	Endo	Flanders, 1959a
Physcus sp. nr. *intermedius* Gahan	Endo	Flanders, 1959a
Physcus sp.	Ecto	McDaniel & Moran, 1972
Prospaltella elongata Dozier	Endo	Flanders, 1959a, 1967
Prospaltella sp. nr. *citrofila* Silvestri	Ecto	Smith 1951, cited by Flanders, 1967, 1969b
Prospaltella sp. 'G'	?	Flanders, 1953b
Prospaltella sp.	?	Rosen, 1965
Prospaltella sp.	?	Cohen, 1969
Prospaltella sp. (from Jamaica)‡	?	Flanders, 1951
Prospaltella sp. (from China)	?	Flanders *et al.*, 1958

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* See Appendix 5.

† More than one species of *Euxanthellus* may be included here.

‡ The authors suggest the males may be hyperparasitic.

APPENDIX 10

Heterotrophic parasitoids: females are primary endoparasitoids of Homoptera, and males are primary endoparasitoids of lepidopterous eggs (Figs 1 & 2)

Species	Female host	Male host	Author
Encarsia lutea (Masi)	*Trialeurodes abutilonea* (Haldeman) & *Trialeurodes vaporariorum* (Westwood)	*Heliothis zea* (Boddie) & *Trichoplusia ni* (Hübner)	Stoner & Butler, 1965
Encarsia sp.	*T. vaporariorum* & *Bemisia tuberculata* Bondar	*Anomis texana* Riley	Beingolea, 1958, 1959
Prospaltella porteri Mercet	*T. vaporariorum* & *Aleurothrixus granelli* Blanchard	*Rachiplusia nu* (Guenée)	Rojas, 1968
Prospaltella sp.	*Aspidiotus juglansregiae* Comstock ?	*Carpocapsa pomonella* (Linnaeus)	Flanders, 1924, 1925, 1926
Prospaltella sp.	?	*Zea grandiosella* (Dyar) & *Heliothis obsoleta* Fabricius	Davies *et al.*, 1933

APPENDIX 11

Heteronomous hyperparasitoids that deposit their haploid eggs indirectly. All diploid eggs are deposited endoparasitically and develop as primary parasitoids

Species	Author(s)
Coccophagus basalis Compere	Flanders in Compere, 1940; Flanders, 1959a; Flanders *et al.*, 1961
Coccophagus gurneyi Compere*	Flanders, 1936b, c, 1937, 1953a, 1959a, 1967
Coccophagus pseudococci Compere	Flanders, 1959a, 1969a (quotes his 1963 paper, but it is not mentioned there)
Encarsia formosa Gahan	Gerling, 1966a; Flanders, 1967
Lounsburyia trifasciata (Compere)	Flanders, 1936c, 1937, 1959a, 1969a
Physcus fulvus Compere & Annecke†	Flanders, 1953b
Prospaltella perniciosi Tower, San José scale race‡	Flanders, 1939a, 1959a, 1967 (in the latter paper he quotes Rice (1937), who does not provide this information)
Prospaltella smithi Silvestri§	Flanders, 1959a, 1969b

*Haploid *Coccophagus gurneyi* eggs may be deposited directly or indirectly.

†Flanders, however, (1952b, 1967) says that it is a direct endoparasitoid.

‡Chumakova & Goryunova (1963) worked on the Far Eastern form and found eggs to be deposited in the primary parasitoid larva (i.e. as a direct endoparasitoid).

§Flanders (1969b) says the haploid (male) egg develops when deposited more or less accidentally inside the prepupa or pupa of either its own or some other parasite species. This could be interpreted as *Prospaltella smithi* being a direct secondary endoparasitoid.

APPENDIX 12

Thelytokous and amphitokous species from genera containing heteronomous parasitoids. It is not certain that the thelytokous species never produce males

Thelytokous species	Author(s)	Amphitokous species	Author(s)
Coccophagus clavellatus Compere?	Annecke & Insley, 1974		
		Encarsia formosa Gahan	Speyer, 1927, 1930; Gerling, 1966a; Flanders, 1967
Prospaltella berlesei (Howard)	Howard, 1912; Flanders, 1959a		
Prospaltella divergens Silvestri	Clausen & Berry, 1932	*Prospaltella perniciosi* Tower, San José scale race (South Atlantic coast strain)	Flanders, 1944b, 1967 (quotes Rice, 1937)
Prospaltella inquirenda Silvestri	Rosen, 1965; Gerson, 1968		
Prospaltella peltata (Cockerell)	Zañartu, 1959		
Prospaltella perniciosi Tower, San José scale race (in European USSR)	Chumakova & Goryunova, 1963	*Prospaltella smithi* Silvestri	Smith, 1951, cited by Flanders, 1967, 1969b
Prospaltella perniciosi Tower, Red scale race	Flanders, 1959a		

CHAPTER 3 : BIOLOGY OF HETERONOMOUS APHELINIDS

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Differences in host relationships between male and female heteronomous parasitoids (Aphelinidae: Chalcidoidea): A review of host location, oviposition and pre-imaginal physiology and morphology

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The male larvae of heteronomous aphelinids have host relationships that are different from those of conspecific females. The adult females have behavioural adaptations that enable them to locate and identify the two 'types' of hosts and to deposit eggs of the appropriate sex. Physiological adaptations of eggs and larvae that are different in males and females are summarized. In some species with hyperparasitic males, hatching of haploid eggs is delayed until the body fluids of the coccoid host are entirely consumed by another parasitoid individual, which is then eaten. Larvae of both sexes may have special resistance mechanisms against the cellular immune responses of their habitual hosts. Morphological differences between conspecific males and females are reviewed. Heteronomous aphelinids, owing to their unusual host relationships, offer unique opportunities in the general study of parasitoid biology, particularly in the fields of parasitoid searching behaviour, sex ratios and resistance mechanisms to host immune responses.

INTRODUCTION

The Aphelinidae (Hymenoptera: Chalcidoidea) parasitize a number of insect groups, but are perhaps best known as parasitoids of aphids, scale insects and whiteflies (Yasnosh 1980; Viggiani 1981). The host relationships of many aphelinid species are unique among parasitic organisms because males have different host relationships from their conspecific females. Female larvae of these heteronomous parasitoids (Walter 1983) are all endoparasitic in Coccoidea or Aleyrodoidea. On the other hand male larvae, depending on their species, are (i) primary ectoparasitoids of Coccoidea or Aleyrodoidea, or (ii) secondary parasitoids of chalcidoid wasps within coccoids or aleyrodids, or (iii) primary endoparasitoids of lepidopterous eggs. Species that fall into the three groups above are called, respectively, diphagous parasitoids, heteronomous hyperparasitoids and heterotrophic parasitoids (Walter 1983). Heteronomous hyperparasitoids can be more precisely defined as: obligate autoparasitoids if the males develop solely on conspecific male or female larvae or pupae; facultative autoparasitoids if males are parasitic on conspecifics or individuals of other species; or alloparasitoids if the males are parasitic only on other species.

The physiology and morphology of the eggs and larvae of each sex is related to the different 'situations' in which they develop. A review of the available literature is necessary because little synthesis of these aspects of heteronomous biologies has been published since Flanders (1936a, 1936b, 1936c, 1937a) first described the phenomenon (but see Nikol'skaya & Yasnosh 1966), and information is often anecdotal. In addition some reports are speculative and need to be placed in perspective, and the detection of possible cryptic (sibling) species complexes may be easier if the subtleties of these insects' developmental biologies are understood.

The aims of this paper are therefore to outline the behavioural adaptations of adult female heteronomous aphelinids that enable them to identify suitable hosts for offspring of either sex and subsequently deposit an egg of the appropriate sex; to review the complexities of pre-imaginal development in heteronomous parasitoids; and to identify topics that require research.

BEHAVIOURAL ADAPTATIONS OF ADULT FEMALE HETERONOMOUS PARASITOIDS

Deposition of male and female eggs

Unmated hymenopterous females of arrhenotokous species can deposit only unfertilized, male-producing eggs. However, mated females hold sperm in their spermathecae and many species can lay fertilized (female) or unfertilized (male) eggs (Brunson 1937, 1938; Moran *et al.* 1969; Weseloh 1969; Crozier 1975; Sandlan 1979). This process has been referred to as selective fertilization (e.g., Flanders 1969a). In some species host size appears to be an important factor in the fertilization or non-fertilization of eggs (van den Assem 1971; Sandlan 1979). Amphitokous species, which produce both males and females parthenogenetically, have no control over the sex of eggs deposited (see Walter 1983), but lay mostly female eggs.

Until Zinna (1962) discovered that mated females of *Coccophagoides moeris* (Walker) could control their spermathecae to produce offspring of either sex, it was believed that mated female Aphelinidae produced only female offspring (e.g., Flanders 1936b, 1936c, 1937a, 1956, 1967). Subsequently, females of a number of aphelinid species have been found to be capable of selective fertilization (Table 1). However, the

TABLE 1. Heteronomous parasitoid species that have females capable of selective fertilization after they have been inseminated.

Species	References	Male Development (Ecto/Endoparasitic)†
Coccophagoides moeris (Walker)	Zinna 1962	ecto
Coccophagus lycimnia (Walker)	B. R. Bartlett in Flanders 1969a	ecto
Coccophagus rusti Compere	Flanders 1969a	endo
Coccophagus semicircularis (Foerster)	B. R. Bartlett in Flanders 1969a; Jarraya 1975, 1977	endo
Encarsia pergandiella Howard	Gerling 1966a	endo
Physcus seminotus Silvestri	Williams 1972	ecto
Physcus subflavus Annecke & Insley	Williams 1972	ecto
Prospaltella clypealis Silvestri	Flanders 1969b	ecto
Prospaltella perniciosi Tower	Chumakova & Goryunova 1963	endo

† Walter (1983) lists the precise host relationships and the authors who described male development.

mated females of at least two species do appear to produce only female offspring (*Coccophagoides utilis* Doutt: Broodryk & Doutt 1966; *Physcus debachi* Compere and Annecke: Fisher 1961). The belief that mated aphelinids could not 'voluntarily' lay male eggs led Flanders (1959a, 1967, 1969a, 1973) to postulate that mated females of certain species (e.g., *Coccophagus ochraceus* Howard) produce males by 'malfunction' of their spermathecae, when oviposition rates are high, but this requires experimental verification.

Under certain circumstances eggs may be deposited in hosts utilized by the opposite sex (Flanders 1969a). Although this seldom occurs (Williams 1972), Flanders (1969a) termed these mistakes 'aberrational' or 'abortive' egg deposition, and in his review of the topic he proposed the term 'alternative differential mortality', presumably because larvae inevitably die in the wrong ('alternative') host.

Location and acceptance of a host for oviposition

Parasitoid searching behaviour and oviposition has been visualized as having three sequential stages that may overlap (Salt 1938; Flanders 1953a). They are termed 'host habitat location', 'host location' and 'host acceptance'. The latter includes oviposition. These aspects of the biology of heteronomous parasitoids have been little investigated, presumably because of the small size of the wasps and the added complexities of their unusual host relationships. Vinson (1975, 1976) reviewed all three steps but confined his attention to 'conventional' parasitoids in which searching behaviour and the relevant stimuli are the same for deposition of eggs of either sex.

Although male and female diphagous parasitoids develop as primary parasitoids and at the expense of the same species of scale insects (Zinna 1961; Flanders 1967), male eggs are deposited ectoparasitically and female eggs endoparasitically. A notable difference here, in the production of male and female offspring, is in the alteration of ovipositional stance (Flanders 1952a, 1959a, 1973).

The hosts of male heteronomous hyperparasitoids are found within scale insects, usually of the species attacked by their female larvae. So females presumably search the same habitats for superficially similar hosts (e.g., see Dowell *et al.* 1981), and the sex of an offspring may only be 'decided' once an acceptable host has been discovered. This raises the interesting question of sex ratio determination in heteronomous hyperparasitoids, which so far has received only theoretical attention (Colgan & Taylor 1981). The criteria for acceptance of parasitoid larvae or pupae for deposition of male eggs must be different from those for female eggs: in fact, hosts for males of some species are accepted even if they are within individuals of homopteran species that are unacceptable as hosts for females (see Valentine 1964). This complication in host searching by aphelinids has not been investigated.

The males of at least one species of alloparasitoid (*Coccophagus malthusi* Girault) appear almost always to develop at the expense of parasitoids within scale insects of a species different from those in which females develop and which, further, are usually found on a different species of plant (Compere 1926; Flanders 1937a; Annecke 1964; Annecke & Insley 1974). Deposition of male and female eggs thus requires different host-searching and host-acceptance behaviour. There are indications that this may also be true of heterotrophic parasitoids (Beingolea 1958, 1959; Stoner & Butler 1965; Rojas 1968), where both sexes are primary parasitoids, the females in whiteflies and the males in lepidopterous eggs.

Detection of previous parasitism by heteronomous hyperparasitoids

Detection of parasitoid larvae or pupae by hyperparasitoid and heteronomous hyperparasitoid females has not been studied as extensively as it has in primary parasitoids. Some hyperparasitoids (Gutierrez 1970; Weseloh & Bartlett 1971) and heteronomous hyperparasitoids (*Encarsia pergandiella* Howard: Gerling 1966a; *Physcus seminotus* Silvestri: Williams 1972; *Coccophagus semicircularis* (Foerster): Flanders 1969a; Jarraya 1975) pierce the primary parasitoid's host with the ovipositor to investigate whether a parasitoid larva or pupa is present or not. Jarraya (1975) proposed that *C. semicircularis* relies on physical contact, because females of this species are more likely to deposit haploid (male) eggs the larger the parasitoid within the scale insect. In contrast, virgin female *Coccophagoides utilis* appear able to detect the conspecific primary parasitoid female larvae before piercing scale insects (Broodryk & Doutt 1966), presumably by sensing chemical traces.

The parasitoid hosts of heteronomous hyperparasitoid males are covered by the scale of the homopterous host. Flanders (1969a) presumed that this scale would stimulate deposition of a female egg when the host is suitable only for males. So he proposed that heteronomous hyperparasitoids would require mechanisms of selective fertilization different from those of other parasitoids. This is not necessarily true because primary parasitoids, both solitary (van Lenteren 1981) and gregarious (Holmes 1972; Wylie 1973), exercise ovipositional restraint until they discover that their intended host is unparasitized.

EGG AND LARVAL PHYSIOLOGY

Inhibited hatching

The eggs of almost all species of heteronomous aphelinids are deposited in a situation potentially favourable for their immediate development. However, the haploid eggs of some exceptional species of heteronomous hyperparasitoids are laid in the body fluids of a scale insect regardless of whether it had been previously parasitized or not. These species are therefore indirect hyperparasitoids (Smith 1912; Flanders 1943, 1963; Walter 1983), and their male eggs do not hatch immediately. The embryo completes development in three or four days and then undergoes a period of quiescence termed 'inhibited hatching' (Cendaña 1937). It may remain in this state for an extended period (85 days recorded by Cendaña) or until a primary parasitoid, which may have been already present as a young larva, or deposited subsequently, has grown large enough to provide sufficient food for the hyperparasitoid. It is thought that consumption of the coccoid's haemolymph by the future host causes the open respiratory system of the aphelinid larva to fill with air, and thus induces hatching (Bess 1939). All species known to have inhibited hatching (Table 2) are indirect heteronomous hyperparasitoids with males that develop ectoparasitically.

The unusual host relationships of heteronomous aphelinids were unknown to Cendaña (1937) when he investigated inhibited hatching in *Coccophagus gurneyi* Compere. In particular, he did not know that the 'quiescent' eggs in the mealybugs he dissected were really young male hyperparsitoid larvae waiting for a host, and he concluded that under no circumstances could they develop further. Also he was unfortunate in never obtaining 'quiescent' eggs from mated females. Therefore he attributed inhibited hatching to the virgin condition of the ovipositing female, and concluded that males could be produced only by mated females. It was only after Flanders' discovery of the true nature of these insects' host relationships that the significance of inhibited hatching to heteronomous hyperparasitoids was appreciated (Flanders 1959a).

TABLE 2. Heteronomous aphelinid species whose haploid eggs undergo inhibited hatching.

Species	References	Male Development (Ecto/Endoparasitic)†
Coccophagus basalis Compere	Compere 1939; Flanders *et al.* 1961	ecto
Coccophagus gurneyi Compere	Cendaña 1937; Flanders 1964	ecto
Coccophagus insidiator (Dalman)*	Flanders 1952b	ecto
Coccophagus pseudococci Compere	Flanders 1959b	? ecto
Coccophagus semicircularis (Foerster)*	Flanders 1967	endo
Lounsburyia trifasciata (Compere)	Cendaña 1937	ecto
Prospaltella perniciosi Tower*	Rice 1937	endo

† Walter (1983) lists the precise host relationships and the authors who described male development.

* Inhibited hatching not conclusively proved in these species.

Differential adaptation of the sexes to overcome immune responses of their hosts

Hosts may be immune to a proportion of individuals of their habitual parasitoid species, but to a larger proportion, or to all individuals of 'non-habitual' parasitoid species (Bess 1939; Lewis & Brazzell 1966; Salt 1975; Blumberg 1977). The immune response is cellular and a capsule usually forms around the foreign body (Salt 1970), in this case an insect egg or larva.

Males and females of some heteronomous species may be differentially adapted to overcome the immune responses of their respective habitual host species, a possibility first mentioned by Bess (1939), although Flanders (1937b) interpreted the same information differently. Flanders thought that larvae of each sex may have different nutritional requirements, so males starve in hosts of females and *vice versa* (e.g., *Coccophagus rusti* Compere in Flanders 1952a). These two phenomena have not been investigated, so both may occur and the extent to which they are related is unknown.

If diploid and haploid eggs of *Coccophagus gurneyi* are deposited into certain pseudococcid hosts (e.g., *Planococcus citri* (Risso)), the female eggs and larvae, but not male eggs, are encapsulated (Flanders 1937b; Bess 1939). This indicates that female eggs and larvae have been 'recognized' as 'foreign' by cells responsible for the immune response. The larvae may therefore not have starved prior to encapsulation. Starvation on its own may take a long time and encapsulation does not necessarily follow (e.g., *Venturia canescens* Gravenhorst in *Hofmannophila pseudospretella* (Stainton) (Salt 1975). In some non-aphelinid parasitoids, resistance to their host's immune response is acquired when a chemical layer is secreted over eggs within the female's reproductive tract (Lewis & Vinson 1968; Salt 1968; Nappi & Streams 1969; Kitano & Nakatsuji 1978). Such a layer may have been applied to male *C. gurneyi* eggs, but not to female eggs; or different chemicals may be secreted over eggs of each sex.

A second possible resistance mechanism was discovered in *Coccophagus capensis* Compere by Cendaña (1937). The embryonic females, but not the males, of this species are enclosed within a cellular membrane, the amniotic membrane or trophamnion (see Hagen 1964). Flanders (1942, 1959a) regarded the trophamnion as a feeding adaptation, but Salt (1968) suggested that it may also be protective by diverting the cellular immune response of the host. Experimental evidence for this function has been gathered indirectly by Salt (1975) who found that braconid teratocytes 'protected' *V. canescens* larvae within a host that normally encapsulates them.

Female heteronomous aphelinids appear to be different from conspecific males in the mechanisms that protect them from their hosts' immune responses. Neither the nature of these mechanisms nor their prevalence in the Aphelinidae is known.

STRUCTURE AND OVIPOSITION OF DIPLOID AND HAPLOID EGGS

Heteronomous aphelinids lay fertilized eggs endoparasitically, and they are often selective about the precise site of oviposition (Flanders 1973). For example, *Coccophagus basalis* Compere selects the suboesophageal ganglion (Flanders *et al.* 1961) and *Prospaltella smithi* Silvestri the Malpighian tubules (Flanders 1973).

Unlaid eggs and deposited fertilized (diploid) eggs are hymenopteriform, with no projections (*Coccophagoides moeris*: Zinna 1962; *Coccophagus capensis*: Smith & Compere 1926; *Euxanthellus philippiae* Silvestri: Smith & Compere 1928; Clausen 1940; *Physcus seminotus* and *P. subflavus* Annecke & Insley: Williams 1972). On the other hand, deposited unfertilized (haploid) eggs may have a pedicel at the anterior end. Hagen (1964) considered it to have a glandular origin, but Flanders (1936d) noted, in *Coccophagus lycimnia* (Walker), that this alteration in shape occurred during oviposition, and he described the process: 'If the parasite locates a prepupa or pupa within the mummified mealybug, she inserts the tip of the ovipositor into the soft integument of the primary inhabitant . . . The large bulbous part [of the egg], in which the embryo develops, issues first and lies on the outer surface of the host. The anterior part of the egg is retained in the ovipositor until after the contents have been ejected into the posterior part. After the bulbous part is deposited, the ovipositor is rotated and withdrawn, leaving the attenuated anterior portion of the egg firmly embedded in the integument of the primary parasite.' (Flanders 1937a). Other species known to deposit haploid eggs with stalks are *Coccophagoides moeris* (Zinna 1962), *Coccophagoides utilis* (Broodryk & Doutt 1966), *Coccophagus caridei* (Brèthes) (Flanders 1939, 1959a), *Coccophagus cowperi* Girault (Flanders in Compere 1940; Wilk & Kitayama 1981), *Coccophagus hemera* (Walker) (Zinna 1961) and *Physcus seminotus* (Williams 1972).

A number of reasons has been postulated for the stalked male eggs of heteronomous aphelinids. These are:

(i) the mechanical prevention of fertilization (Flanders 1969a; Williams 1972), because the micropyle is the sperm entrance and is at the end that is constricted;
(ii) that the stalk serves as a reservoir for the egg contents while the body of the egg is squeezed down the ovipositor, as in those species whose ovipositor valves cannot bulge to accommodate the bulk of the passing egg (Askew 1971); and
(iii) the stalk assists in the firm attachment of the egg to the outside of the host, as suggested for *Coccophagoides utilis* by Broodryk & Doutt (1966), but in general for ectoparasitoids by Cushman (1926), Clausen (1940), Hagen (1964) and Flanders (1973).

The first suggestion is unlikely because some heteronomous hyperparasitoids do not have sexual dimorphism of the egg and yet are capable of selective fertilization without distorting their haploid eggs (e.g., *Coccophagus semicircularis*: Flanders 1937a; Jarraya 1975, 1977), and the second because diploid eggs are deposited down the same ovipositor bore and are unstalked. There are, on the other hand, two lines of support for the third suggestion: (i) all species that have sexual dimorphism of the egg are diphagous parasitoids or ectoparasitic heteronomous hyperparasitoids (Flanders 1973; Walter 1983: Appendices 5 to 9), and (ii) the eggs of endoparasitic males do not have this modification (e.g., *Coccophagus gurneyi*: Cendaña 1937; Flanders 1964; *C. semicircularis*: Flanders 1937a; *Lounsburyia trifasciata* (Compere): Cendaña 1937).

Euxanthellus philippiae eggs do not have the conventional sexual dimorphism reported in other species of heteronomous parasitoids. The haploid egg of this species has a very short pedicel on the side (Smith & Compere 1928), and is probably formed differently from that of other heteronomous species: it may be of glandular origin (Hagen 1964), as described in *Agriotypus* sp. (Clausen 1931) or it may be formed in the ovariole as postulated for *Euplectrus* sp. (Neser 1973), but this needs investigation. The stalk apparently 'glues' the haploid egg to the host (Smith & Compere 1928: Fig. 16).

LARVAL MORPHOLOGY AND DEVELOPMENT

Female larval morphology and development

Heteronomous host relationships are manifested morphologically in the considerable sexual dimorphism of the larvae of many species (Flanders 1936d, 1937a). Generally, the most distinctive larval stage in the life cycle of any parasitoid species is the first instar; subsequent stages are usually grub-like, or hymenopteriform, without any conspicuous projections from the body (Hagen 1964). This heteromorphosis occurs also in heteronomous aphelinids, and sexual dimorphism is restricted to the first instar and, less markedly, to the second. There may, however, be sexual differences in the hard cuticular structures of the final instar, for example spiracular diameter (Williams 1972). There is no obvious adaptive significance to heteromorphosis, although Hagen (1964) has suggested that it may be associated with locomotory requirements in younger larvae.

The female larvae of heteronomous parasitoids are endoparasitic and develop in a fluid medium. The great majority are solitary parasitoids (exceptions are *Coccophagus caridei* (Flanders 1939), *C. lycimnia* (Howard, 1891), *C. semicircularis* (Cendaña 1937; Jarraya 1975) and *C. smithi* (? = *Prospaltella smithi*) (Anonymous 1937)). There are two morphological categories of first-instar female heteronomous parasitoids, although these seem to intergrade.

(i) Hymenopteriform larvae have a spherical or spindle-shaped body (Clausen 1940), e.g., *Coccophagoides utilis* (Fig. 1). All known examples are listed in Table 3. These larvae retain their hymenopteriform shape until they pupate.

(ii) Caudate larvae have an elongate, segmented body with the last segment forming a tail (Clausen 1940), e.g., *Coccophagus capensis* (Fig. 2) (Smith & Compere 1926; Cendaña 1937). See Table 3 for known representatives. Some of these have very short tails (e.g., *Lounsburyia trifasciata*: Cendaña 1937), but are nevertheless included. Caudate larvae become hymenopteriform after one or two moults. A full-grown caudate larva is represented in Fig. 3 to demonstrate the extent of heteromorphosis.

Female larvae usually have three instars (Table 3), and spiracles (4–9 pairs, Table 3) appear only in the final instar. Young endoparasitic larvae take up dissolved oxygen through the cuticle (see Fisher 1971), and later, when the host is consumed, the larva is exposed to air and respires by means of spiracles. The tracheal system may, however, start to develop earlier (Taylor 1935; Broodryk & Doutt 1966; Williams 1972; Zinna 1961, 1962). First-instar larvae have thirteen segments, excluding the head. The number of segments is retained throughout larval development in most species, but the third-instar *Coccophagus gurneyi* larva has twelve body segments (Cendaña 1937). Taylor (1935) recorded fourteen segments in first-instar *Physcus intermedius* Gahan but Williams (1972) regards this as being incorrect.

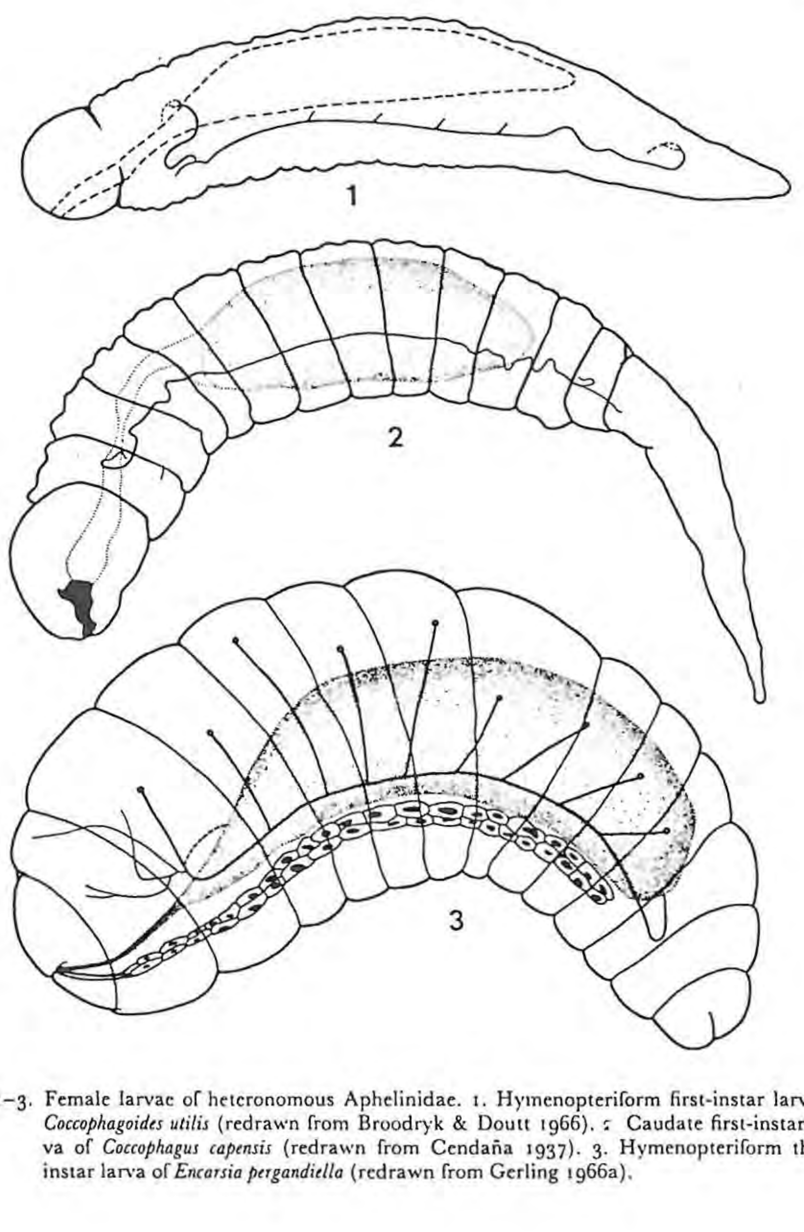
TABLE 3. Larval morphology of female first-instar heteronomous aphelinids. See text and Figs 1 & 2 for a description of the larval types.

SPECIES	NUMBER INSTARS	PAIRS SPIRACLES PER INSTAR	REFERENCES
A. HYMENOPTERIFORM LARVAE			
Coccophagoides moeris (Walker)	4	0, 0, 0, 9	Zinna 1962
Coccophagoides utilis Doutt	4	0, 0, 0, 9	Broodryk & Doutt 1966
Coccophagus basalis Compere	3	?	Flanders *et al.* 1961
Coccophagus gurneyi Compere	3	0, 0, 7	Cendaña 1937
Coccophagus hemera (Walker)	3	0, 0, 9	Zinna 1961
Coccophagus lycimnia (Walker)	3?	0, 0, 9	Smith & Compere 1928
Encarsia tricolor (Foerster)	4	?	Stüben 1949; Arzone 1976
Physcus intermedius Gahan	3	0, 0, 9	Taylor 1935
Physcus seminotus Silvestri	?	?, ?, 9	Williams 1972
Physcus subflavus Annecke & Insley	?	?, ?, 9?	Williams 1972
Prospaltella lahorensis Howard	3	0, 0, 9	Viggiani & Mazzone 1978
B. CAUDATE LARVAE			
Coccophagus capensis Compere	3?	0, 0, 9	Smith & Compere 1926; Cendaña 1937
Coccophagus semicircularis (Foerster)	3	0, 0, 9	Cendaña 1937
Encarsia pergandiella Howard	3	0, 0, 9	Gerling 1966a
Lounsburyia trifasciata (Compere)	3	0, 0, 4	Cendaña 1937
Prospaltella perniciosi Tower	3	0, 0, 8	Tower 1914; Rice 1937 Chumakova & Goryunova 1963
C. UNKNOWN LARVAL MORPHOLOGY			
Coccophagus caridei (Brèthes)	3	0, 0, 9	Flanders 1939
Physcus debachi Compere & Annecke	4	0, 0, 0, ?	Fisher 1961
Prospaltella inquirenda Silvestri	2	?	Gerson 1968

Varied duration of female larval development

The larval development of many hymenopterous parasitoid species may be prolonged if the host is too young. Generally, the duration of the first instar is extended, and this probably allows the host to grow to a suitable size (Salt 1941; Askew 1971). Heteronomous aphelinids also show this characteristic; it has been recorded in *Coccophagoides utilis* (Broodryk & Doutt 1966) and *Encarsia formosa* Gahan (Nechols & Tauber 1977).

In addition, Broodryk & Doutt (1966) found, under constant temperature conditions and in scale insect hosts of the same age, that development of *C. utilis* females varies considerably in duration. A similar situation has also been recorded in



Figs 1–3. Female larvae of heteronomous Aphelinidae. 1. Hymenopteriform first-instar larva of *Coccophagoides utilis* (redrawn from Broodryk & Doutt 1966). 2. Caudate first-instar larva of *Coccophagus capensis* (redrawn from Cendaña 1937). 3. Hymenopteriform third-instar larva of *Encarsia pergandiella* (redrawn from Gerling 1966a).

Coccophagus capensis, C. gurneyi, C. semicircularis, Lounsburyia trifasciata (Cendaña 1937) and *C. caridei* (Flanders 1939) although the effect of host age on developmental rate in these species was not mentioned. Broodryk & Doutt (1966) indicated that the ability to produce offspring with different intrinsic larval growth rates was genetically fixed in every individual they investigated. Males too may have variable developmental periods, but they are not as extended as that of females (*C. utilis*: Broodryk & Doutt 1966; *C. gurneyi*: Compere & Smith 1932; *C. caridei*: Flanders 1939).

Staggered development of females has been considered important in new, isolated colonies of aphelinid wasps because it is thought to increase the probability of females being mated (Flanders 1939; Broodryk & Doutt 1966). Specifically, if a mated *C. utilis* female located a suitable patch of unparasitized scale insect hosts, she could lay only female eggs (incapable of selective fertilization). On emergence these virgin females, which are unlikely to meet males, require *C. utilis* larvae for their haploid eggs (*C. utilis* is an obligate autoparasitoid). The only suitable hosts for these male eggs would be those sister wasps still in the larval or pupal stage. These males may then emerge at the same time as, and mate with, females that took even longer in their development. This interpretation needs examination in the field.

Male larval morphology and development

Male larvae, regardless of whether they are primary parasitoids or hyperparasitoids, can be divided into two groups: those that develop endoparasitically and those that develop ectoparasitically. The principal difference between these two groups clearly lies in their method of respiration. Endoparasitic males resemble females in the development of their respiratory system (Flanders 1937a; Gerling 1966a), while ectoparasitic males respire by means of spiracles during all instars.

Endoparasitic male larvae constitute three morphological types (Table 4):

TABLE 4. Larval morphology of endoparasitic male first-instar heteronomous aphelinids. See text and Figs 4 & 5 for a description of the larval types.

SPECIES	NUMBER INSTARS	PAIRS SPIRACLES PER INSTAR	REFERENCES
A. TELEAFORM LARVAE			
Coccophagus capensis Compere	3	0, 0, 9	Flanders 1937a
Coccophagus semicircularis (Foerster)	3	0, 0, 9	Flanders 1937a
Coccophagus rusti Compere	?	?	Flanders, in Compere 1940
B. CAUDATE LARVAE			
Coccophagus scutatus Howard	?	?	Flanders 1937a
Encarsia pergandiella Howard	3	0, 0, 9	Gerling 1966a
Prospaltella perniciosi Tower	?	?	Rice 1937; Chumakova & Goryunova 1963
C. HYMENOPTERIFORM LARVAE			
Prospaltella lahorensis Howard*	3	0, 4, 9	Viggiani & Mazzone 1978

* Becomes ectoparasitic in the second instar.

(i) The teleaform type which has an elongate rather than the typical spherical abdomen of most teleaform larvae (Flanders 1937a; Clausen 1940), e.g., *Coccophagus capensis* (Fig. 4).

(ii) Caudate larvae e.g., *Coccophagus scutatus* Howard (Fig. 5), unlike teleaform larvae, have distinct segmentation of the elongate body (Clausen 1940). The final segment is elongate.

(iii) *Prospaltella lahorensis* Howard males are hymenopteriform (Viggiani & Mazzone 1978) like the female larvae of some species of heteronomous aphelinids.

There are two types of ectoparasitic males in heteronomous aphelinids (Table 5). They were first described by Flanders (1937a).

TABLE 5. Larval morphology of male ectoparasitic first-instar heteronomous aphelinids. See text and Figs 6 & 7 for a description of the larval types.

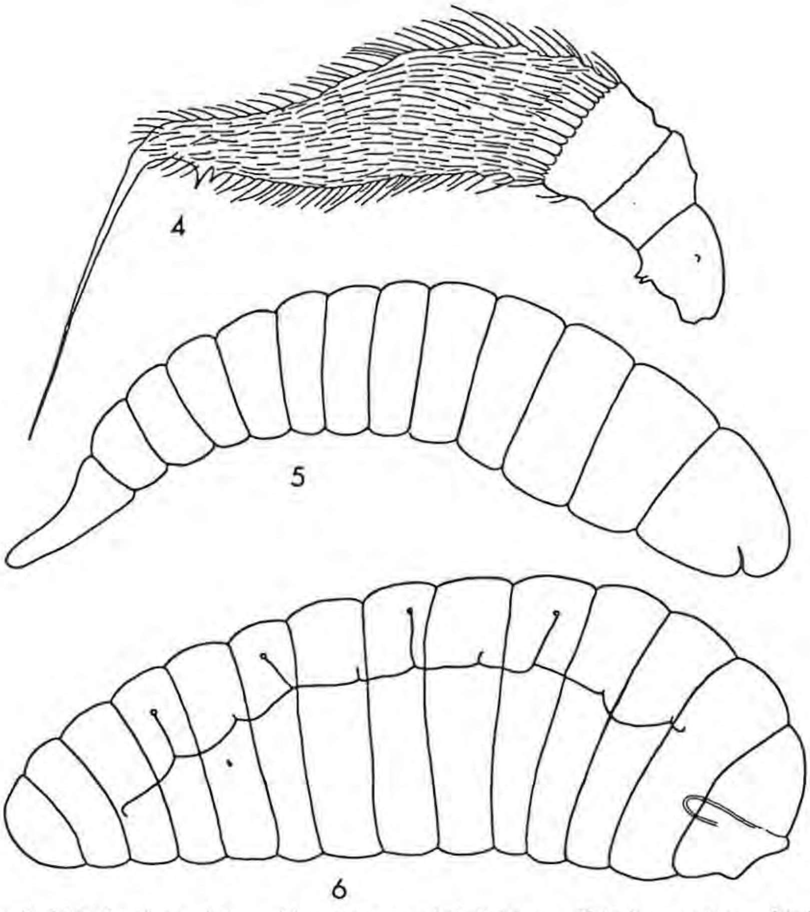
SPECIES	NUMBER INSTARS	PAIRS (SPIRACLES) PER INSTAR	REFERENCES
A. HYMENOPTERIFORM LARVAE			
Coccophagoides moeris (Walker)	4	6, 6, 9, 9	Zinna 1962
Coccophagoides utilis Doutt	3?	?, ?, 6	Broodryk & Doutt 1966
Coccophagus caridei (Brèthes)	3	4, 4, 9	Flanders 1939
Coccophagus hemera (Walker)	3	5, 9, 9	Zinna 1961
Coccophagus lycimnia (Walker)	3	4, variable, 9	Flanders 1937a
Coccophagus ochraceus Howard	3	?, 10, 9	Cendaña 1937; Flander 1937a
Physcus debachi Compere & Annecke*	3	5, ?, 9	Fisher 1961
Physcus seminotus Silvestri	4	4, 6, 9, 9	Williams 1972
Physcus subflavus Annecke & Insley	4?	?	Williams 1972
B. PLANIDIAL LARVAE			
Coccophagus basalis Compere	3?	1, ?, ?	Flanders, in Compere 1940; Flanders *et al.* 1961
Coccophagus gurneyi Compere	3	2, 2, 7	Flanders 1937a, 1964
Lounsburyia trifasciata (Compere)	3	2, 2, 8	Flanders 1937a

* Presumably has hymenopteriform first-instar males.

(i) Most common is the ectoparasitic hymenopteriform type (e.g., *Physcus seminotus* (Fig. 6)).

(ii) The planidium larva is found only in indirect heteronomous hyperparasitoids (e.g., *Coccophagus gurneyi* (Fig. 7)). They are not as heavily sclerotized as 'conventional' planidia (Flanders 1937a; Clausen 1940), and their locomotory adaptations are not as extensive as those found in the planidia of other families (e.g., Perilampidae: Tripp 1962), presumably because they hatch very near their future host.

By the final instar, all males, regardless of initial form, have undergone some degree of heteromorphosis and are hymenopteriform. For example, compare the third-

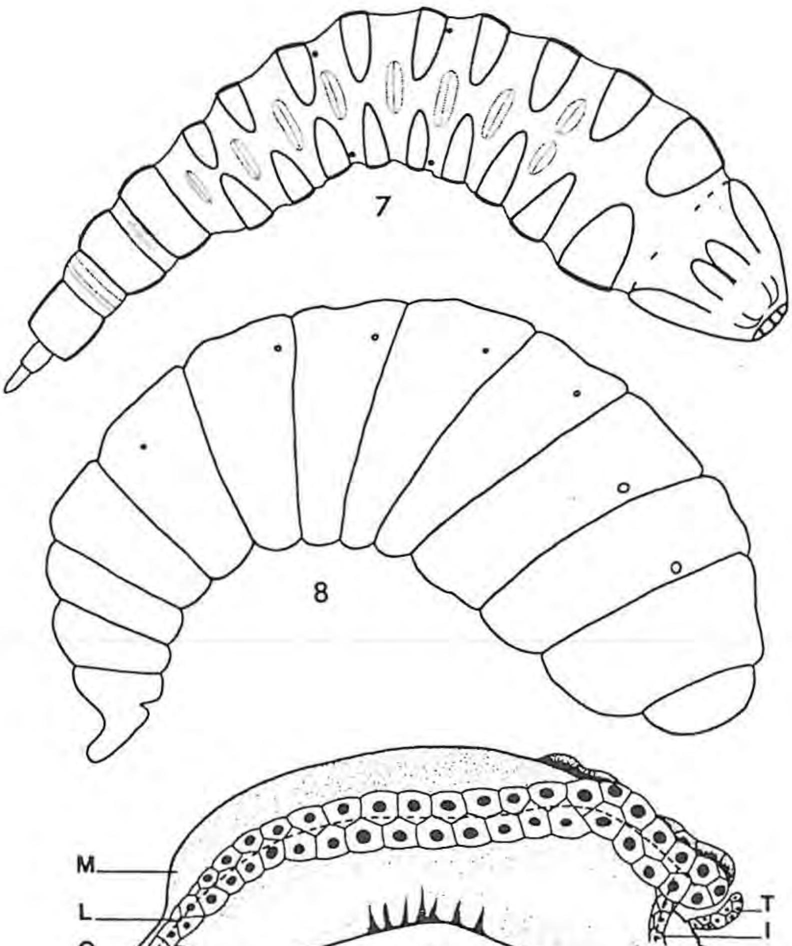


Figs 4–6. Male first-instar larvae of heteronomous Aphelinidae. 4. Teleaform endoparasitic larva of *Coccophagus capensis* (redrawn from Flanders 1937a). 5. Caudate endoparasitic larva of *Coccophagus scutatus* (redrawn from Flanders 1937a). 6. Hymenopteriform ectoparasitic larva of *Physcus seminotus* (redrawn from Williams 1972).

instar male *Coccophagus gurneyi* (Fig. 8) with the first-instar planidium (Fig. 7). Tables 4 and 5 also include information on the number of larval instars (usually 3) in male heteronomous aphelinids, and the number of spiracles per instar is listed. In the second-instar larvae of some species e.g., *Coccophagus caridei* (Flanders 1939), *Lounsburyia trifasciata* (Flanders 1937a) and endoparasitic *C. gurneyi* larvae (Flanders 1964), not all of the spiracles are functional.

A special case of heteronomous hyperparasitism: 'dual ontogeny of the male'

The biology of *Coccophagus gurneyi* is particularly interesting because the males follow either of two morphological and physiological pathways. They may develop



Figs 7–9. Larvae of heteronomous Aphelinidae. 7. Planidial ectoparasitic first-instar larva of *Coccophagus gurneyi* (redrawn from Flanders 1937a). 8. Hymenopteriform third-instar male larva of *Coccophagus gurneyi* (redrawn from Flanders 1937a). 9. Alimentary canal and ileo-labial glands of female fourth-instar *Coccophagoides moeris* larva (redrawn from Zinna 1962) (H–Hindgut; I–Ileac gland; L–Labial gland; M–Midgut; O–Oesophagus; T–Third ileac gland).

either ectoparasitically or endoparasitically. This phenomenon, termed 'dual ontogeny of the male' (Flanders 1964), was first documented by Compere & Smith (1932) although heteronomous host relationships were unknown to them. Before publication of Flanders' (1964) paper, the exact nature of the biology of *C. gurneyi* was obscure (see Compere & Smith 1932; Cendaña 1937; Flanders 1937a).

Zinna (in Flanders 1964) demonstrated that if the unfertilized egg is deposited in a mealybug, hatching is inhibited until a second parasitoid larva consumes the mealybug and stimulates hatching of the *C. gurneyi* egg. Development is then entirely *ectoparasitic* and these males may be considered indirect hyperparasitoids. On the other hand, if oviposition takes place into a primary parasitoid (direct hyperparasitism), development is *endoparasitic*, except for the final stages of the third instar when the larva feeds ectoparasitically.

Endoparasitic larvae are not morphologically very different from ectoparasitic larvae. They both have two pairs of spiracles in the second instar, but in the endoparasitic type they are non-functional (Flanders 1964). Endoparasitic larvae moult into the second instar within the unhatched egg (Cendaña 1937; Zinna in Flanders 1964), and it is these second-instar larvae that hatch into the host's haemolymph. By having non-functional spiracles, they presumably gain, for their tracheal system, some form of protection from the host's body fluids. Gerling (1966a) recorded moulting within the egg in the heteronomous hyperparasitoid *Encarsia pergandiella*, which deposits male eggs directly into a host. However, in this species the first-instar larva does not have open spiracles.

It is not known how any given haploid egg of *C. gurneyi* has the potential to follow either of these very different developmental pathways. The possibility that we are looking at two cryptic (sibling) species should be checked.

Comments on larval sexual dimorphism

Sexual dimorphism in heteronomous aphelinids is clear, and the extent of this dimorphism varies between genera, and even within genera. The larvae of the two sexes in both *Coccophagoides utilis* and *Encarsia pergandiella* are very similar. On the other hand there are species that have great differences between the sexes, a good example being *Coccophagus capensis*, which has caudate females and teleaform males (Figs 2 & 4). It is unknown whether morphological differences between sexes are adaptive or not. Differences within a few species clearly are: for example there are species that have typically hymenopteriform or caudate female larvae and male larvae that are indirect hyperparasitoids and planidial in form. Here female larvae are adapted to an essentially 'aquatic' and confined existence within a host insect. They have no special locomotory organs and when, in the final larval instar, they are exposed to atmospheric air, their respiratory system becomes functional. On the other hand, male planidia have obvious adaptations for locomotion and protection from desiccation and they also have a functional respiratory system as early as the first instar.

COCOONS AND THE PUPAL STAGE

Most chalcidoid species do not have a cocoon (Hagen 1964). However, some ectoparasitic species pupate in exposed situations and construct silken cocoons (Flanders 1938; Hagen 1964). Certain endoparasitic chalcidoid species, including some heteronomous parasitoids, also construct cocoons. Larvae of these species, before they pupate, secrete cocoon-forming substances from the labial gland-Malpighian tubule complex (or ileo-labial gland: Fig. 9) (Flanders 1938; Zinna 1959, 1961, 1962). Earlier authors (e.g., Timberlake 1913; Smith & Compere 1926) erroneously thought that the cocoon comprised the exuviae of the final-instar larva. Although very few cases of male heteronomous parasitoids spinning cocoons have been documented, there are examples of species in which (a) larvae of both sexes produce cocoons (*Encarsia pergandiella*: Gerling 1966a); (b) only females do so (*Coccophagoides moeris*: Zinna 1962); and (c) only males secrete cocoons (*Coccophagus gurneyi*: Cendaña 1937; Flanders 1964).

Table 6 lists heteronomous species with females known to produce cocoons and the nature of the environment (dry or moist) in which they pupate. Originally Timberlake (1913) and Compere & Smith (1926) thought cocoons were protective shrouds to prevent pupae from drying out, but Table 6 includes a number of species that pupate within hosts that still contain body fluids. Also many species that pupate in a dry environment apparently have no cocoon (Table 7). (Although the authors cited in Table 7 made no mention of cocoons in the aphelinid life cycle, the works included are sufficiently detailed for cocoons not to have been missed.) Zinna's (1962) work on *Coccophagoides moeris* clarifies Flanders' (1938) alternative explanation for cocoon function in endoparasitic chalcidoids, that it assists pupal respiration. Female *C. moeris* larvae in the third (penultimate) instar secrete the cocoon. It is filled with air and this presumably enters through the host's tracheal system, parts of which are incorporated into the cocoon. Zinna suggested that the cocoon's main function is provision of an air source for diffusion of oxygen through the larval cuticle. This is conceivable because oxygen is known to diffuse very slowly through haemolymph. The cocoon would also provide atmospheric conditions within the still-moist host for the air-breathing final-instar larva. The cocoon may no longer serve any function after pupation takes place in the dried-out host remains, and in *Coccophagus rusti* it often breaks on desiccation of the host (Flanders 1938). However, in those species (Table 6) that do pupate in still-moist hosts, the cocoon probably aids pupal respiration. Larvae of species that produce no cocoon probably consume, before moulting into the final-instar larva, enough host haemolymph to expose their spiracles to the atmosphere.

So the cocoon of heteronomous parasitoids seems to be a respiratory aid rather than a protective case for larva and pupa, and it is questionable whether it should be referred to as a cocoon. In those species that pupate in a moist host, it may also keep host haemolymph, and thus potentially lethal fungal or bacterial growth, from contaminating the pupa.

Although *Coccophagoides moeris* and *Coccophagus hemera* larvae of each sex do not secrete cocoons, they both possess ileo-labial glands (Zinna 1961, 1962). The tissues and organs of hosts parasitized by either sex of these species disintegrate, and Zinna presumed this to be due to secretion of proteolytic enzymes from the ileo-labial glands.

Pupal morphology does not differ greatly between the sexes. Those differences recorded are very minor and similar to those in chalcidoid parasitoids that have normal host relationships (see Williams 1972).

DISCUSSION

Only a few long-term studies have been conducted on heteronomous aphelinids. This is unfortunate because, despite their small size (often less than 1,5 mm in length) and the complexities of their host relationships, they offer unique opportunities for the study of parasitoid biology generally. For example, the fact that hosts chosen by heteronomous females for female eggs are different from those chosen for male eggs

TABLE 6. Heteronomous species with female larvae known to produce a cocoon, and the nature of the environment in which the female pupates. The stage of the life cycle in which the cocoon assists in respiration is indicated: (i) apneustic penultimate-instar larva, (ii) pneustic final-instar larva and (iii) pupa.

SPECIES	ENVIRONMENT	REFERENCES	STAGE ASSISTED
Coccophagoides moeris (Walker)	DRY	Zinna 1962	(i), (ii)
Coccophagus basalis Compere	MOIST	Flanders *et al.* 1961	(i), (ii), (iii)
Coccophagus capensis Compere	MOIST	Smith & Compere 1926; Cendaña 1937	(i), (ii), (iii)
Coccophagus caridei (Brèthes)	DRY	Flanders 1937a, 1939	(i), (ii)
Coccophagus hemera (Walker)	DRY	Zinna 1961	(i), (ii)
Coccophagus lycimnia (Walker)	DRY	Smith & Compere 1928; Cendaña 1937	(i), (ii)
Coccophagus rusti Compere	DRY	Flanders 1938, 1953b	(i), (ii)
Coccophagus saintebeauvei Girault	MOIST	Compere 1931; Compere in Cendaña 1937; Clausen 1940	(i), (ii), (iii)
Coccophagus semicircularis (Foerster)	MOIST	Timberlake 1913; Cendaña 1937	(i), (ii), (iii)
Encarsia pergandiella Howard	DRY	Gerling 1966a	(i), (ii)
Lounsburyia trifasciata (Compere)	MOIST	Cendaña 1937	(i), (ii), (iii)

TABLE 7. Heteronomous species that probably have no cocoon in the female life cycle.

SPECIES	ENVIRONMENT	REFERENCES
Coccophagoides utilis Doutt	DRY	Broodryk & Doutt 1966; Kennett *et al.* 1966.
Encarsia formosa Gahan	DRY	Speyer 1927; Gerling 1966b
Encarsia tricolor (Foerster)	DRY	Stüben 1949; Arzone 1976
Physcus intermedius Gahan	DRY	Taylor 1935
Physcus seminotus Silvestri	DRY	Williams 1972
Physcus subflavus Annecke & Insley	DRY	Williams 1972
Prospaltella lahorensis Howard	DRY?	Viggiani & Mazzone 1978
Prospaltella perniciosi Tower	DRY	Tower 1914; Rice 1937; Chumakova & Goryunova 1963

raises questions about host-searching in parasitoids, especially about the 'decisions' that searching females must make. In addition, sex ratios can be determined at oviposition because the sex of deposited eggs can be recognized by an ovipositing female's behaviour. This behaviour is unique amongst parasitoids, and sex ratios in other species are evident only after offspring have emerged and pre-imaginal mortality may have altered the ratio. Heteronomous aphelinids may be useful too in the study of parasitoid larval physiology, since females often have morphological and physiological differences from males additional to the usual sexual differences. Each sex may provide a natural control in observations on the opposite sex. Here resistance mechanisms to host immune responses and the secretion and function of cocoons by endoparasitic chalcidoids come to mind.

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CHAPTER 4 : MATERIALS AND METHODS

Collection of parasitoids. Parasitoids were collected from the field for experimental purposes, or in order to start cultures. Parasitised scale insects were dissected from leaves or twigs and isolated in gelatin capsules. This ensured the virgin condition of the emergent adults. In addition, quantitative information on population sex ratios in the field was obtained. Saissetia-infested leaves were placed in emergence boxes (Fig. 4.1A) made of the thick cardboard 'sleeves' used for protecting wine bottles. After insertion of plant material, the open end was closed and made light-proof with the bottom end of a second sleeve. A perforated vial top was glued over a hole in the bottom of the first sleeve so that vials could easily be detached for capture of live parasitoids, and removal of spiders or other predators.

Regular samples were taken from ten Saissetia-infested Trichilia emetica Vahl trees in Grahamstown. Samples were taken at approximately 3-week intervals. The intervals were slightly longer when scale insects were mostly in the adult stage and few C. bartletti emerged, and slightly shorter when hosts were suitable for parasitism by C. bartletti. On each sampling occasion, ten Saissetia-infested compound leaves of Trichilia were taken, one from each tree. The number of samples was restricted in this way, because any more of the unavoidably destructive sampling from this small population would have adversely affected the number of scale insects on the trees. Before the leaves were enclosed in an emergence box, scale insects were counted and classified into arbitrary size groups (see chapter 8). All parasitoids that emerged were identified and counted.

Host plant and insect cultures.

(i) Potato sprouts. Although Saissetia coffeae (Walker) and C. bartletti occur naturally on Trichilia in Grahamstown, a substitute host plant was desirable because potted Trichilia plants (Fig. 4.1B) are large, unwieldy and cannot be manipulated beneath a microscope. Green potato sprouts have often been used to culture various Saissetia

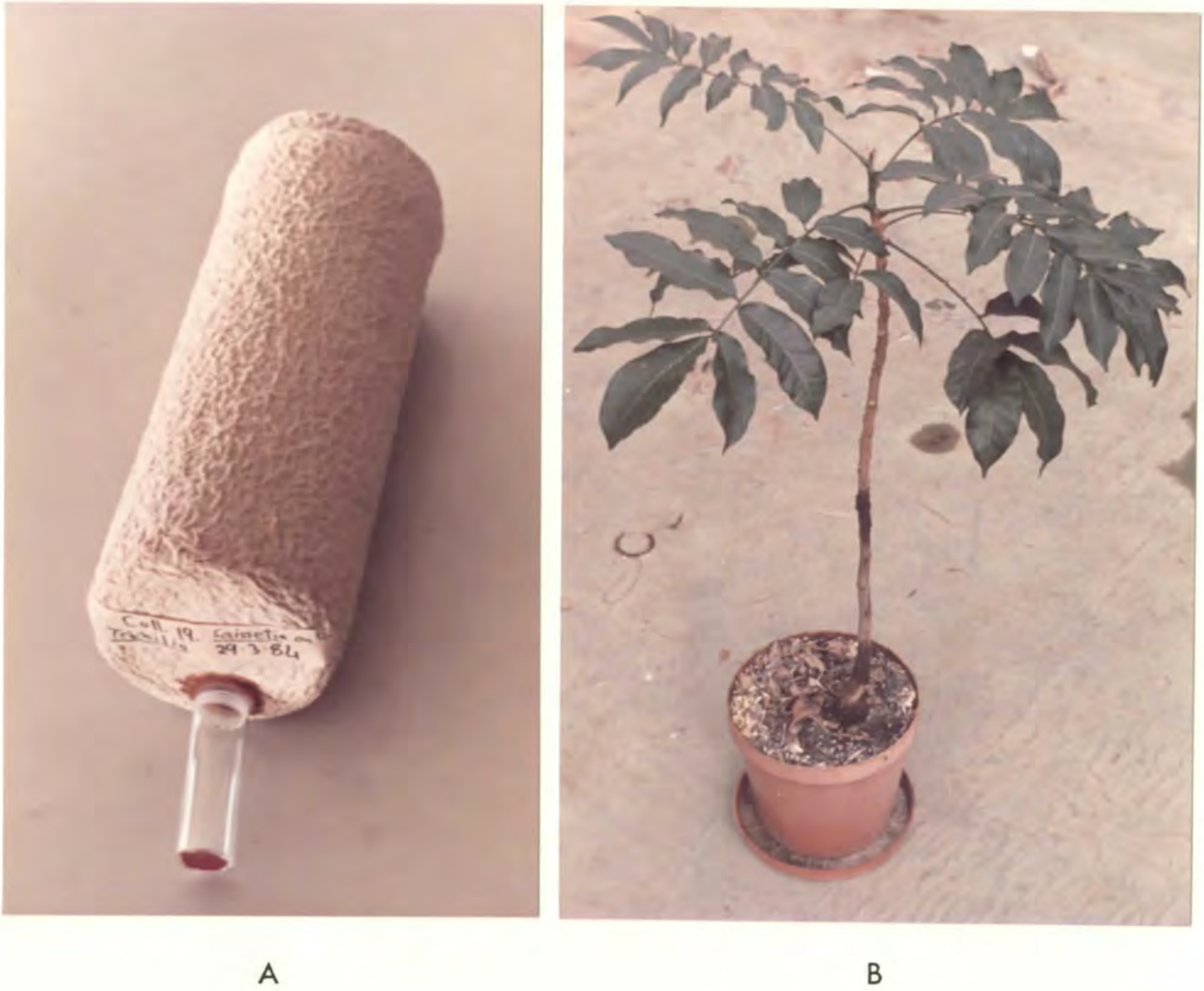

Fig. 4.1 : A. Wine sleeves converted into an emergence box. B. Potted Trichilia emetica plant.

species (Smith 1921; Flanders 1942a; Finney & Fisher 1964; Blumberg & Swirski 1977). The most suitable sprouts for manipulation under a microscope are those that are tall (150-200 mm), straight and almost leafless. In addition, if colonies of black scale insects are to be maintained on a sprout for longer than a generation (2-3 months), the sprouts should be thick (> 6 mm diameter), green and succulent (Fig. 4.2A). Thin sprouts usually wither before scale insects attain

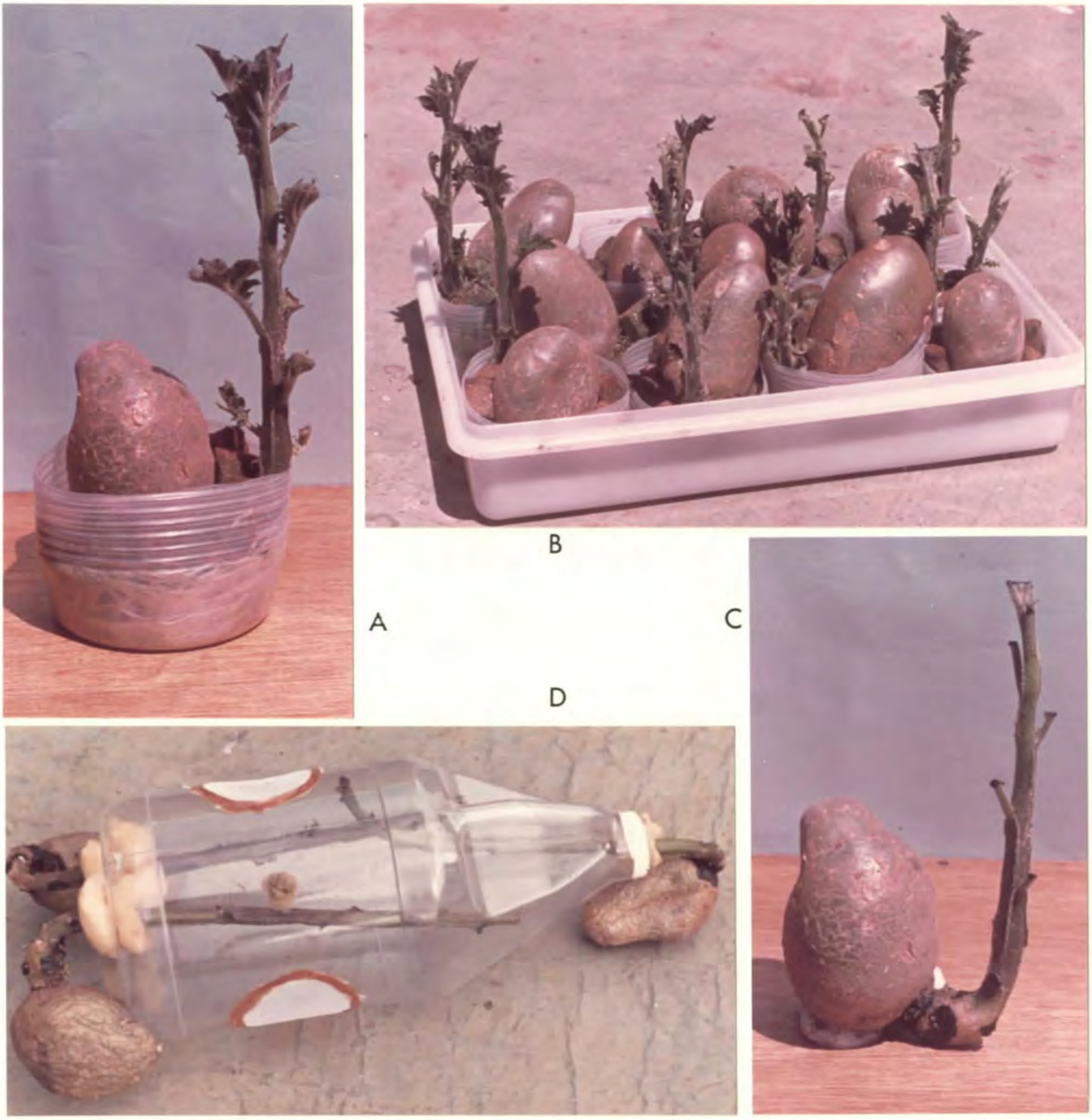

Fig. 4.2 : Green potato sprouts used for culture of *Saissetia coffeae* and *Coccophagus bartletti*. Experimental work on *C. bartletti* was also conducted on these sprouts. A. Sprouted potato in container in which it was grown. B. Containers were packed into trays that held excess water and aided handling of sprouts. C. Potato sprout with roots and leaves trimmed off and wounds sealed with bitumen. D. Trimmed sprouts, settled with suitable scale insects, in a plastic bottle containing a *C. bartletti* culture.

adulthood and before small parasitoids like C. bartletti develop fully.

The medium- to large-sized tubers chosen should be young, healthy, undamaged and of good quality (Flanders 1942a; Blumberg & Swirski 1977). Local cultivars (see Visser 1979) found to produce suitable sprouts were Cedara, Sebago, Up-to-date, Pentland Crown and Vanderplank. Sackfiller and BP_1 were unsuitable for these purposes. The most convenient containers and substrate for producing good sprouts were found to be the perforated lower portions (80 mm tall) of 1 litre plastic milk bottles filled with small cubes of 'Oasis' fibre (Fig. 4.2A). To facilitate manipulation of the containers a number of them could be placed in plastic trays, which also held drained water (Fig. 4.2B).

'Oasis' was chosen as a substrate because it retains only enough water to keep the tuber moist without it rotting. Good drainage and controlled watering is also necessary to prevent an undesirable flush of growth (Flanders 1942a; Finney & Fisher 1964). For this reason, too, relative humidity must be maintained at approximately 60-65% (Flanders 1942a; Finney & Fisher 1964). To encourage slow growth, the temperature should be held between 22°C and 24°C. Leafiness can be manipulated to a certain extent by controlling the amount of light to which growing sprouts are exposed. A light regime of 12 hours light and 12 hours dark was most satisfactory.

Once sprouts had achieved a suitable size, the roots were cut off. This limited further growth. If necessary the few leaves produced could be removed and the severed stalks covered with a bituminous coating (trade name 'Tree Seal') to prevent desiccation (Fig. 4.2C). Tubers in culture that became withered before producing sprouts were discarded and replaced. So, too, were tubers that produced multiple sprouts or sprouts that withered from the tip (a presumed viral infection: see Jandrell 1981).

Sprouts ready for inoculation with Saissetia were leant upon old, crawler-producing sprouts. The crawlers are positively phototactic, so the newer sprout was placed above the older one and on the window side of the laboratory. Good infestations were achieved at room temperatures and humidities. Relative humidities above aproximately 80% encouraged fungal attack and infestations of mites (*Tyrophagus putrescentiae* (Shrank)) and thrips (including *Heliothrips haemorrhoidalis* (Bouché)). Although *Tyrophagus* is generally a scavenger it does seem to attack scale insects. *Heliothrips* scars the surface of sprouts, and crawlers seem not to settle on these areas. Immediately after inoculation, sprouts were placed in muslin cages to prevent unwanted parasitism and were held at 23 ±1°C and approximately 60-65% RH.

After 2-3 weeks the scale insects on the sprouts were ready for parasitism by *C. bartletti*, and were used either in experiments or for culture maintenance. For the latter, the sprout itself was placed in a 2 litre plastic bottle (Fig. 4.2D) through a 25 mm diameter hole in the bottom, or through the open top. The gap between sprout and bottle was closed with a foam rubber stopper. Large holes were cut into two sides of the container and covered with muslin for ventilation. Field-collected parasitoids were introduced into the bottle, or old sprouts containing parasitised hosts were introduced through an adjacent hole. Cages were held at room temperature.

To prevent excessive inbreeding, four such culture bottles were maintained simultaneously. At two-day intervals each sprout was moved to the next bottle in line until two complete circuits had been made. Then a new sprout with young, unparasitised *S. coffeae* was placed in each container, and the cycle repeated. The old sprouts were removed to a muslin holding cage until the parasitoids reached the pupal stage.

Sufficient parasitoid pupae for experimental purposes were dissected from the sprouts and placed individually in gelatin capsules until they emerged. This ensured they remained unmated. Sprouts with the remaining pupae were returned to the culture bottles. Parasitoids collected in the field were regularly added, to augment the cultures. *C. bartletti* was maintained in culture so wasps would continuously be available for experimental purposes. Whenever possible, however, field-collected individuals were used in preference to laboratory-cultured insects.

(ii) Leaf discs. Willard (1976) successfully cultured to maturity some diaspidid and coccid scale insect species on leaf discs floated upon distilled water, a method previously found appropriate for culturing or experimenting upon other Homoptera (Hughes & Woolcock 1965; White 1968). Willard found difficulty in establishing coccid crawlers (including Saissetia oleae (Olivier)) on leaf discs. Likewise, I found S. coffeae reluctant to settle on discs of Trichilia emetica, so crawlers were allowed to settle on leaves before the discs were cut. Survival was thereby increased and only those scale insects that lifted

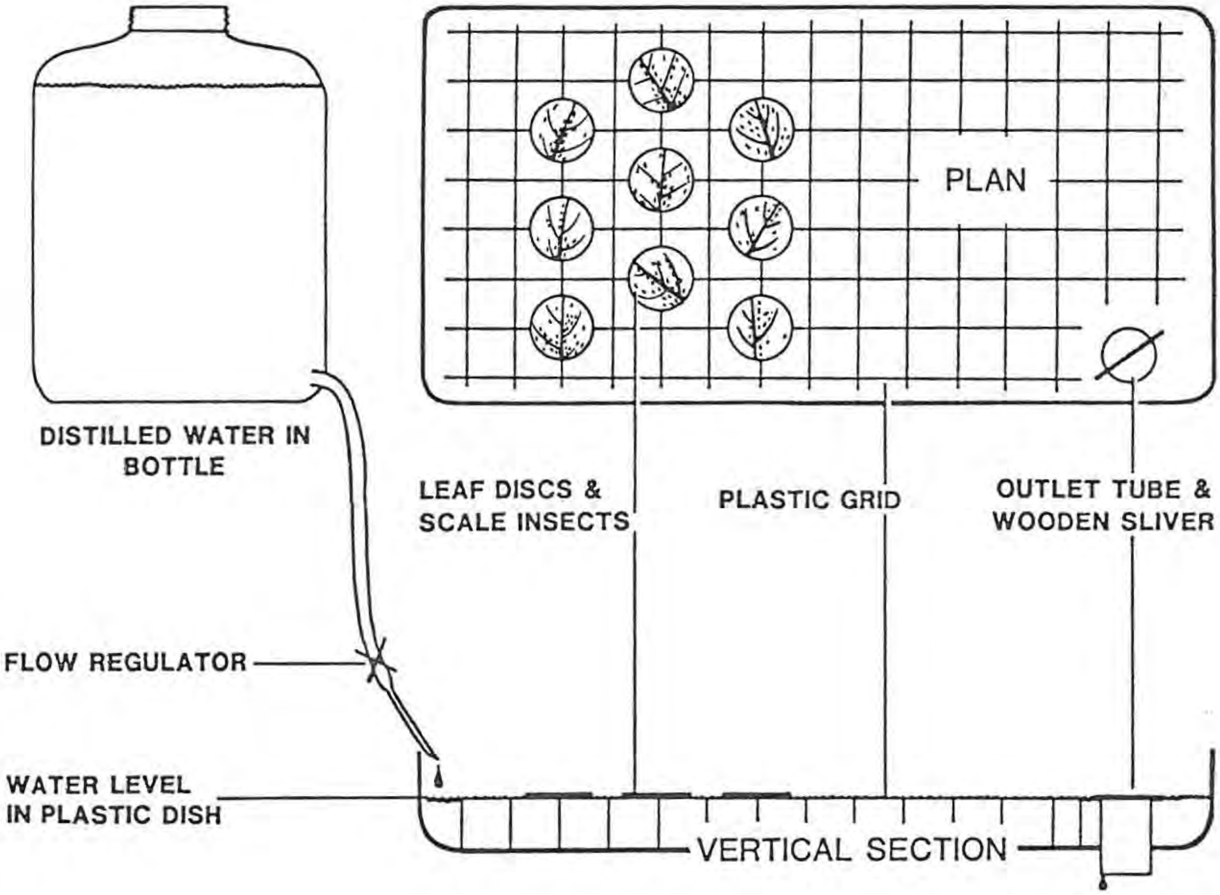

Fig. 4.3 : Tray in which leaf discs that contained parasitised scale insects were held. The drip irrigation system was designed to maintain a constant water level, thus ensuring that the discs were always.afloat, but that they did not move around and touch other discs.

and wandered were in danger of drowning. Leaf discs containing suitably-sized scale insects were used in the behavioural observations on C. bartletti. The discs remained viable longer than the 5-8 week period necessary for larval development of the parasitoid, especially if the cut edge was sealed with wax.

Willard's methods were slightly modified in other ways to facilitate the handling and storage of leaf discs. Discs were placed on a plastic grid that was housed in a tray (320 x 220 mm) (Fig. 4.3). Water level was maintained so that the top of the grid was just covered. Therefore the discs held their position. A drip system (Fig 4.3) provided continuous water input, and a wide-mouthed (25 mm) tube was glued to the level of the grid to drain excess water through the bottom of the tray. In this way, fewer discs were lost as a result of waterlogging. Wooden slivers were balanced across the tube to break the surface tension of the water against the glass. In addition, 2.5g benomyl fungicide (trade name 'Du Pont Benlate') per litre was added to the reservoir of the drip. This retarded fungal growth at the edges of the leaves where honeydew tended to accumulate.

Experimental cages. Because C. bartletti is so small (about 1.5 mm in length), all behavioural observations had to be made with the aid of a binocular microscope. The simplest and most effective transparent enclosure for caging females on potato sprouts under the microscope was found to be a 150 mm length of glass tubing (internal diameter = 23 mm) (Fig. 4.4A). The gap between the bottom end of the glass tube and the stem of the enclosed sprout was closed off with a circular foam rubber bung, and the top was sealed with a perforated, muslin-covered 'polytop' stopper. Visual distortion, brought about by the curvature of the glass, was a problem when observation under higher magnification was necessary.

Initially these cages were used in long-term experiments but transpiration by the sprout and the accumulation of honey-dew caused the humidity to reach unacceptable levels. Fungus, particularly, became troublesome under these conditions. Mites and thrips also flourished.

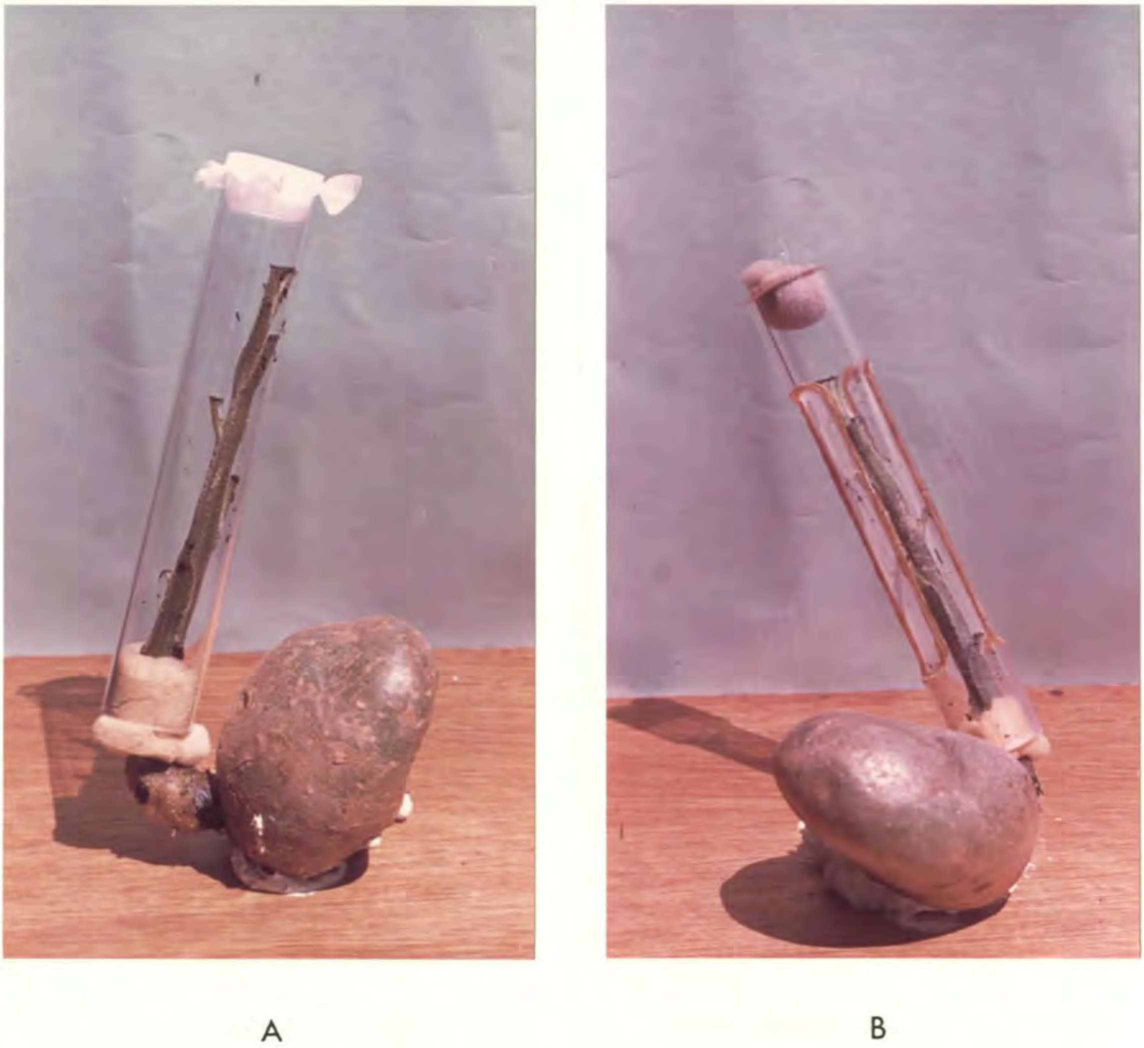

Fig. 4.4 : Cages designed to hold potato sprouts and *C. bartletti* females for experimental purposes. Glass cages (A) were replaced by perspex ones (B) that contained muslin-covered ventilatory holes. Ventilation precluded fungal growth in honeydew accumulations.

To overcome these difficulties, perspex tubes, with large muslin-covered cut-outs were used (Fig. 4.4B). Fungus never developed in these cages, even if large amounts of honeydew were secreted.

After the leaf disc method of culturing *S. coffeae* was perfected, they were used in preference to potato sprouts for observation purposes. The leaf disc was held, during observation, on a piece of moist filter paper. A short section of glass tubing formed a cage that was enclosed on top with a piece of glass (Fig. 4.5). Although the activity pattern of female C. bartletti wasps on the two substrates was identical, leaf discs proved easier to use because the scale insects were all on one plane and observation could take place through a flat glass surface.

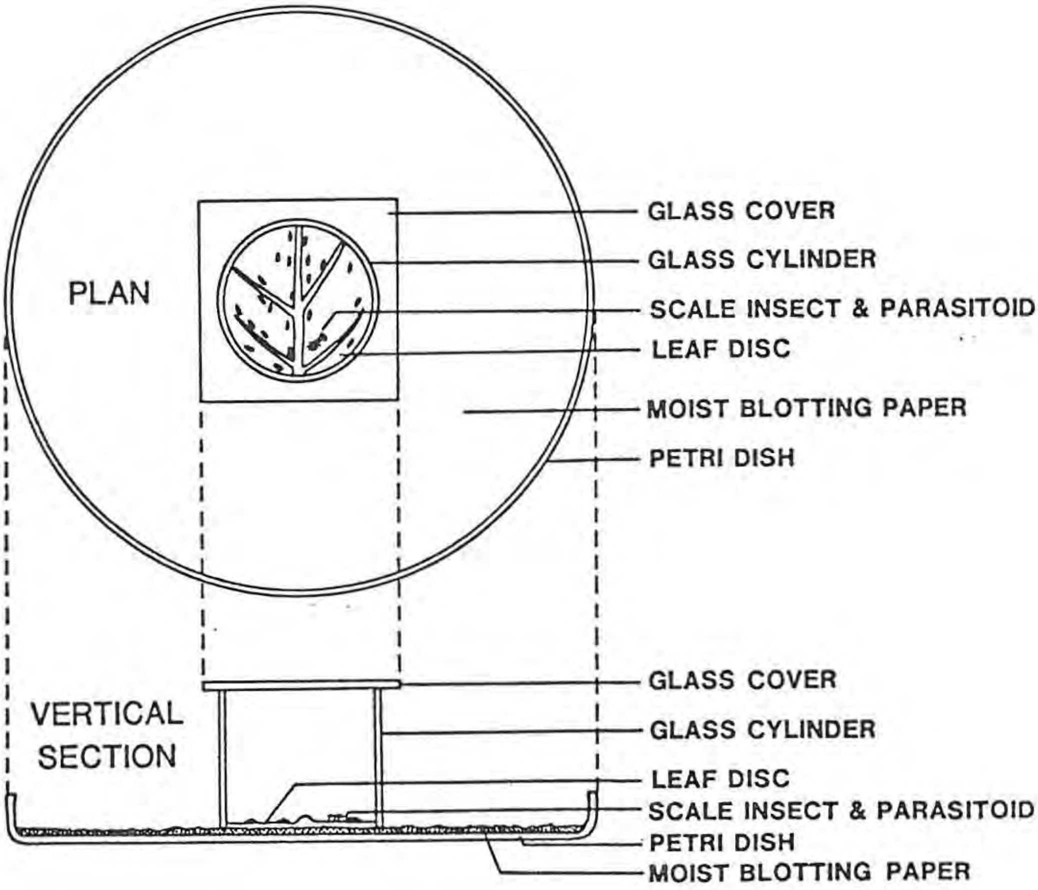

Fig. 4.5 : Behavioural observations on parasitoids were conducted with the aid of a binocular microscope. During observations the parasitoid was housed in this observation chamber. The advantage of this system lay in observations being conducted through a flat sheet of glass, which did not distort the image.

Collection of data. Detailed commentary on the behaviour of wasps (eg mating behaviour) was recorded on tape. Times were read from a digital stopwatch. Less detailed observations (eg daily activity patterns) were recorded on a paper chart that was run (at a constant known velocity) through a flat-bed recorder connected to a six-channel activity recorder. Particular experimental conditions and methods will be given in each section. Unless otherwise stated, experiments were conducted at 23 ± 1°C, and the resultant larvae (if any) were held at room temperature to complete development. When it was necessary to count offspring, they were dissected from the sprouts in the pupal stage and isolated in gelatin capsules. Thus, oviposition by the next generation of wasps, and therefore possible distortion of results, was prevented.

Dissection and marking of insects. Because of the small size of hosts and parasitoids, special care had to be taken when marking individual hosts, and when dissecting the ovaries from parasitoids, or when dissecting parasitoid eggs from hosts. The marking system used for scale insects is illustrated in Fig. 4.6. Indian ink was applied by means of a single hair glued to a handle, or with a finely-sharpened matchstick. The former was more successful and frequent wetting of the hair improved adhesion of the ink to the cuticle. Marking did not hamper either movement or development of scale insects.

Male (ectoparasitic) eggs were located by lifting the host with the aid of a minuten pin mounted in a matchstick. Dissection of female (endoparasitic) eggs from hosts proved more difficult. The most reliable method of dissection was to cut across the width of the scale insect, just anterior to the anal plates, with a minuten pin (Fig. 4.6). This was made easier if the body contents were gently squeezed forward with a second pin which then could be used to hold the insect down whilst the cut was made. After severing the posterior end of the scale insect, a pin was rolled from the front to the back of the insect to express the body contents and parasitoid egg, if present. Cutting across the body broke the gut and made retrieval of eggs easier, presumably because they are deposited into the digestive system. Dissection was performed under saline.

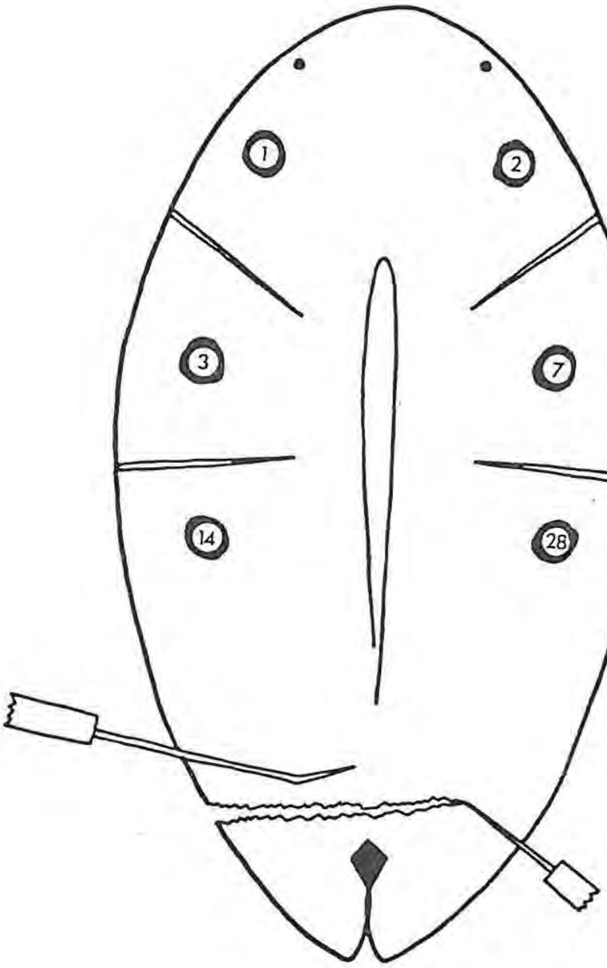

Fig. 4.6 : System of marking used to enable individual identification of scale insects. The number of an individual was indicated with a small spot, or combination of spots, of Indian ink in the appropriate place (numbered on diagram). To locate female (endoparasitic) eggs of Coccophagus bartletti, a bent, blunt pin was used to gently squeeze the body contents forward. A second, sharp pin was used to tear across the body just anterior to the anal plates. The body contents were then easily squeezed out.

The abdomen of C. bartletti females is tiny (about 0.33 mm in length) and dissection is difficult. It was made somewhat easier if performed under 35-70% alcohol: the ovaries whitened and became more visible without becoming too brittle. Strong illumination and a dark background helped. Dissection was easiest from the ventral side. A minuten pin was pushed through the thorax to anchor the insect. A second pin was used to pull the abdomen tip away from the body. With practice the ovaries could be pulled from the abdomen in one piece. Alternatively, a slit was torn down the side of the abdomen. This usually exposed the gonads, and the remaining tergites and sternites could be pared away.

This chapter describes the materials and methods that were used generally. Specific details varied from experiment to experiment and are given in the appropriate section.

CHAPTER 5 : IDENTITY OF HOST AND PARASITOID.

C. bartletti exists in sympatry with C. lutescens, and these species are almost identical morphologically. This raised the possibility that either C. lutescens or other, as yet unrecognised, cryptic species may be present in the material of this study. To ensure that only C. bartletti wasps were used for experimental purposes, and were counted in field samples, the following investigation was undertaken.

Host. C. bartletti was originally described as a primary parasitoid of Saissetia oleae from Stellenbosch (Annecke & Insley 1974). In Grahamstown, collections of C. bartletti have been made from Trichilia emetica on which various species of soft scale insects occur, including S. coffeae, S. somereni (Newstead) and Coccus sp. The black scale insect that established most readily on green potato sprouts in the laboratory was S. coffeae, and C. bartletti readily accepted it for oviposition and successfully completed development. So S. coffeae is presumably an habitual host of this parasitoid in the field, and perhaps S. somereni is too. Saissetia species were identified from the keys of De Lotto (1965) and Williams & Kosztarab (1972). Microscope slides were prepared according to the method of Cilliers (1967).

Parasitoid. The small (about 1.5 mm) yellow Coccophagus species reared in large numbers during preliminary sampling of Saissetia spp. on Trichilia was initially identified by D.P. Annecke and M.J. Mynhardt as C. lutescens. This identification was tentative and based on coloration as no slide material was prepared. Compere (1931) had described C. lutescens females as follows: 'General color pale yellow on the dorsum, fading to white on the sides and venter of thorax and on the face and cheeks.' Later, Annecke & Insley (1974) differentiated C. bartletti from C. lutescens on the basis of the female's distinct brown pronotum and anterior mesoscutum, as well as dusky suffusions elsewhere on the thoracic and abdominal dorsum. Small differences in antennal structure were also detected.

Because specimens in my collections were variable in the amount of brown on their dorsal surface, and because even those with extensive brown markings were identified by Annecke & Mynhardt as *C. lutescens*, the possibility existed that more than one (cryptic or sibling) species of *Coccophagus* was present and/or that *C. lutescens* and *C. bartletti* were synonyms. The mating behaviour of parasitoids from the study site and other areas was therefore investigated. Details of morphology and coloration important in the classification of these species were also noted (see Compere 1931; Annecke & Insley 1974). Offspring of known parentage were examined to establish the extent of intraspecific colour variation. Microscope slides were prepared according to the methods of Prinsloo (1980) and Noyes (1982).

(i) Mating behaviour. Parasitic Hymenoptera often have an elaborate and protracted courtship. In their review of the topic Gordh & DeBach (1978) divided mating behaviour into three arbitrary categories: precoital courtship, coitus and postcoital mount behaviour. Signals between the sexes may include display of colours, pheromone release, wing vibration, antennation, head movement, mouthpart manipulations and/or leg tapping (see van den Assem 1974; Matthews 1975; van Veen 1982), but there seems to be no clear phylogenetic pattern, except that species within a genus (eg *Monodontomerus* (Torymidae): Goodpasture 1975; *Aphytis*: Gordh & DeBach 1978; *Achrysocharoides* (Eulophidae): Bryan 1980), and sometimes subfamily (eg Pteromalinae: van den Assem 1974; Tetrastichinae: van den Assem *et al.* 1982a), tend to have similar courtship behaviour.

Table 5.1 provides a review of the duration of the three categories of mating behaviour in Aphelinidae, and the signals used in pre- and postcoital display. Postcoital mount behaviour is not a general habit, but where it does occur the postcoital display is by far the longest part of mating behaviour. Mating is generally fairly short in species that do not have a postcoital display (except for *Encarsia tricolor* (Foerster)), and is sometimes only a few seconds long (Table 5.1). The latter species, in addition, do not show elaborate courtship behaviour and the signals between male and female must be all, or mainly, pheromonal. Most aphelinids may have mating behaviour of this nature, which may explain why, despite their common use in biological control

TABLE 5.1 : Analysis of mating behaviour in aphelinid species that parasitise Coccoidea and Aleyrodoidea. Mating behaviour is divided into precoital courtship, coitus and postcoital courtship, and the duration of each category is presented. In some cases, authors did not distinguish between categories, so the time taken is inserted between the column for precoital courtship and the one for coitus. Signals used in pre- and postcoital display are differentiated. Females of those species indicated with an asterisk are known to be monandrous.

SPECIES	DURATION (SECS)			SIGNAL USED				AUTHOR(S)
	PRECOITAL COURTSHIP	COITUS	POSTCOITAL COURTSHIP	PHEROMONES	WINGS	ANTENNAE	LEGS	
**Aphytis lingnanensis* Compere	3	4	105	?Pre	Post	Pre & Post	Post	Gordh & DeBach 1978
**Coccophagoides utilis* Doutt	5 - 11		0?	Pre	-	-	-	Broodryk & Doutt 1966
**Coccophagus atratus* Compere	2-10	2	0	Pre	-	Pre	-	Clark 1984; Donaldson 1984
**Coccophagus bartletti* Annecke & Insley	15	4	90	Pre	Post	Pre	-	This study
Coccophagus capensis Compere	<1		0?	?Pre	?	?	?	Flanders 1937
Coccophagus hemera (Walker)	Few secs		0	?Pre	-	Pre	-	Zinna 1961
**Coccophagus lutescens* Compere	5	3	48	Pre	Post	Pre	Post	This study

CONTINUED

TABLE 5.1 : CONTINUED

SPECIES	DURATION (SECS)			SIGNAL USED				AUTHOR(S)
	PRECOITAL COURTSHIP	COITUS	POSTCOITAL COURTSHIP	PHEROMONES	WINGS	ANTENNAE	LEGS	
Coccophagus _ochraceus_ Howard	Few secs		0?	?Pre	-	-	-	Cendaña 1937
Encarsia _lahorensis_ (Howard)	Few secs		0?	?Pre	?	Pre	?	Viggiani & Mazzone 1978
Encarsia _perniciosi_ (Tower)	7-16	?	180-360	?Pre	Post	Pre & Post	Post	Tower 1914
*_Encarsia_ _partenopea_ Masi	Few secs		?	?Pre	-	Pre	-	Mazzone 1983
Encarsia _tricolor_ (Foerster)	180-240	>1	0	?Pre	Pre	Pre	-	Stüben 1949
Lounsburyia _trifasciata_ (Compere)	30-60		0?	?Pre	?	?	?	Cendaña 1937; Flanders 1937
*_Physcus_ _seminotus_ Sivestri & *_P._ _subflavus_ Annecke & Insley	Few secs		0?	?Pre	-	-	-	Williams 1972
Pteroptrix _smithi_ (Compere)	20-30	3-5	0?	?Pre	-	Pre	-	Bar & Gerling 1971

(see Clausen 1977), few details have been published about their courtship. There is little incentive to publish details of a brief and straightforward encounter between the sexes. Methods for comparing species (for taxonomic purposes) at a pheromonal level may be necessary.

Analyses of mating behaviour such as those in Table 5.1 and in Gordh & DeBach (1978) may therefore omit cryptic signals between males and females. Other, more subtle, forms of communication may even go unnoticed, for example the gentle strumming of a female's abdominal setae by a male's tarsi (described below), and perhaps even the precise position in which the male stands on the female. For observations on mating behaviour to be useful for comparative and, particularly, taxonomic purposes, it must be descriptive and detailed and, where possible, quantified.

Fortunately the mating behaviour of the Coccophagus species investigated is relatively elaborate, and is potentially useful taxonomically, as for example in Aphytis (Gordh & DeBach 1978), the pteromalid Muscidifurax (van den Assem & Povel 1973), Monodontomerus (Goodpasture 1975) and the eulophid Melittobia (van den Assem et al. 1982b). Wasps used for observations on mating behaviour were taken from the field, or were first-generation insects from cultures. They were stored, after mating, for morphological examination.

The mating behaviour of Coccophagus wasps (identified later as C. bartletti) from my study site (n = 41) was remarkably consistent and followed a definite pattern that falls into nine sequential categories that are illustrated and numbered in Fig. 5.1. This figure also shows the duration of each behavioral act, and where appropriate, the number of repetitions. Stimulation of males, marked by their questing behaviour, is probably pheromonal (category number 1) because it occurs at a distance and in response only to virgin females. (Second matings seldom, if ever, occur). In the nearby presence of a virgin female, males walk in a zigzag pattern. Their antennae are held wide apart and the scape is clearly visible. Once the male locates the female, he touches her body briefly with his antennae (category number 2) and mounts her (category number 3) if she stands still.

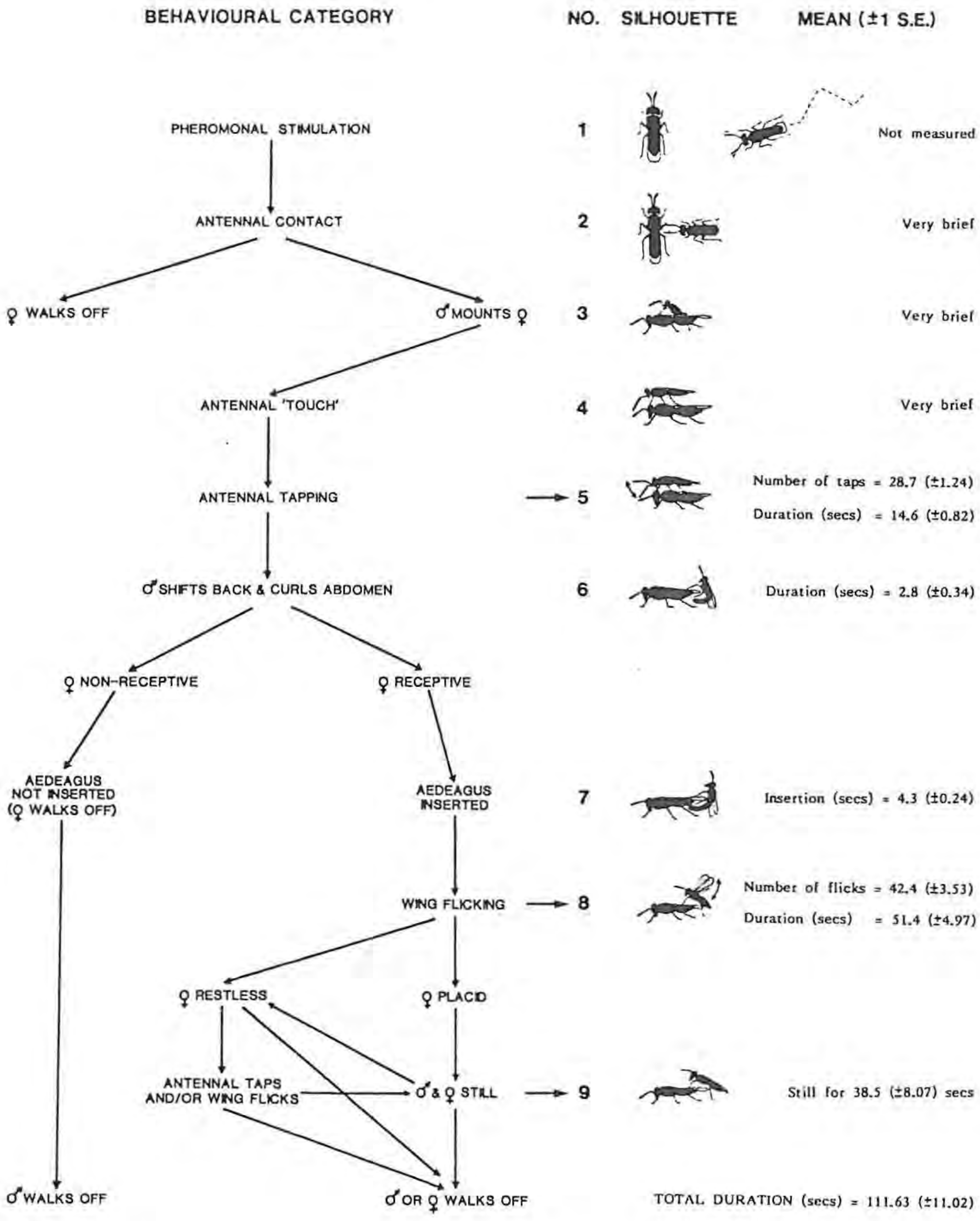

Fig. 5.1 : Diagrammatic representation of mating behaviour in Coccophagus bartletti. Behavioural categories are presented in flow chart form. The number of each category is that used for descriptive and comparative purposes in the text. Arrowed numbers indicate those categories in which C. bartletti differs from C. lutescens (Fig. 5.2). Behaviours described as 'very brief' usually lasted less than one second.

If the female walks away, the male never follows her although he does continue searching. Once mounted, the male briefly touches the female's antennae with his own (category number 4). This is followed immediately by a lengthy antennal-tapping performance (category number 5) in which the male rapidly raises and lowers his antennae over those of the female. The antennae may or may not touch: if they do their strike is diagonal to and displaces the female's antennae. During this performance, the stance of the male is characteristic. His prothoracic legs are placed upon the vertex or the compound eyes of his partner. Her wing bases are stroked by the inwardly-directed tarsi of the male's mesothoracic legs, and stroking is intensified if she refuses to stand still. The hind legs of the male rest on the front edge of the female's wings. This stimulation usually renders the female receptive and passive, and the male then moves back and curls his abdomen tip under the female (category number 6) to insert his aedeagus (category number 7) for a few seconds. There is no visible indication of receptivity by the female, as is found in other species (van den Assem 1974; Matthews 1975; Gordh & DeBach 1978; van den Assem et al. 1982a, b). Males (3 of 41 observed) repeated antennal tapping if insertion was taking too long, or if the female became restless. For simplicity these repeated behaviours are not represented in the statistics of Fig. 5.1, and their inclusion would add less than one second to the mean duration of mating. After insemination and withdrawal the male detains the female by remaining in the 'insertion' posture and flicking his wings open and closed (category number 8). His mesothoracic legs are moved slowly backward and forward. The tarsi often brush or strum the female's abdominal setae. If the female becomes restless after insemination, the male moves back onto her dorsum and quietens her with a 'touch' of his antennae or with a number of antennal taps. Males on females that remain passive (63%; n = 41) eventually become inactive too, and both partners stand still (category number 9), until eventually one or the other walks off. Therefore, like many other parasitic wasps, holding the female stationary for a period seems important to the male.

The pattern of mating behaviour (n = 16) of C. lutescens (collected

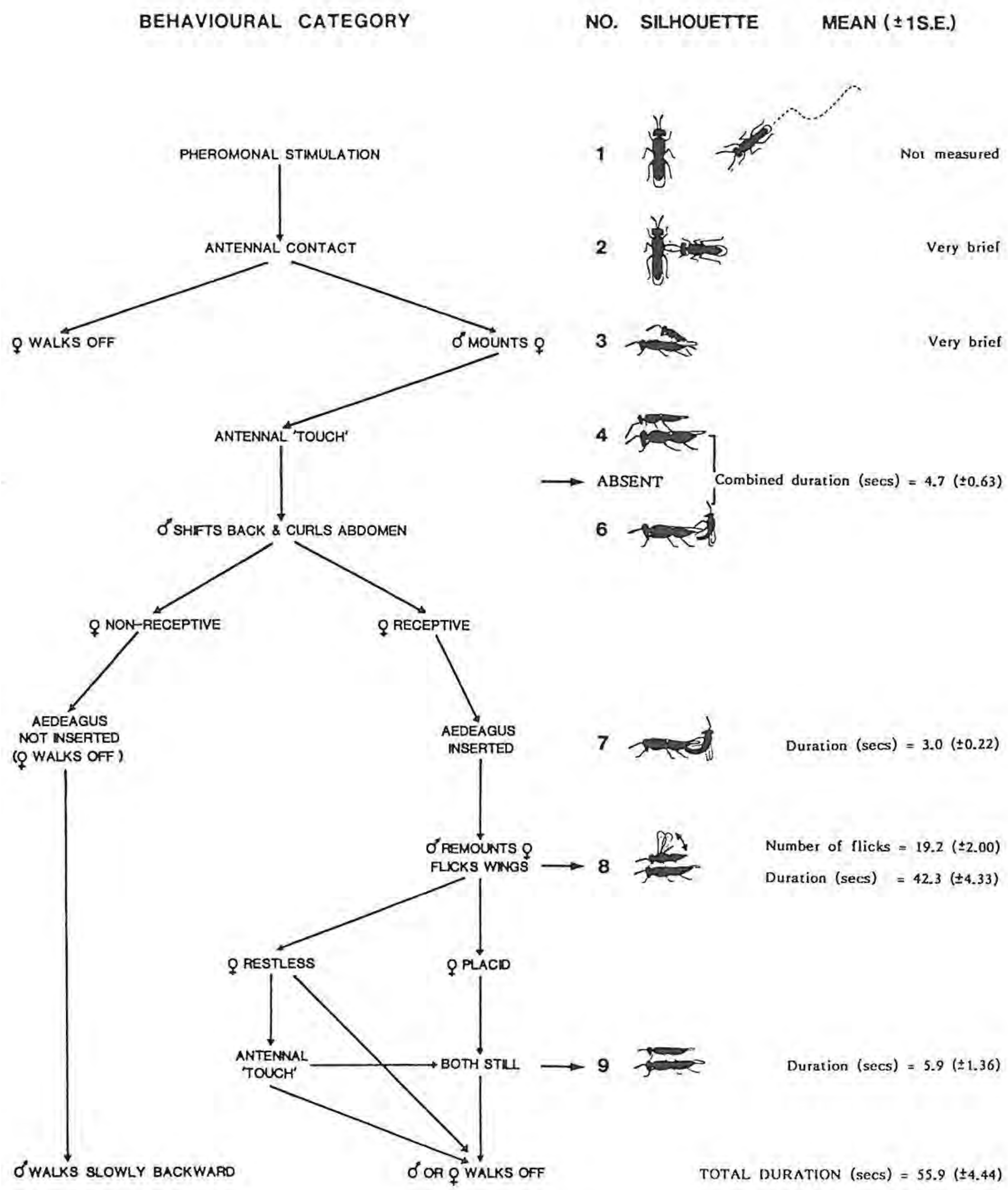

Fig. 5.2 : Diagrammatic representation of mating behaviour in Coccophagus lutescens. Behavioural categories are presented in flow chart form. The number of each category is that used for descriptive purposes in the text. Arrowed numbers indicate those categories in which C. lutescens differs from C. bartletti (Fig. 5.1). Behaviours described as 'very brief' usually lasted less than one second.

from *S. oleae* on *Exomis microphylla* (Thunberg) Aellen about 5 km north of Grahamstown) shows many of the basic elements seen in *C. bartletti*, but consistent differences do exist (Fig. 5.2). Location of a female (category number 1), and the subsequent mounting and pacifying behaviours (category numbers 2-4), are essentially similar to the equivalent steps in *C. bartletti*. The first major difference is the absence of any antennal-tapping (category number 5) in *C. lutescens*. If females of *C. lutescens* are receptive, a single touch to her antennae by those of the male is sufficient to stimulate acceptance. It is possible that the male, like males of some *Aphytis* species (Khasimuddin & DeBach 1975), releases a pheromone that identifies the individual as conspecific. Only two of the sixteen males had to repeat the antennal touch.

Precopulatory behaviour in *C. lutescens* is significantly briefer ('t' = 7.81, d.f. = 55; $P \ll 0.01$) and simpler than in *C. bartletti*. Females of the latter species refuse conspecific males if they omit antennal-tapping. Aedeagal insertion (category number 7) in the two species follows the same pattern, but that of *C. lutescens* takes significantly longer ('t' = 3.18, d.f. = 55; $P < 0.01$). The greatest difference in courtship behaviour between the two species occurs in the post-copulatory stage (category numbers 8 & 9). Male *C. lutescens* immediately after withdrawing the aedeagus, walk back onto the female and adopt the 'antennal-tapping' stance of *C. bartletti*. They never tap with their antennae, but, with their prothoracic tarsi, tap the female's vertex and compound eyes. Periodically the male 'licks' each tarsus by running it through his mouthparts. At the same time the male flicks his wings in similar fashion to *C. bartletti*. *C. lutescens* flicks its wings significantly fewer times than *C. bartletti* ('t' = 4.0, d.f. = 55; $P \ll 0.001$), and its post-copulatory behaviour is of shorter duration.

Postcoital guarding of females is common in insects, and has often been interpreted in terms of sperm precedence (sperm competition) (eg Parker 1970, 1974; Thornhill & Alcock 1983; Waage 1979). That is, males guard females because sperm of the female's most recent partner is considered to be used with greater frequency. Results from parasitic Hymenoptera, however, contradict the theory (Wilkes 1966; Holmes 1974), and other investigations have yielded ambivalent results generally (both situations discussed in Walker 1980). Nevertheless, mate guarding by males with polyandrous females does theoretically confer a selective advantage on males that do guard (Parker 1970, 1974; Thornhill & Alcock 1983). The aphelinids with guarding behaviour are mostly monandrous (Table 5.1), but Gordh & DeBach (1978) postulate that males guard females until they do become refractory to other suitors. The number of species that do not show guarding behaviour in Table 5.1 raises questions of whether (i) their females become refractory sooner than those of other species, (ii) the female is 'guarded' by means of an antiaphrodisiac pheromone (as in Tenebrio beetles (Happ 1969) and Heliconius butterflies (Gilbert 1976)), (iii) their males are rare relative to females and a high probability exists that males will encounter another mate during the period they would have spent guarding (Thornhill & Alcock 1983, p. 346), or (iv) postcoital mounting serves another function, or is merely an effect (sensu Williams 1966) of some other adaptive feature of mating behaviour.

(ii) Morphology and coloration. C. bartletti (NCI and Grahamstown specimens) is most different from C. lutescens (NCI specimens) in antennal structure. The most important difference is in the relative sizes of their three funicle segments (Fig. 5.3; Table 5.2). The second funicle segment of C. bartletti is relatively short (about the same size as the small first segment) and never has rhinaria (n = 79). That of C. lutescens, however, is large in relation to the first funicle segment, and is almost as long as the third funicle segment, and at least one rhinarium is always present (n = 39). In addition, the most distal septum of the antennal club is oblique in C. lutescens, but not so in C. bartletti (Annecke & Insley 1974; Fig. 5.3).

The Grahamstown C. lutescens specimens seemed to have the second funicle segment intermediate in length between the short first and long third segments. I tested whether the ratios (length of first segment/length of 2nd segment) and (length of 2nd segment/length of 3rd segment) of the Grahamstown specimens were significantly different from specimens identified as C. lutescens in the National Collection of Insects. The latter specimens were from a variety of South African

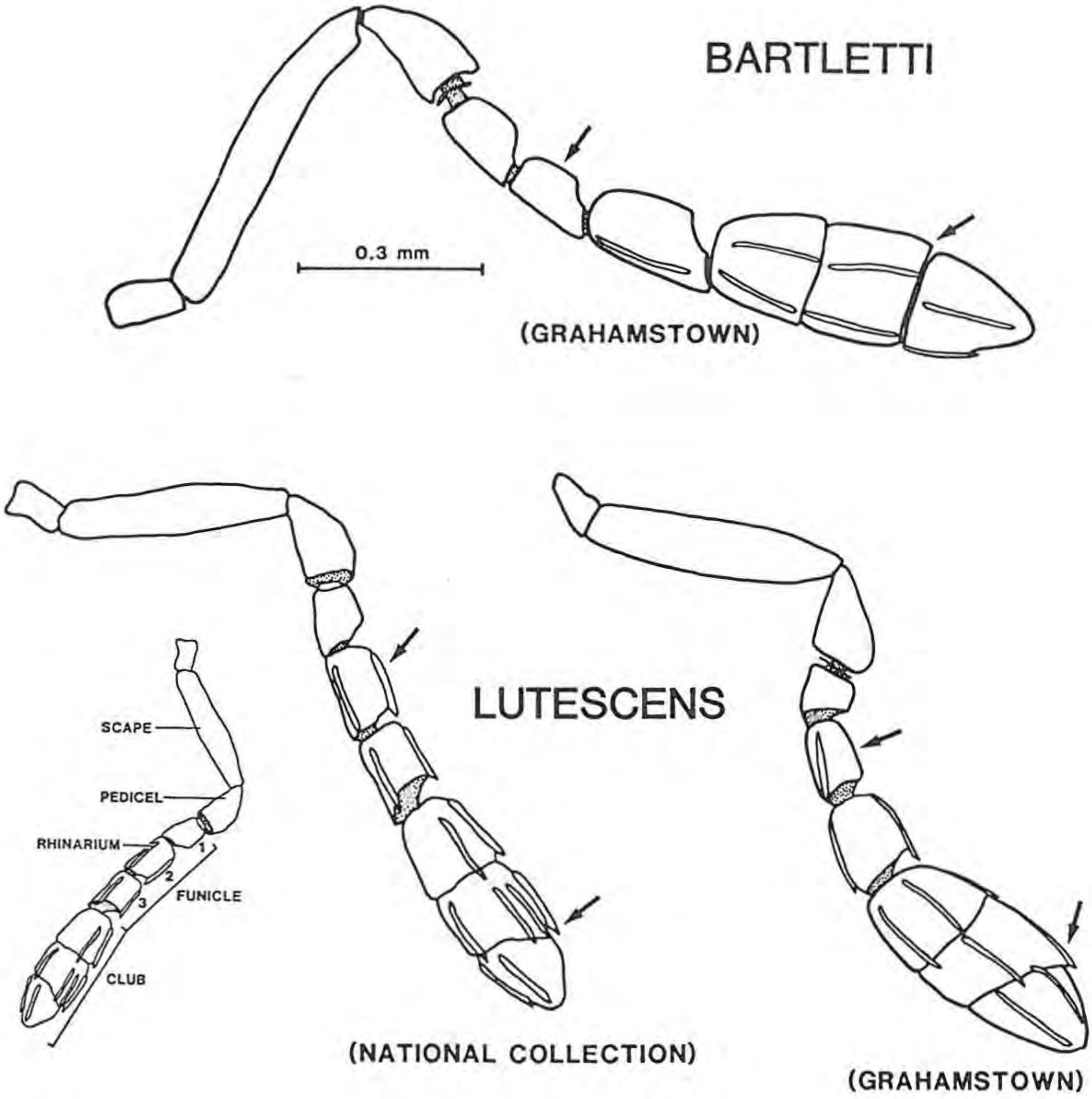

Fig. 5.3 : Arrows indicate those features in which the antennae of Coccophagus bartletti differ from those of C. lutescens (see Table 5.2 and text). Antennae of two C. lutescens specimens are illustrated. Grahamstown individuals differ slightly from specimens in the National Collection of Insects, Pretoria (see Fig. 1.2) in the relative lengths of their funicle segments (Table 5.2). The scale bar does not apply to the National Collection C. lutescens specimen, which was redrawn from Annecke & Insley (1974).

Table 5.2 : Ratios ($\bar{x} \pm 1$ S.E.) of funicle segments of two samples each of <u>Coccophagus</u> <u>bartletti</u> and <u>C.</u> <u>lutescens</u>. One sample comprised named specimens in the National Collection of Insects (Pretoria) and the other specimens are from Grahamstown. The ratios (length of 1st segment/length of 2nd segment) and (length of 2nd segment/length of 3rd segment) from one sample of each species were compared with the same ratios from the other sample of that species. Because the data were not normally distributed, even after arcsine and logarithmic transformation, a Wilcoxon two-sample test (Sokal & Rohlf 1981, pp.432-437) was used.

SOURCE OF SPECIMENS	NATIONAL COLLECTION			GRAHAMSTOWN			P & STATISTICAL SIGNIFICANCE	
SEGMENT RATIOS	1/2	2/3	n	1/2	2/3	n	1/2	2/3
<u>Coccophagus</u> <u>bartletti</u>	1.14 (±0.03)	0.67 (±0.01)	15	1.11 (±0.01)	0.66 (±0.008)	64	$P > 0.1$ N.S.	$P > 0.2$ N.S.
<u>Coccophagus</u> <u>lutescens</u>	0.77 (±0.01)	0.94 (±0.02)	18	0.83 (±0.02)	0.84 (±0.01)	21	$P < 0.01$ **	$P \ll 0.001$ **

localities (Fig. 1.2). A Wilcoxon two-sample test indicated that the two samples represented discrete populations (Table 5.2).

The ratios obtained for Grahamstown <u>C.</u> <u>lutescens</u> may have differed from NCI <u>C.</u> <u>lutescens</u> only because the measurements were taken at different times, or because the two samples were mounted by different individuals at different times. To determine whether this was likely, funicle segment ratios of Grahamstown <u>C.</u> <u>bartletti</u> specimens were compared with NCI <u>C.</u> <u>bartletti</u> specimens. No statistical difference between the latter two populations (Table 5.2) indicated that the observed differences in antennal structure between the <u>C.</u> <u>lutescens</u> populations are probably not artefacts.

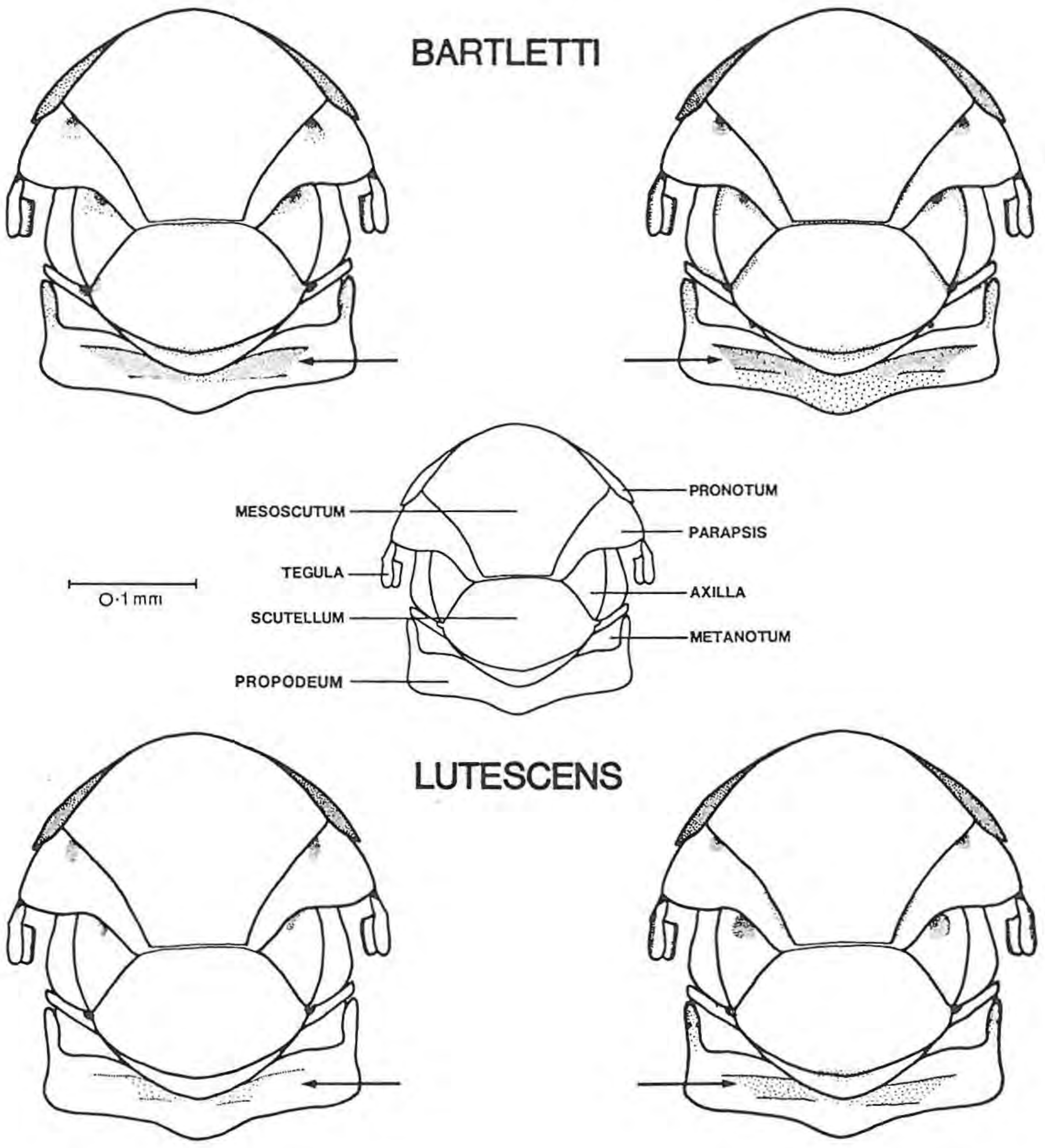

Fig. 5.4 : Thoraces of light (left) and dark (right) specimens of Coccophagus bartletti and C. lutescens. Brown markings are indicated with dots, the density of which are proportional to the intensity of coloration. The only consistent difference lies in the propodeal mark (arrowed).

The dorsal thoracic surfaces of females from both species have brown markings (Fig. 5.4). Males were not examined as they were not used in any experimental work. Thoracic markings were not anticipated for *C. lutescens* which was described as an entirely yellow species by Compere (1931) and Annecke & Insley (1974). The marks on the pronotum, tegulae, parapses, axillae and posterior edge of the scutellum of *C. bartletti* are similar in shade and correspond with those in *C. lutescens* (Grahamstown specimens illustrated; NCI specimens vary, and some are entirely yellow). However, the markings on the posterior edge of the mesoscutum, lateral points of the scutellum, and on the propodeum (Fig. 5.4) of *C. bartletti* are generally more extensive and darker than in *C. lutescens*. The only difference lies in the propodeal mark (arrowed in Fig. 5.4), which is usually lighter in *C. lutescens*. Little variation in coloration was noticed within broods of *C. bartletti*, and no specimens were lighter than the light one illustrated in Fig. 5.4 (top left).

The genae of dead *C. lutescens* specimens are slightly darker than those of *C. bartletti*. This is not evident in most *C. lutescens* specimens in the National Collection of Insects, and Compere (1931) and Annecke & Insley (1974) described the face and cheeks as fading to white. The darker cheek of Grahamstown *C. lutescens* is visible even in live specimens, which, in common with *C. bartletti*, have a pale metallic blue face. The genae are more intensely coloured than the rest of the face, but the blue fades soon after death. This phenomenon has been described in various brown *Prococcophagus* species (Annecke & Mynhardt 1979), but not in *Coccophagus*.

Although coloration is a fair indication of specific distinctness within this group of species, it is probably unwise to use it alone in species identification.

The discrepencies between Compere's (1931) description of *C. lutescens*, specimens identified by Annecke & Insley (1974) as *C. lutescens*, and the Grahamstown *C. lutescens* raise two possibilities.

a) *C. lutescens* may be more variable than described in the literature.

b) The Grahamstown population of C. lutescens may represent an additional cryptic species within the ochraceus group of Coccophagus. Until this possibility is investigated further, the name C. lutescens will be retained, and where necessary the Grahamstown population will be specified.

(iii) Searching behaviour. Differences between C. bartletti and C. lutescens probably occur in their host-searching behaviour. Consistent differences between the species may exist either in the host species they attack most commonly, or in the height of vegetation through which they search. C. lutescens has usually been found at or near ground level (S. Neser, pers. comm.; this study) and C. bartletti has so far been found only in tree canopies (Annecke & Insley 1974; this study). Other species that seem to search at particular levels in vegetation are discussed in papers by Ullyett (1953), Weseloh (1972, 1976) and Shaw (1983).

(iv) Conclusions. The comparative study of mating behaviour demonstrated that consistent differences occur between C. bartletti and C. lutescens. This, and the constancy of the mating behaviour of individuals collected from Saissetia on Trichilia in Grahamstown, indicated strongly that individuals of only one species, C. bartletti, were present. In addition, all specimens from these collections that were examined microscopically (n = 69; at least 5 specimens collected during each month of the year) proved to be C. bartletti. Samples from my Grahamstown study site could therefore be analysed as containing only C. bartletti.

Having established the Grahamstown study population as representing a single species, C. bartletti, the basic ovipositional and host-feeding biology could be studied preparatory to assessing daily activity patterns of females, the ratio of male to female eggs deposited, and, ultimately, the response of ovipositing females to previously-parasitised hosts.

CHAPTER 6 : BIOLOGY OF COCCOPHAGUS BARTLETTI

Introduction. Before observations could be carried out on the daily activity patterns of C. bartletti females, or on their progeny sex ratios and reactions to parasitised scale insects, it was necessary to determine the behaviour and postures associated with female and male egg deposition, and with host-feeding. In some parasitoids, including Aphytis species (Luck et al. 1982) and the ichneumonids, Poecilostictus cothurnatus (Gravenhorst) (van Veen 1981) and Itoplectis maculator (Fabricius) (Cole 1981), egg deposition is associated with a typical, identifiable activity in the oviposition sequence, and this 'signal' was sought, too, in C. bartletti. Initially though, I ascertained that ectoparasitism and endoparasitism is obligatory for males and females respectively. Based on observations of C. bartletti, it is clear that certain interpretations of the biology of diphagous parasitoids have been incorrect and attention is drawn to these misinterpretations in this chapter.

Diphagous host relationships. Walter (1983a) listed nine parasitoid species known to have diphagous host relationships. However, Encarsia partenopea Masi should be removed: it has recently been discovered that BOTH sexes are primary endoparasitoids of white flies (Mazzone 1983). This species will be further dealt with in the discussion (chapter 10). Coccophagus matsuyamensis Isihara (Viggiani 1980) and Prococcophagus varius Silvestri (Mazzone & Viggiani 1983) are additions to the list. Methods that were originally used to determine the nature of these species' host relationships are not clear, particularly in Flanders' (1942b, 1953, 1959, 1967, 1969) brief mention of the developmental habits of C. ochraceus Howard, the first species known to be a diphagous parasitoid. Flanders relied, perhaps, on the data of Cendaña (1937), who had dissected larvae and described ectoparasitic development in C. ochraceus, but without knowing that only males of the species developed ectoparasitically. More recent reports seem also to have been based largely on dissections of eggs, larvae and pupae from hosts (eg Zinna 1961; Mazzone & Viggiani 1983).

Published information about the host relationships of *C. bartletti* is scant. There is reference to ectoparasitic males and endoparasitic females in a paper by Flanders (1959; based on unpublished studies by T.W. Fisher). Confirmation of the obligatory nature of these host relationships in *C. bartletti* was therefore necessary initially.

C. bartletti females lay eggs ectoparasitically and endoparasitically, and this is easily distinguishable by the female's behaviour at oviposition (see the following section). To determine whether ectoparasitic eggs were always male and endoparasitic ones all females, scale insects, each with a parasitoid egg of known oviposition site, were individually marked. When the developing parasitoid reached the pupal stage, the host was lifted from the substrate and isolated in a gelatin capsule to await emergence of the wasp, which was then sexed. Eleven hosts with 'endoparasitic' eggs and 14 with 'ectoparasitic' ones yielded adult wasps. The former were all females and the latter males. Two further observations support these data. Virgin females, which produce only male offspring, lay all their eggs ectoparasitically. Secondly, mummies (dried out host remains) that contained female wasps have their sternites intact whereas those that hold males no longer have sternites. This suggests that the developing male larvae destroyed the sternites when they entered the host towards the end of the larval feeding period (see Cendaña 1937). If mummies are lifted carefully from the host plant, the sex of the departed parasitoid can be determined in this way.

Oviposition, host size and host-feeding. Female (endoparasitic) eggs are deposited with a single probe into the host, and appear to lie in the gut but sometimes in the haemolymph. This activity lasts, on average, more than two minutes (Table 6.1), which is a long time compared with the few seconds taken by *C. hemera* (Walker), the other diphagous parasitoid examined (Zinna 1961). Some heteronomous hyperparasitoids (*C. capensis* Compere, *C. semicircularis* (Foerster) and *Lounsburyia trifasciata* (Compere)) even take less than a second or two to deposit a female egg (Cendaña 1937). This act lasts only six seconds in *C. atratus*, but much longer (usually more than a minute) when male

TABLE 6.1 : Oviposition by *C. bartletti*. Eggs can be sexed at oviposition and data ($\bar{x} \pm 1$ S.E.) are presented separately for female and male eggs. Duration of probes with the ovipositor, egg lengths and host dimensions are listed. Data were obtained by observing individual wasps, timing their behaviour and subsequently dissecting the hosts. Statistical probabilities were determined with a Wilcoxon two-sample test because variances in all pairs of samples were not equal : a 't'-test gave similar results.

	FEMALE EGGS	n	MALE EGGS	n	P & SIGNIFICANCE
DURATION PROBE 1	155(±10.2)secs	50	131(±8.4)secs	50	$P>0.1$;N.S.
DURATION INTERVAL	-	-	42(±10.3)secs	50	-
DURATION PROBE 2	-	-	133(±8.9)secs	50	-
EGG LENGTH	0.13(±0.001)mm	68	0.11(±0.001)mm	37	$P \ll 0.001$**
INITIAL HOST LENGTH	0.66(±0.03)mm	50	0.62(±0.02)mm	50	$P>0.5$;N.S.
INITIAL HOST WIDTH	0.36(±0.02)mm	50	0.33(±0.01)mm	50	$P>0.5$;N.S.
FINAL HOST LENGTH	0.96(±0.02)mm	25	0.97(±0.03)mm	25	$P>0.2$;N.S.
FINAL HOST WIDTH	0.51(±0.01)mm	25	0.53(±0.02)mm	25	$P>0.9$;N.S.

eggs are deposited (Clark 1984; Donaldson 1984). Oviposition rate is probably not influenced by large egg size relative to body size in *C. bartletti*, as this ratio approximates that in *C. hemera* (Zinna 1961). Other small heteronomous hyperparasitoids (eg *C. gurneyi* Compere: Cendaña 1937; *Coccophagoides moeris* (Walker): Zinna 1962; *Physcus seminotus* Silvestri: Williams 1972; *Encarsia formosa* Gahan: van Lenteren *et al.* 1976; Agekyan 1982; *E. opulenta* (Silvestri): Dowell *et* al. 1981) also probe the host for nearly two minutes and even longer before an egg is deposited.

Male (ectoparasitic) eggs are deposited beneath the host, and are attached by a short stalk to a sternite. They are most often found behind a metathoracic leg, as in C. ochraceus (Cendaña 1937). When depositing a male egg the wasp probes first into the host and only after retracting her ovipositor does she push it into position beneath the scale insect. Surprisingly, the first probe lasts, on average, as long as a diploid egg oviposition, and the second one is of similar duration (Table 6.1). The first probe may serve either or both of two functions. It is perhaps a 'test' probe that enables the wasp to assess the quality of the host, or a moult-inhibiting chemical may be injected. van Lenteren & DeBach (1981) discuss the similar ovipositional behaviour of ectoparasitic Aphytis species. Some scale insects, beneath which the parasitoid female clearly intended to deposit a male egg, walked away before the egg could be attached, so the first probe does not induce paralysis, as found in some caterpillar parasitoids (eg Neser 1973; Shaw 1983). The pattern of male egg oviposition described for C. bartletti is unlike that of the diphagous parasitoids C. ochraceus (Cendaña 1937; Flanders 1967) and C. hemera (Zinna 1961), which do not probe the host first.

Walter (1983b) reviewed the reasons postulated to explain the existence of a stalk on male eggs of several heteronomous aphelinid species. He agreed with Broodryk & Doutt (1966) that it assists in attachment of the egg, because only ectoparasitic and never endoparasitic male eggs have a stalk. Firm anchorage of the egg, even if the host is largely sessile, is necessary because black scale insects walk around at various stages of their development, even in the early stages of the final instar (Smith 1944; and this study). Drawing out of a stalk on the end of the egg causes male eggs to be significantly shorter than female ones (Table 6.1; Zinna 1961; Mazzone & Viggiani 1983). First-instar larvae of both sexes are hymenopteriform (defined in Clausen 1940; Hagen 1964). Unlike many heteronomous aphelinids, they show no larval sexual dimorphism other than young male larvae having spiracles and females none (see Walter 1983b), and this seems common to diphagous parasitoids (see Cendaña 1937; Flanders 1937; Zinna 1961).

A mistaken belief that mated female Aphelinidae produce only female offspring led Flanders (1953, 1959, 1967, 1969, 1973) to postulate that mated females of certain species (eg *C. ochraceus*) produce males only by malfunction of their spermathecae, which is said to occur when oviposition rates are high. Flanders (1959) suggested that rapid oviposition results in depletion of the spermathecal gland, consequent lack of sperm activation, and no fertilisation. However, mated *C. bartletti* females never laid more than 17 eggs in a day, and activity was spread over five hours in the presence of suitable hosts. This slow rate of oviposition is unlikely to have affected spermathecal function and yet males were produced. Further, the first egg laid at the start of the day was sometimes male, and subsequent ones female, so spermathecal 'malfunction' seems a most unlikely explanation.

Hosts accepted for female egg deposition were not significantly different in size from those in which male eggs were deposited (Table 6.1). Parasitised hosts, in turn, were not significantly different in size from scale insects drilled for host-feeding (Table 6.2). Drilling for host-feeding is unlikely to be mistaken for an oviposition probe. It is both quantitatively (Table 6.2) and qualitatively different. Host-feeding drills take much longer, and in the process, the wasp often raises her body full length on her legs, and usually rotates her stance through at least 180°, at the same time raising and lowering her body to slide the ovipositor to all parts of the scale insect's body. Drilling complete, she preens and wanders around in the vicinity of that host before returning to it to feed. She often loses it (as does *Encarsia formosa*: Agekyan 1982) but is clearly seeking that particular one, hence the long interval (Table 6.2) between drilling a host and drinking from it. Host-feeding killed the scale insects and left them desiccated. Even if hosts were not re-located after being drilled, they died. Eggs were never laid in these hosts. *C. ochraceus* (Cendaña 1937) and *Encarsia formosa* (van Lenteren *et al.* 1980; Agekyan 1982; Kajita 1983) also drill hosts specially for feeding, which seems to be done regularly. Other species (*Lounsburyia trifasciata*: Cendaña 1937; *Physcus seminotus*: Williams 1972; *Encarsia opulenta*:Dowell *et al.* 1981)

TABLE 6.2 : Host-feeding behaviour of Coccophagus bartletti (n = 30). Drilling is qualitatively (see text) and quantitatively different from oviposition behaviour (data from Table 6.1: only statistical results from a Wilcoxon two-sample test are presented).

	HOST-FEEDING	OVIPOSITION	
		FEMALE EGG	MALE EGG
DRILLING	334(±22.5)secs	$P \ll 0.001$**	$P \ll 0.001$**
INTERVAL	117(±24.4)secs	-	-
DRINKING	227(±25.2)secs	-	-
HOST LENGTH	0.67(±0.04)mm	$P > 0.5$; N.S.	$P > 0.5$; N.S.
HOST WIDTH	0.36(±0.03)mm	$P > 0.5$; N.S.	$P > 0.9$; N.S.

feed, upon occasion, from oviposition wounds, and some (eg C. gurneyi: Cendaña 1937; C. atratus: Donaldson 1984) never host feed.

Predicting oviposition after a probe into a host. Diploid eggs could not be seen through the hosts' cuticle. Dissections showed that some hosts probed only once by C. bartletti contained no egg. To determine how predictable oviposition is in this species following a probe with the ovipositor, a frequency histogram was drawn up that represents the number of consequential and inconsequential probes of different duration (Fig. 6.1). Only those probes equal to or longer than 76 seconds regularly resulted in oviposition. (Less than 4% of ovipositions were of shorter duration than this). However, only 60% of probes longer than 76 seconds yielded eggs, so diploid egg deposition cannot be accurately predicted. Hosts, except for those probed for less than 50 seconds and which never contained eggs, must be dissected to ascertain parasitisation.

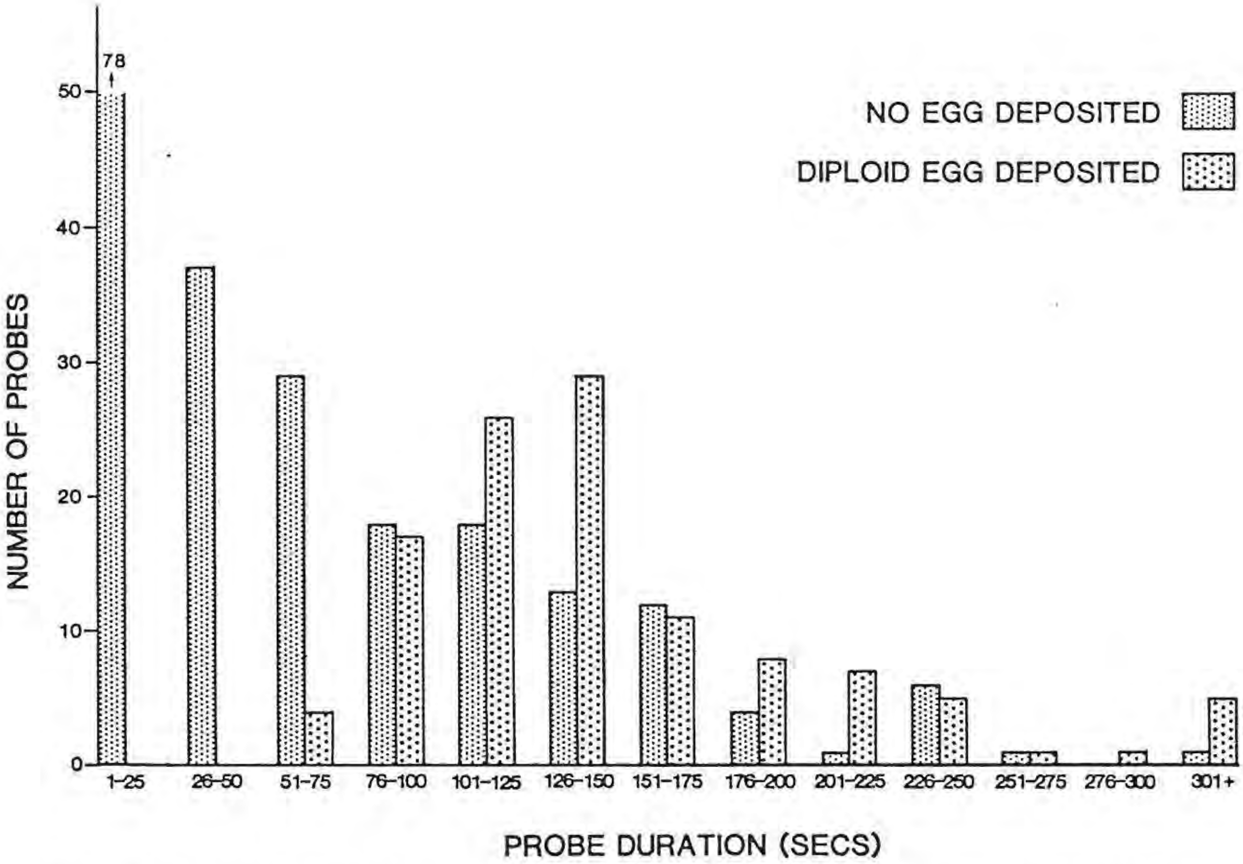

Fig. 6.1 : Host-probing and oviposition of female eggs by Coccophagus bartletti. Frequency of occurrence of different length probes that resulted in either egg deposition (coarse stippling) or no egg deposition (fine stippling). Data taken from observations on 6 females and dissection of the hosts they probed. Three hundred and thirty-two probes were recorded, but 8, in which the host walked away and was lost, were excluded, as were 13 in which the host contained an egg but had been probed more than once.

Male eggs could be seen after deposition, but not invariably so. In addition, the probe beneath the scale insect sometimes did not result in oviposition, or the first probe was omitted. So all hosts that had been probed were initially checked by looking underneath the scale insect for male C. bartletti eggs.

Despite this uncertainty about oviposition, and therefore the necessity for dissecting 'stung' hosts, the ability to sex eggs of *C. bartletti* was most useful in interpreting the behaviour of ovipositing females. Knowing the ovipositional and host-feeding characteristics of *C. bartletti* meant that the daily activity of females could be observed, and patterns of oviposition sought, which was preliminary to studying sex ratios and superparasitism in this species. Daily activity patterns in *C. bartletti* are discussed in the next chapter.

CHAPTER 7: ACTIVITY PATTERNS OF COCCOPHAGUS BARTLETTI

Recently emerged hymenopteran parasitoids, in the presence of an abundance of suitable hosts, usually respond in a characteristic way. Responses are generally species-specific and may be affected by mating. Females of some species contain a full or partial complement of eggs and are immediately prepared for oviposition. Others need time, and usually need proteins, to mature eggs. Longevity may be short, or prolonged. Many species with a long life need regular protein, and sometimes carbohydrate meals, to maintain egg production. If hosts remain unavailable, eggs may be resorbed to conserve proteins. Species that illustrate one or more of the points made in this introductory section have been studied by the following authors (Flanders 1935, 1942c; Quednau 1967a; Neser 1973; Quednau & Guevremont 1975; Dowell 1978; Sugimoto & Ishii 1979; Olaifa & Akingbohungbe 1982; Sugimoto *et al.* 1983; Dahms 1984). Reviews of the topic are also available (Flanders 1950; Bartlett 1964; Doutt 1964; Bell & Bohm 1975).

The nutrient requirements of parasitoid females and the nature of their reproductive systems may influence the amount and timing of ovipositional activity, even when hosts are abundant. Parasitoids of different species may therefore have different patterns of activity, which, in some species are repeated on a daily basis (eg Force & Messenger 1964; Quednau 1964, 1967b; Waloff 1974; Quednau & Guevremont 1975; Hofsvang & Hågvar 1975a, b; Sugimoto & Ishii 1979). In studies of daily activity patterns a clear distinction must be drawn between flight (dispersal) activity and ovipositional activity. Each may require special sampling or observational methods, as stressed by Weseloh (1976). This distinction was not made by Ekbom (1982), who interpreted activity patterns of *Encarsia formosa* in relation to oviposition, whereas the sampling method used was more appropriate to studying dispersal by this aphelinid.

Most of the authors cited above were concerned principally with the daily fecundity of parasitoids. They therefore did not take account of the duration or changes in the intensity, with time, of ovipositional activity. The daily pattern of ovipositional activity in a species may be an important consideration in (i) the design of experiments that include periods of ovipositional activity, and (ii) the standardisation procedures used to prepare parasitoids for experiments. Experiments in which sex ratios are studied may be particularly affected. For example, Donaldson & Walter (1984: Appendix 1) found that interpretation of sex ratios in Spalangia endius Walker (Pteromalidae) would have been aided if information on daily activity patterns of mated and virgin females was available. If eggs are deposited during discrete bouts of activity, then a study of the sex ratio of a species should include an analysis of the sex ratio of offspring deposited during each bout of activity. Information of this nature would be useful in identifying whether male and female eggs are deposited in a set temporal pattern, as described in a few parasitoid species (see Waage 1982a, b).

In parasitoids, such as C. bartletti, which host-feed frequently and so acquire proteins to provision developing eggs, it seems likely that oviposition will occur in discrete bouts during which a batch of matured eggs will be deposited. Such behaviour may be more pronounced if there is enforced abstention from hosts during the periods when eggs are maturing. This may occur, for example, if oviposition does not take place at night, or if there is considerable flight activity between encounters with patches of hosts.

The behaviour of C. bartletti was examined for consistent patterns in daily activity. In another experiment individual females, subjected to different regimes of host-feeding or starvation, were dissected to establish possible links between activity patterns, host-feeding and egg production. An investigation of daily patterns of ovipositional activity in C. bartletti was necessary before sex ratios could be investigated and before experiments could be designed to test the reaction of C. bartletti females to parasitised hosts.

Activity of a single female. In initial observations on the activity of C. bartletti, a single female was observed daily, starting 1-2 days after emergence, for fourteen successive days. Observations were started each day at approximately 09h00 and were continued for 5 hours, despite increasing periods of inactivity after 3 hours exposure to hosts. An excess of hosts was provided.

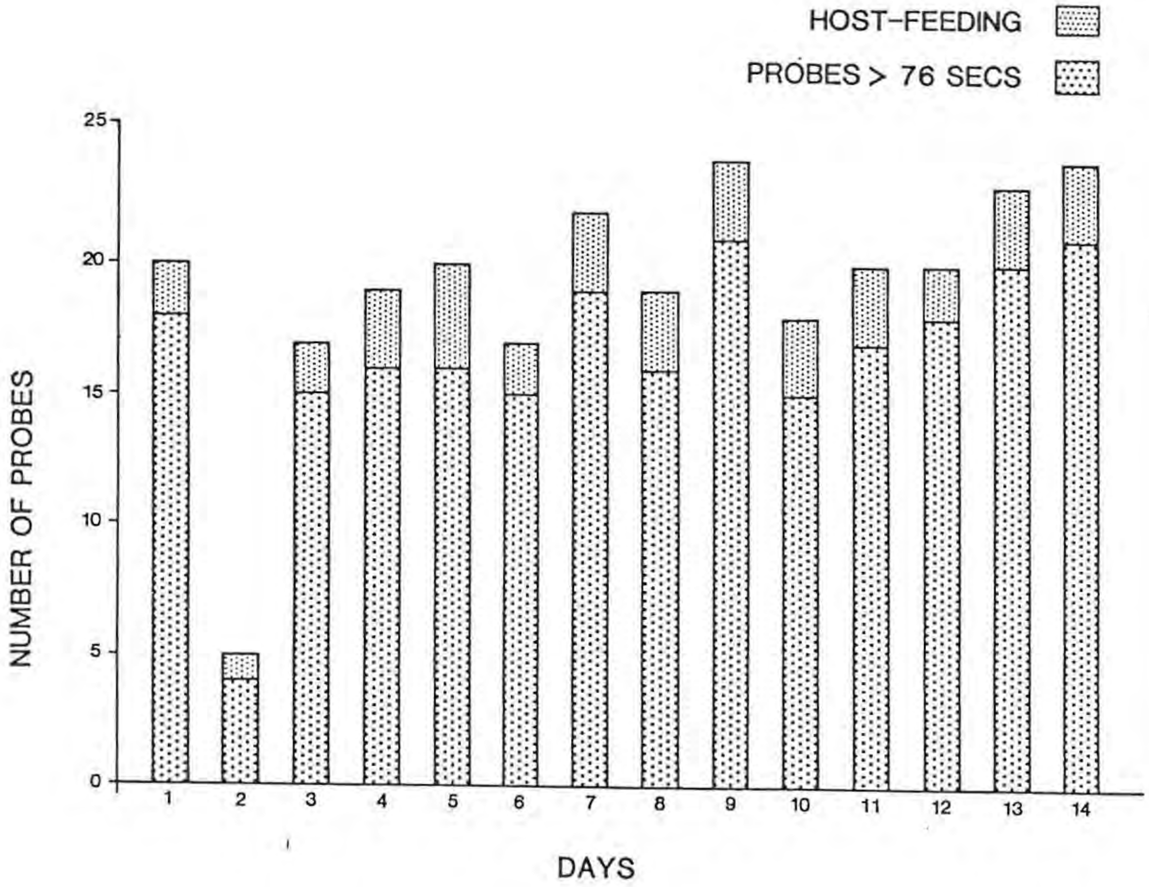

Fig. 7.1 : Number of probes (per day) by a single <u>Coccophagus bartletti</u> female that was observed for 5-hour periods on 14 successive days. Observations started 1-2 days after emergence, before this individual female had been exposed to hosts. Probes of 76 seconds, or longer, were included, and double probes were counted as one.

A frequency histogram shows the number of probes that potentially resulted in oviposition (ie probes longer than 76 seconds: see chapter

6) each day (Fig. 7.1). The number of probes daily was consistently between 15 and 21, except for day 2. Egg production possibly lagged up to that stage whilst the first proteins imbibed during the previous days host-feeding were assimilated. A lag between host-feeding and subsequent oviposition was found in the eulophid Chrysocharis pentheus Walker (Sugimoto & Ishii 1979). In general, the amount of ovipositional activity by the C. bartletti individual studied was similar on successive days.

The data for all 14 days were then grouped, and activity was analysed per half hour period (Fig. 7.2). All probes were included, but they were divided into 3 groups, those equal to or longer than 76 seconds (including double probes), those shorter than 76 seconds, and host-feeding probes. Fig. 7.2 shows that most activity occurs in the first five half-hour periods. It then drops to a much lower level (half-hour 6) after which it remains consistently low. Of the longer probes, 84% occurred in the first 3 hours, and this period appears to constitute a discrete bout of activity. Short probes were also less common after 2½ to 3 hours, when the parasitoid spent relatively long intervals standing still and sometimes preening herself. Host-feeding was approximately evenly distributed throughout the observation period, but was most common in the first half hour.

The delimitation of activity bouts in studies of animal behaviour has recently received statistical attention (Fagen & Young 1978; Slater & Lester 1982). If there is a random distribution of events in time, short intervals between events would be more common than long intervals. If a frequency histogram of such data is plotted, a negative exponential distribution is obtained (Fagen & Young 1978). Usually the logarithm of the frequency (of all intervals longer than each successive interval) is plotted on these 'log survivor' functions. During a bout of any given activity, intervals between the activities also have a negative exponential distribution. Between bouts of activities, isolated occurrences of the behaviour under observation do occur, so 'between-bout' intervals of much longer duration than 'within-bout' intervals are occasionally measured. Inclusion of all these intervals on the log survivor plot results in a change in slope

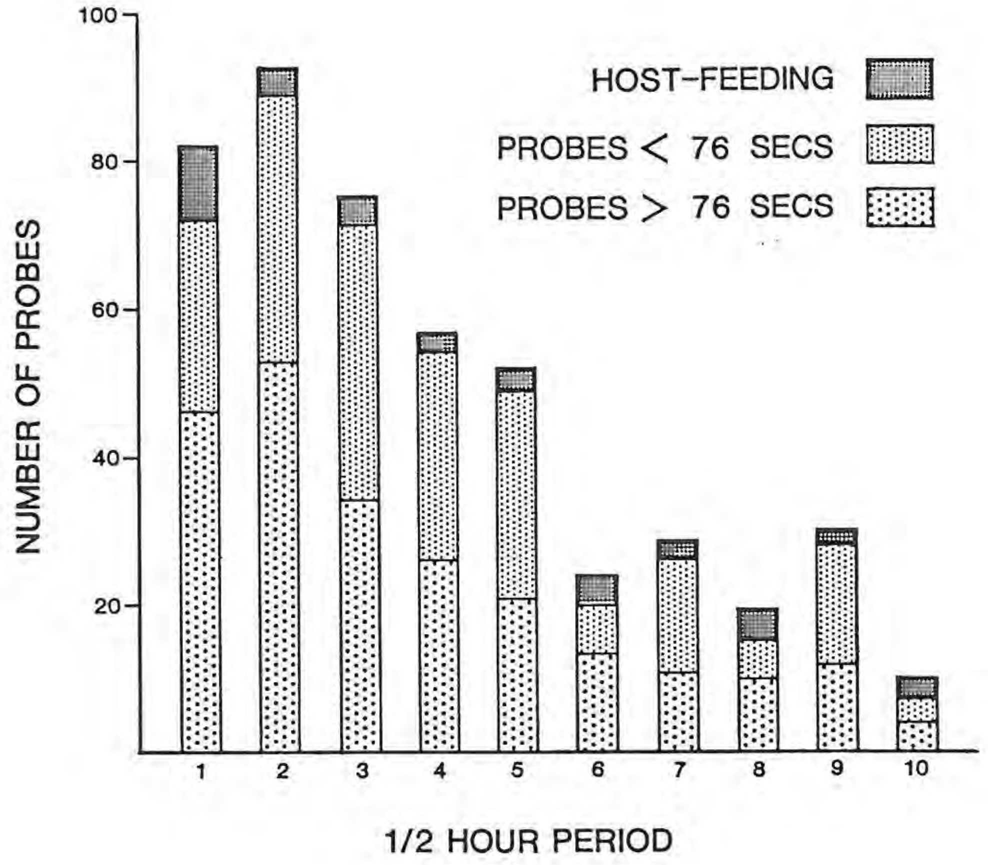

Fig. 7.2 : Frequency of probes per ½-hour period, during 14 5-hour periods of observation on a single Coccophagus bartletti female. Observations took place on successive days. Probes shorter than 76 seconds probably did not result in egg deposition. 84% of probes longer than 76 seconds (including double probes) occurred in the first 3 hours.

of the plot where the longer between-bout intervals occur (Fagen & Young 1978; Slater & Lester 1982). A change in slope signals a change in probability of an event occurring at any given time after the previous event (Fagen & Young 1978). This was the method employed to delimit bouts of specific activities in C. bartletti. Of course, long durations of behavioural observation are necessary to ensure inclusion of between-bout intervals.

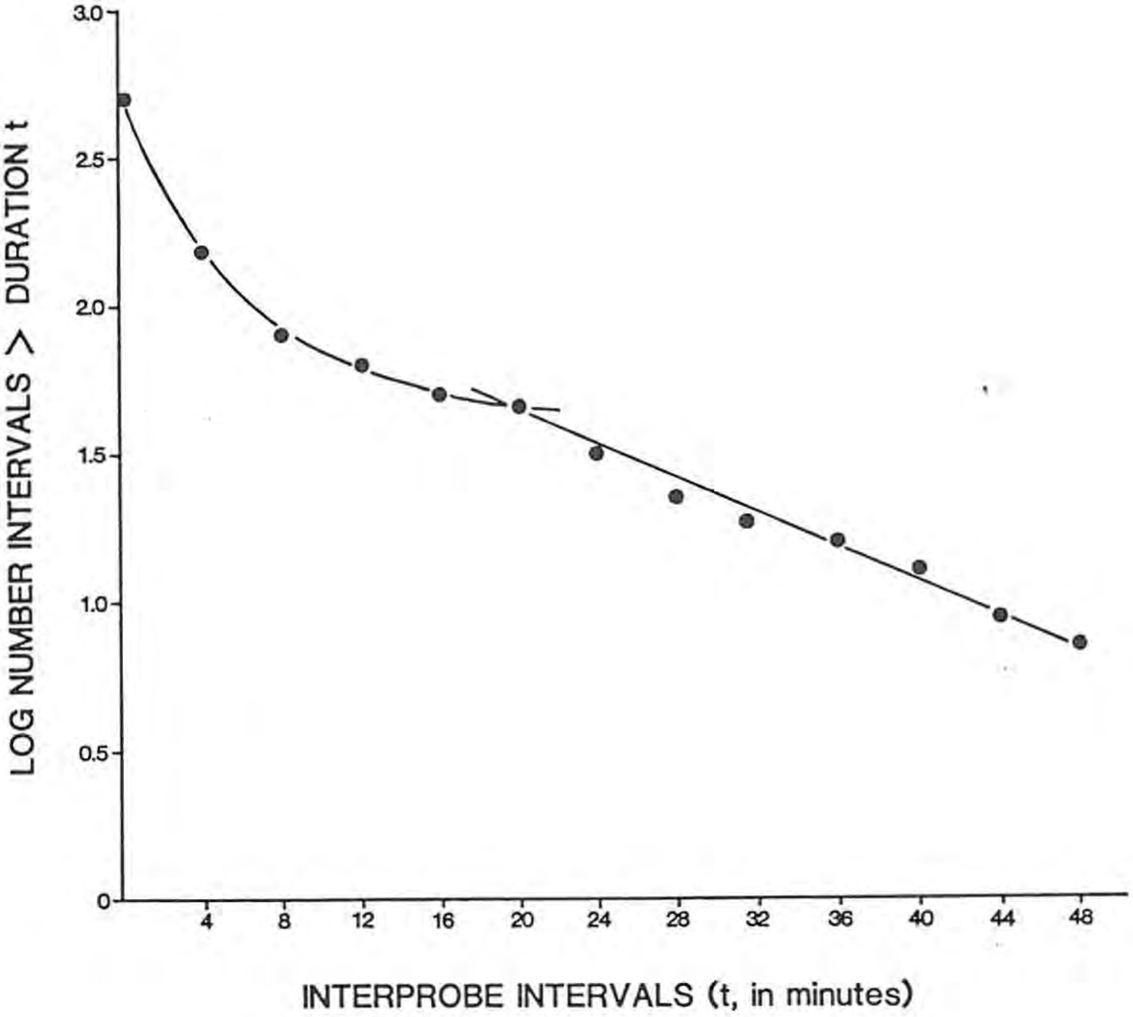

Fig. 7.3 : The log survivor plot derived from 14 5-hour observation periods on a single Coccophagus bartletti female. Intervals between successive probes were noted, and frequencies of interprobe intervals were determined. Interprobe intervals (t) are plotted with increments of 4 minutes, along the x-axis. Along the y-axis, the frequency of intervals is plotted in terms of the log of the number of intervals longer than the duration of each interprobe interval (t). That point where the slope of the graph alters is taken to indicate the interval duration that delimits a bout of activity. Here it occurs at the 20 minute interval. Curves fitted by eye. The figure is based on the methods of Fagan & Young (1978) and Slater & Lester (1982) which are discussed in the text.

A log survivor plot, from data obtained during the 14 5-hour observation periods on a single female (Fig. 7.3), indicates that interprobe intervals longer than 20 minutes are probably between-bout intervals. If an oviposition bout of C. bartletti is completed within the first 3 hours of its exposure to hosts, one would expect that intervals between probes longer than 20 minutes (= between-bout intervals) would be most frequent in and after the 3rd hour of observation. A frequency plot of these long between-bout intervals (Fig. 7.4) shows this to be true, and confirms the impression that 3 hours is sufficient time to include most (84%) of C. bartletti's ovipositional activity.

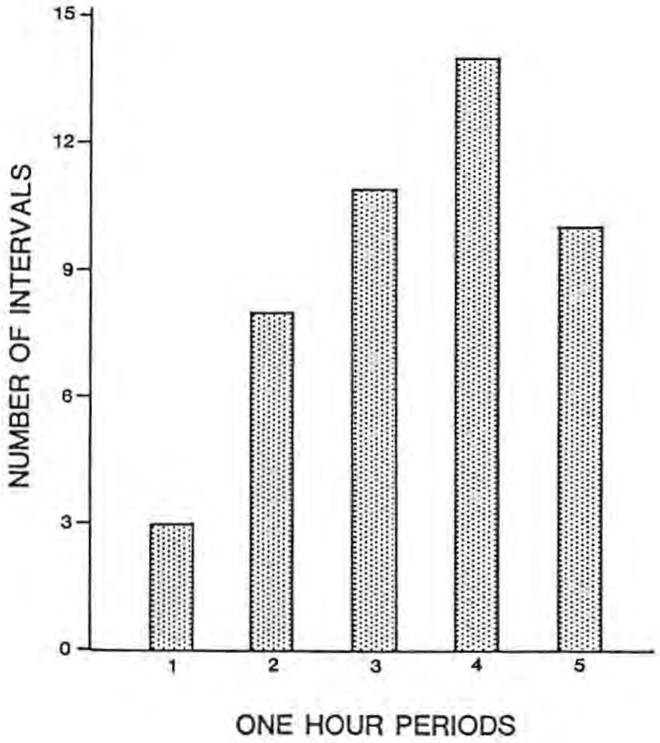

Fig. 7.4 : Frequency of inter-probe intervals longer than 20 minutes, plotted per hour of observation. Fourteen 5-hour periods of observation were carried out on a single Coccophagus bartletti female, each on successive days. Intervals, between probes, longer than 20 minutes indicate the end of a bout of oviposition.

These data were derived from preliminary observations on a single female. Confirmation of the conclusions were sought in three ways: experiments were conducted to test when most activity occurred, further observations were performed on a number of individuals, and ovaries of wasps that had been exposed to prescribed conditions were dissected.

Activity experiments. The observations, described above, on a single C. bartletti female indicated a peak of ovipositional activity beginning immediately the wasp was placed with hosts and lasting approximately 3 hours. Because preliminary observations were continued for only 5 hours, and were conducted only during the morning, additional experiments were made on groups of 5 wasps to determine (i) whether, after their oviposition peak, they have another peak of activity later in the day, or whether they continue oviposition at the extremely low rate previously observed (Fig. 7.2), and (ii) whether, given the choice of morning or afternoon, their activity peak still occurred in the morning.

Wasps were exposed to an excess of hosts for 6 hours in the morning, and immediately thereafter transferred to a second large group of scale insect hosts for a further 6 hours. Night hours were spent away from hosts, but honey was provided. Offspring obtained from each group of hosts were counted and sexed.

Results are presented in Table 7.1, which shows that 81% of offspring resulted from eggs deposited during the morning exposure to hosts. So C. bartletti females do seem to have one main peak of activity during any day, and this, other observations revealed, always occurs after first exposure to hosts. Subsequently the wasps continue ovipositing, but at a much reduced rate. In addition, the sex ratio of offspring produced in the morning is not significantly different from that of the afternoon's activity ($\chi^2 = 1.35$; $0.5 > P > 0.1$).

Under natural conditions in the field, C. bartletti females may be exposed to hosts throughout the day and night. If oviposition continues, even at a low rate, during the night, there may be

TABLE 7.1 : Number of offspring of each sex produced by 10 C. bartletti females. The wasps were exposed to an excess of hosts for 6 hours during the morning and then transferred to a second set of hosts for 6 hours during the afternoon. Two replicates were carried out, each with 5 wasps and lasting for 3 days. The temperature was 24°C.

		NUMBER OF OFFSPRING PRODUCED		
		FEMALE	MALE	TOTAL
PERIOD OF EXPOSURE	07h00 - 13h00	48	38	86
	13h00 - 19h00	14	6	20

insufficient eggs for the females to have any activity peak the following morning. To test the effect of darkness on oviposition, a group of wasps was exposed to an excess of hosts at night. Control groups of wasps were exposed to scale insects under day conditions. During the rest period of each group, honey was provided.

Table 7.2 shows that if female C. bartletti wasps were withheld from hosts during the day, they did oviposit at night, albeit 'reluctantly'. The 'nocturnal' wasps produced only 17% as many offspring as 'diurnal' wasps. The sex ratios of 'nocturnal' wasps was significantly different from that of 'diurnal' wasps ($\chi^2 = 9.98$; $P \ll 0.005$), the former producing proportionately many more males despite the apparently greater complexity involved in the deposition of haploid eggs.

The experiment to test whether C. bartletti females oviposit when it is dark was performed in a constant environment room in total darkness during simulation of night conditions. An experiment was designed to check whether wasps in the field, where it is seldom completely dark, also suffer the same ovipositional constraints at night. Each of two

TABLE 7.2 : Number of offspring of each sex produced by 2 groups of 10 Coccophagus bartletti females. One group of wasps was exposed to hosts only during the day and the other was exposed only at night. Exposures lasted 12 hours and wasps that were isolated from hosts were fed honey and left under the same conditions as the other group of wasps. There were two replicates, each with a day and a night group of 5 C. bartletti females. The temperature was 23±1°C and each experiment lasted 5 days.

		NUMBER OF OFFSPRING PRODUCED		
		FEMALE	MALE	TOTAL
PERIOD OF EXPOSURE	07h00 - 19h00	126	88	214
	19h00 - 07h00	11	25	36

groups of female C. bartletti was exposed to hosts during the day for 9 hours and to hosts at night for 9 hours. The experiment was performed indoors (to allow temperature regulation) with the transparent cages (Fig. 4.4B) exposed to the sky. A group of 4 wasps was given hosts during the day initially, and then a new batch of hosts at night. The cycle was reversed for a second group, which was exposed initially to scale insects at night, to determine whether egg-laden wasps would oviposit if hosts became available at night.

If oviposition occurs readily during the night in the field, one, or both, of two results would be expected. Wasps of the first group would produce approximately equal numbers of offspring during day and night and/or the second group would produce more, or an equal number of offspring during the night.

Although the results are disappointing in terms of the low numbers of offspring obtained, Table 7.3 shows that neither expectation for nocturnal oviposition in the field is upheld. *C. bartletti* therefore appears to lay eggs principally during the day, and with a peak of activity immediately hosts are contacted and providing sufficient hosts are available.

TABLE 7.3 : Number and sex of offspring produced by each of 2 groups of 4 *Coccophagus bartletti* females. Each group was alternatively, for 3 days, exposed to hosts during the day (9 hours) and night (9 hours). Group 1 was active first during the day, then the night, and these conditions were reversed for the second group. The experiment was conducted indoors with the transparent cages exposed to the sky. Day temperatures varied between 18.5 and 24.5°C, night ones from 19 to 26°C.

		NUMBER OF OFFSPRING PRODUCED		
		FEMALE	MALE	TOTAL
		GROUP 1 - DAY FIRST		
PERIODS OF EXPOSURE	08h00 - 17h00	11	1	12
	20h00 - 05h00	0	0	0
		GROUP 2 - NIGHT FIRST		
	20h00 - 05h00	0	4	4
	08h00 - 17h00	8	7	15

Behavioural observations on other individual females. Initial observations on activity patterns of C. bartletti involved only a single female, so the conclusion drawn, that a three-hour peak of activity occurs immediately hosts are contacted, needs confirmation using other females. In the experimental work, periods of exposure to hosts were too long to be useful in this regard. Initial attempts to use shorter periods failed, probably because wasps were disturbed too often. Therefore, the activity patterns of other individual females were examined, over both 3-hour and 5-hour periods.

The results are plotted in Fig. 7.5, and they confirm that C. bartletti females make most of their long probes (85% in this case) during their first three hours with hosts. In addition, the number of long (greater than 76 seconds) and double probes per ½ hour made by parasitoids exposed to hosts for 3 hours is very similar to the pattern of oviposition shown by those females exposed for 5 hours (χ^2 on untransformed data = 3.27; $0.75 > P > 0.5$). However, the number of short probes and host feeding probes was more variable. The number of short probes varied, perhaps, with the proportion of unsuitable hosts amongst those presented to the wasps. The frequency of host-feeding may be dependant on the number of small hosts used for feeding. Most host-feeding, though, is also done during the first three hours exposure to hosts (74%). Occasionally more than 3 hours was necessary before most of a wasp's ovipositional activity had been completed, but these extensions of the activity period usually occurred if the wasp did not start ovipositing immediately on being exposed to hosts.

Log survivor plots (Fig. 7.6) derived from these data have a pattern similar to that obtained from observations on a single female (Fig. 7.3), again with a change in slope at the 20 minute interval. Frequency data for intervals shorter than 20 minutes were transformed to their reciprocals, and linear regression equations were derived for them (Fig. 7.7). Data from Fig. 7.3 is also included. Because the slope of the curve predicts the probability of a future event occurring, the linear regression lines, theoretically, should not differ significantly in slope. However, an analysis of covariance shows that they do (F = 17.3122; D.F. = 2 & 12; $P < 0.0005$), probably because observations on

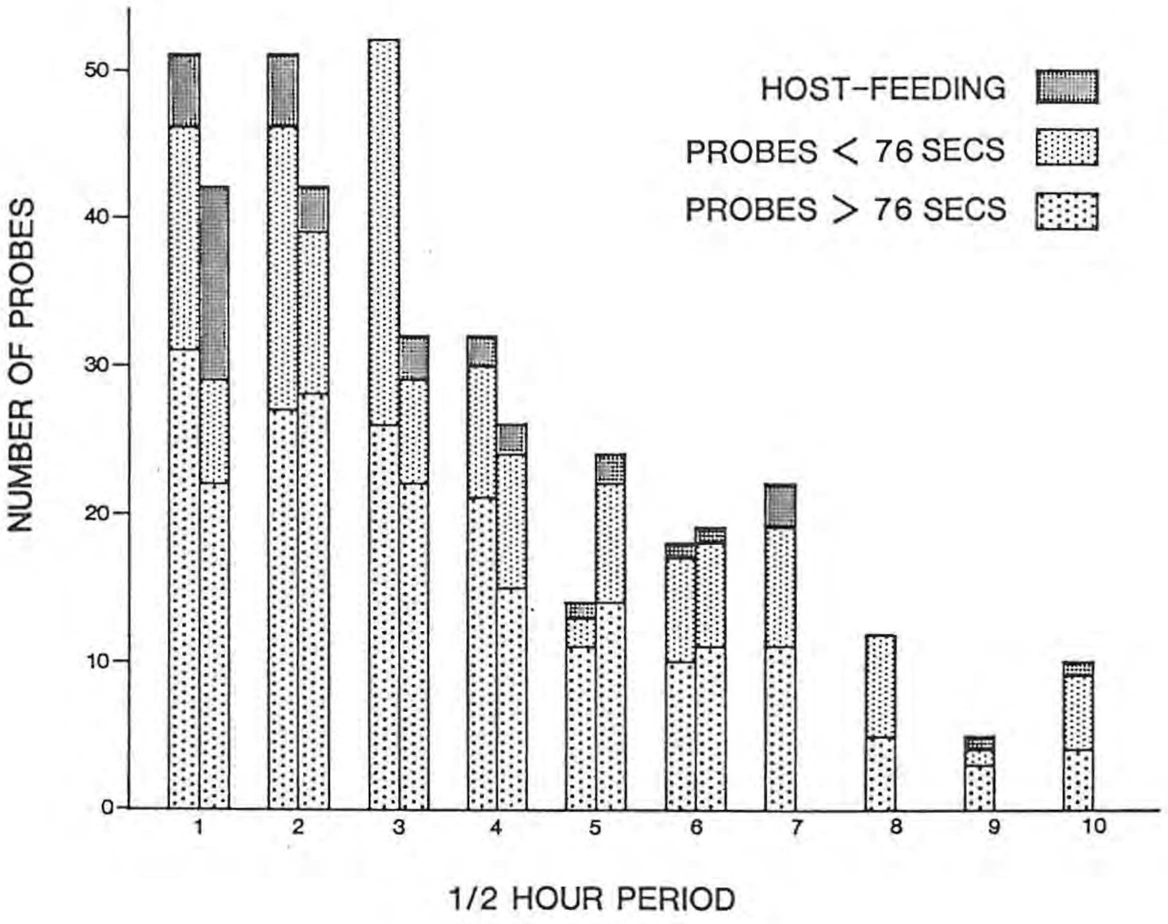

Fig. 7.5 : Activity patterns of mated <u>Coccophagus bartletti</u> females. The total number of probes per ½-hour period is plotted, and the probes are classified in three categories (probes longer than 76 seconds and double probes, probes shorter than 76 seconds, and host feeding probes). Observations were conducted over 5-hour periods (left hand bars) and 3-hour periods (right hand bars). For the former, n = 9 (3 females used), and the latter n = 15, but corrected to 9 observation periods (6 females used). All observations on wasps took place 48 hours after a previous observation period. On initial exposure to hosts, wasps fed readily, but laid few eggs, so these observations are excluded from the analysis.

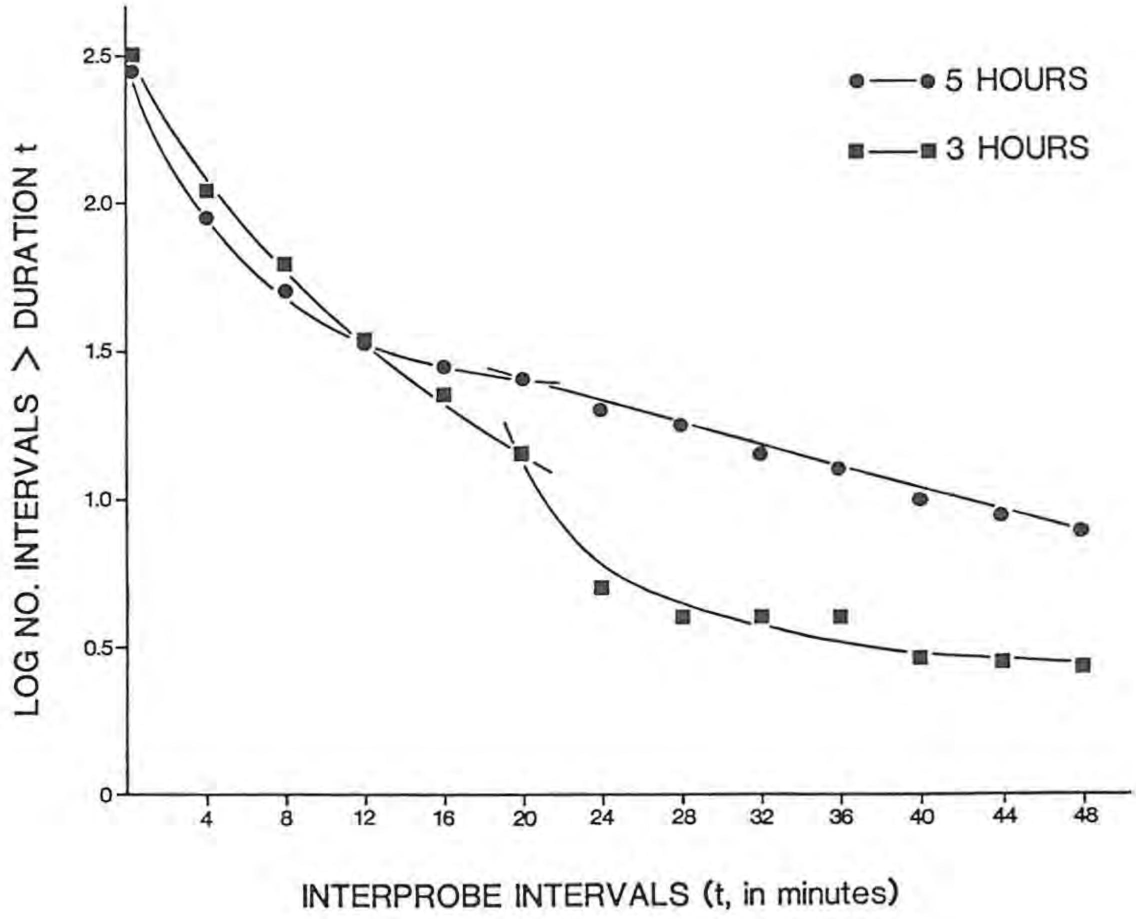

Fig. 7.6 : Log survivor plot (for explanation see Fig. 7.3) derived from observations reported in Fig. 7.5. Curves fitted by eye.

the single female took place on potato sprouts, whilst data pooled from a number of females derives from observations of females on leaf discs. In addition, some females were observed for only 3 hours, so 'between-bout' intervals were recorded less frequently than in 5 hour observation periods.

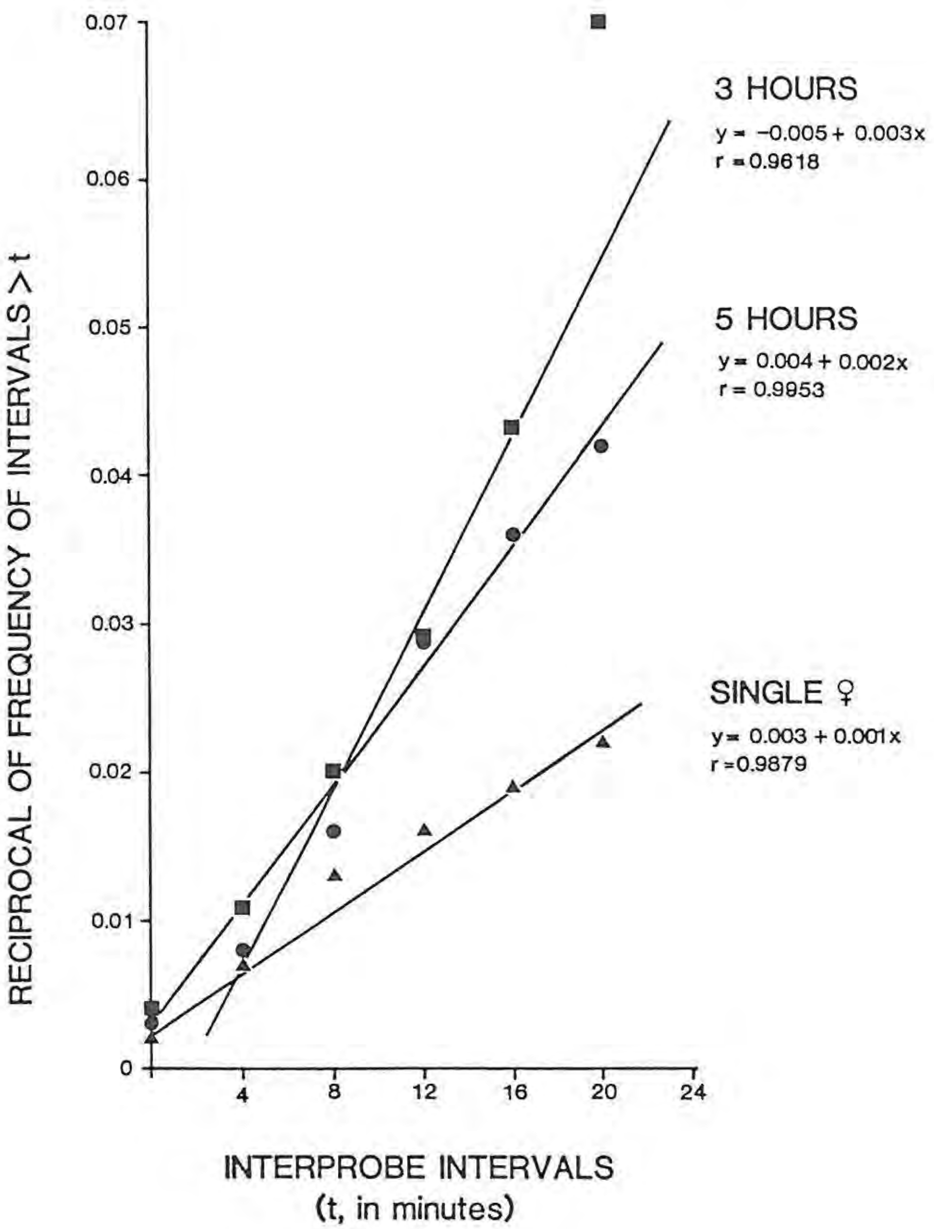

Fig. 7.7 : Linear regression lines of frequency data from Figs 7.3 & 7.6, for intervals of 20 minutes and shorter. Original data transformed to reciprocals. In all cases the slope is significantly different from 0.

The frequency of intervals of 20 minutes or longer was plotted per hour of activity (Fig. 7.8). Again, most of these 'between-bout' intervals occur in the fourth hour and later, and this confirms that 3 hours is a good approximation of the duration of oviposition bouts in C. bartletti. During subsequent observations, wasps were observed for longer than 3 hours if they still showed signs of activity, although this situation was rare.

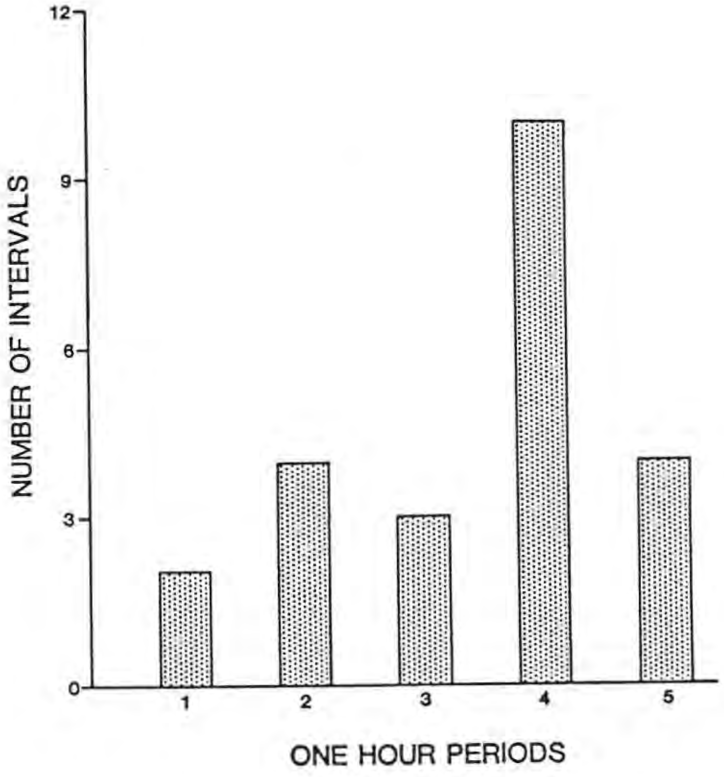

Fig. 7.8 : Frequency of inter-probe intervals longer than 20 minutes, plotted per hour of observation. Only data derived from those wasps observed for 5 hours were included. Most long intervals occur in the fourth hour, indicating that 3 hours approximates the duration of the normal bout of oviposition activity in these wasps.

After activity observations were completed, all hosts probed by C. bartletti females were dissected to establish whether an egg had been deposited or not. Fig. 7.9 confirms that oviposition follows a pattern that correlates with that for long probes (greater than 76 seconds and which usually resulted in female egg deposition) and double probes (which resulted in male egg deposition). It also shows that 83% of eggs are laid during the first three hours of activity.

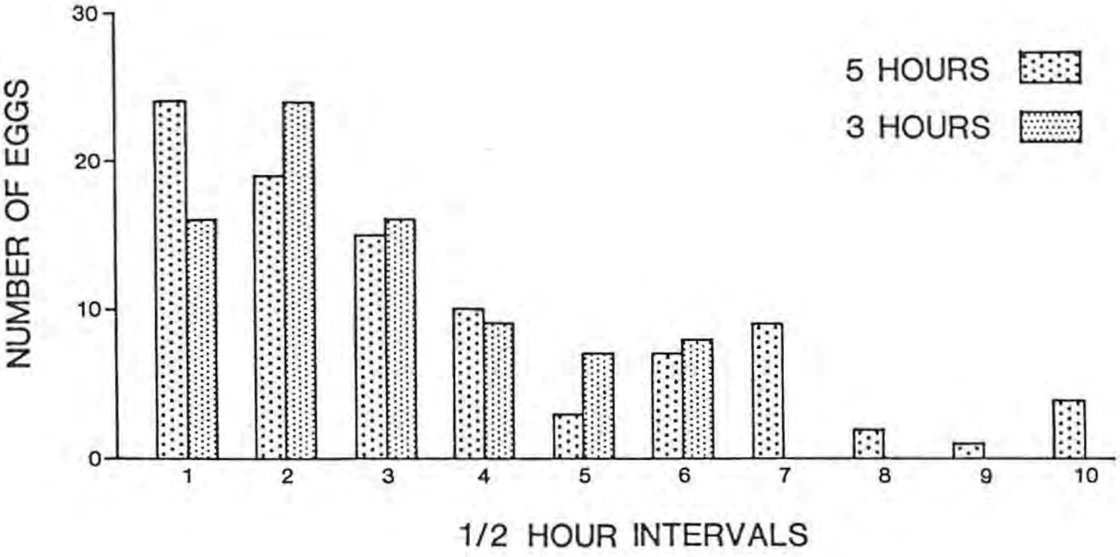

Fig. 7.9 : Total number of eggs (per ½-hour) deposited by <u>Coccophagus bartletti</u> females during 3-hour or 5-hour exposures to hosts. The data are derived from the same source as those in Fig. 7.5 (all hosts probed by wasps were dissected).

These results confirm the conclusions reached from observations on the activity of a single <u>C. bartletti</u> female. Three-hour periods of observation on these wasps were sufficient to include almost all of their ovipositional activity for the day, if observation started immediately they were provided with hosts. Occassionally more than three hours was necessary (see Fig. 7.5, ½ hour number 7) but this usually occurred if the wasp did not start oviposition immediately.

Ovarian egg production. To determine whether the bouts of ovipositional activity of C. bartletti females are related to the number of eggs in the ovaries at any one time and the rate at which deposited eggs can be replaced, individuals that had been subjected to different conditions were dissected, and the number of ovarian eggs counted (Table 7.4). Only those eggs longer than 0.10 mm were included. Although 0.10 mm is shorter than the length of deposited diploid eggs (0.13 mm; Table 6.1), it is likely that most of the shorter eggs mature and would be deposited shortly after the wasps encountered hosts. There is supportive evidence for this statement. No significant difference was found between the mean number of eggs deposited per 5-hour observation (10.4 ±1.19) and the number of eggs in the ovary after 48-hours' abstinence from oviposition ($t = 0.7263$; D.F. = 23; $0.5 > P > 0.4$).

TABLE 7.4 : Mean number of eggs, longer than 0.10 mm, within the ovaries of Coccophagus bartletti females. During all treatments honey or honey dew was available to the wasps, and they were exposed to a temperature of 24°C during a 12 hour photophase, and 18°C during the scotophase.

NUTRITIONAL STATUS	DISSECTED	$\bar{x}$(±1 S.E.)	RANGE	n
Not host-fed	1hr after emergence	2.3(±0.28)	0-4	15
	24hrs after emergence	5.1(±0.29)	2-7	19
Host-fed	24 hrs with no hosts	7.4(±0.75)	4-13	15
	48 hrs with no hosts	11.6(±1.02)	5-19	16
	120 hrs with no hosts	13.5(±0.96)	4-18	15
	240 hrs with no hosts	9.2(±0.66)	4-14	15
Host-fed, 48hrs with no hosts	5 hrs with hosts	1.1(±0.35)	0-5	15
	10 hrs with hosts	2.0(±0.59)	0-6	12

Table 7.4 shows that ovipositional activity of C. bartletti is governed by the state of the ovaries. Host-feeding is necessary for egg production, except for a small first batch of eggs. In addition, more eggs are present in the ovaries after 48-hours post-feeding isolation than after 24-hours isolation. Increasing this interval, even to 120 hours, increased the mean number of eggs slightly, but not significantly ($t = 1.3163$; D.F. $= 29$; $0.2 > P > 0.1$). Wasps were therefore left for 48-hour intervals between observations to ensure maturation of a batch of eggs. Nevertheless, C. bartletti was highly variable in the number of eggs contained in the ovaries (and in the number of eggs deposited).

During a 5-hour exposure to hosts, almost all eggs available in the ovaries are deposited, and this is true, too, of wasps exposed to hosts for as long as 10 hours (Table 7.4). So it seems that although egg maturation is a slow process, oviposition will continue, during daylight, if hosts are present. However, after a period on hosts, usually of 3-hours or longer, wasps tend to move from the area containing hosts. They jump more frequently too. These activities may move the parasitoid from the host patch and lead to migration in search of more hosts, in which case the ovaries are likely to contain a large complement of eggs at each new encounter with hosts. Alternatively, the female parasitoids may merely be seeking a nearby scale-insect-free, and therefore ant-free area, to 'rest' (which they often do, for protracted periods).

Finally, if female C. bartletti wasps are withheld from hosts for about 10 days, resorption of eggs begins (Table 7.4), and after about a month very few eggs remain ($\bar{x} = 0.9$; range 0-2; $n = 7$). This is supposedly a protein-saving mechanism (Bell & Bohm 1975).

Both quantified observation and experimentation on C. bartletti females show that oviposition occurs in discrete bouts (of approximately 3 hours) in the laboratory. The termination of oviposition was usually signalled by females paying no further attention to hosts for protracted periods of 30 minutes or longer. They often left the plant substrate. Observations were therefore continued for 3 hours, or until the individual's behaviour signalled that oviposition had been completed. Dissections of ovaries confirmed this interpretation, and also indicated that maturation of a full batch of eggs took at least 48 hours (at temperatures to which wasps were exposed). The effect of age upon the females' abilities to deposit eggs is dealt with in the following chapter.

Although the size of batches of *C. bartletti* eggs in the field are unknown, the batches observed in the laboratory are considered acceptable for an analysis of the temporal pattern of male and female egg deposition in *C. bartletti*. In the ultimate test of the hypothesis proposed in chapter one, parasitised scale insects will be exposed to mated *C. bartletti* females to determine whether these wasps preferentially lay male eggs on them. Therefore information on the patterns of oviposition in *C. bartletti* is important: if a propensity exists for male eggs to be deposited in a particular stage of the oviposition sequence, this would have to be accommodated when designing observational experiments on the reaction of *C. bartletti* to parasitised hosts. Oviposition was studied, in the next chapter, for temporal patterns of male and female egg deposition in batches of eggs.

CHAPTER 8 : SEX RATIOS OF *COCCOPHAGUS BARTLETTI*

The expectation (chapter 1) that *C. bartletti* females would deposit male eggs on all or most parasitised hosts encountered could be investigated, and meaningfully interpreted, only once information on the sex ratios of this species was available. Therefore, data on three aspects of *C. bartletti* sex ratios were gathered. Firstly, the ratios of female to male eggs deposited under known conditions during the 3-hour bouts of intense ovipositional activity (chapter 7) were examined. Any consistent patterns (see Waage 1982a, b) in daily male and female production, which may have influenced reactions of *C. bartletti* females to previously-parasitised hosts, were sought. Secondly, possible changes, with age of the parental female, in the sex ratio of offspring produced was investigated because Donaldson & Walter (1984) detected that there may be a shift away from male production in older *Spalangia endius* females. Kirkland (1982) and Avilla & Albajes (1984) found a similar shift in the braconid species they studied. Finally, samples of *C. bartletti* were regularly taken from the field to assess (i) the relevance of laboratory results and (ii) whether changing environmental conditions (eg ratio of small acceptable hosts to larger acceptable hosts, or number of conspecifics present) affected the sex ratio. The latter may also have provided some clues about the evolution of diphagous host relationships.

Sex ratios during bouts of oviposition. Twenty-six bouts of oviposition (each of at least 3-hours duration), based on observations of 8 mated females, were analysed. Twenty-three of these bouts yielded more female than male eggs (range 54.5-100% females) (Fig. 8.1). Only three had male-biased sex ratios (range 41.2-46.2% females). The mean sex ratio (± 1 S.E.) was 77.5 (± 3.3)% females.

To detect any possible temporal pattern of female and male egg deposition, the results from all observations were pooled, and the sex ratio in the groups of all first-oviposited eggs, second-oviposited eggs, and so on, determined. A bar diagram of results is depicted in

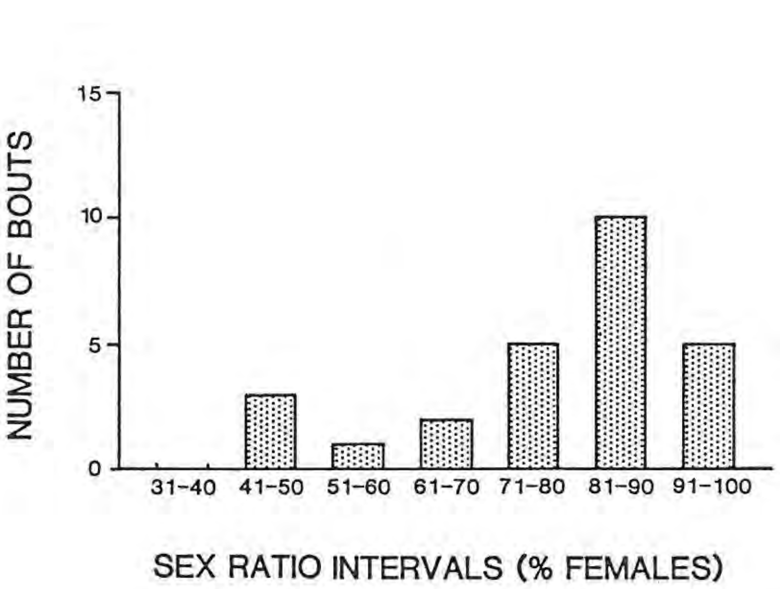

Fig. 8.1 : The frequency at which sex ratios of <u>Coccophagus bartletti</u> eggs occurred in 26 bouts of oviposition (each of at least 3-hours duration). Data from 8 mated females.

Fig. 8.2. Expected numbers of female eggs were calculated based on the hypothesis that male or female eggs are deposited randomly with respect to their position in the oviposition sequence. No difference (see Fig. 8.2 for details of statistical test) was found between the observed and expected number of female eggs produced. So there is no obvious temporal pattern for deposition of male and female eggs. In addition, the temporal pattern of male and female egg deposition for each female, with one exception, was also random (runs test for randomness: Sokal & Rohlf 1981, p. 782). The exceptional female had deposited relatively few male eggs, with these interspersed individually amongst the female eggs.

Therefore, eggs of <u>C. bartletti</u> are generally deposited in a random sequence with regard to sex, but with females approximately three times more numerous than males.

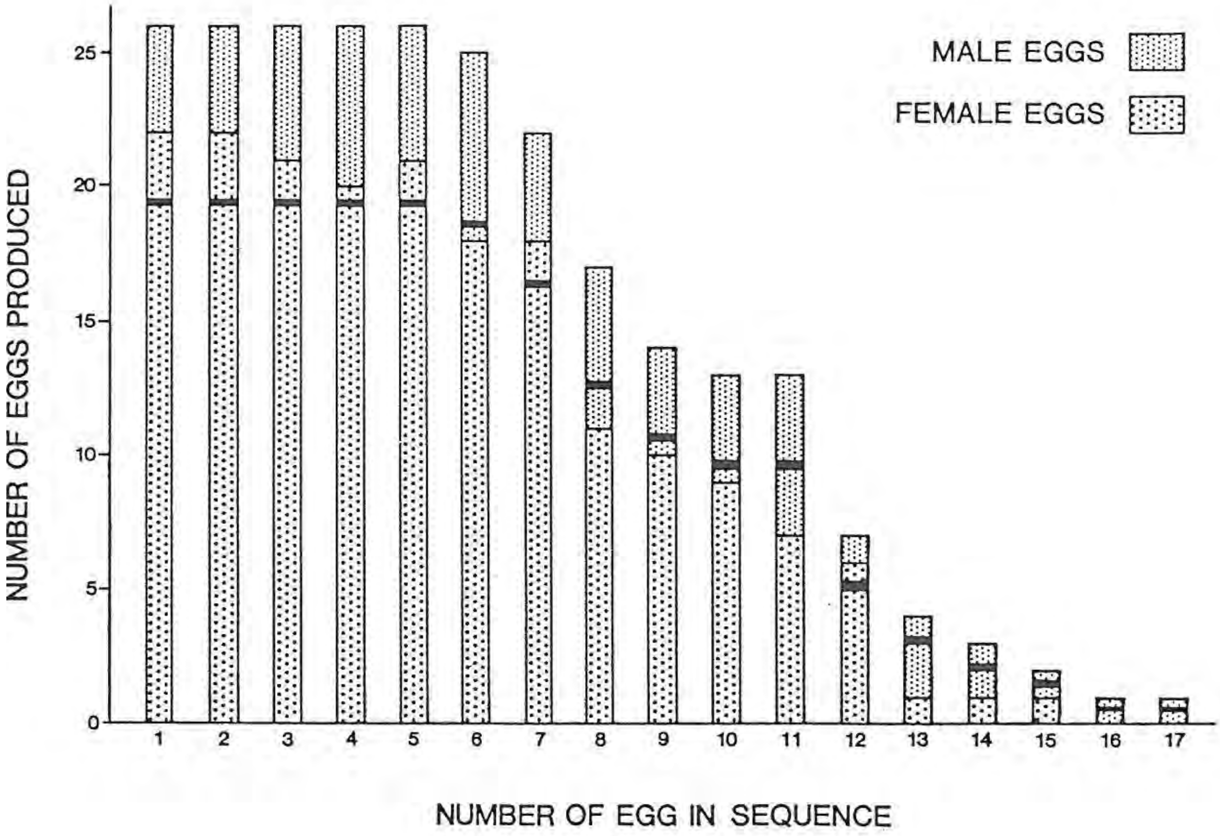

Fig. 8.2 : Twenty-six bouts of oviposition activity (each of at least 3 hours in duration) by 8 Coccophagus bartletti females were analysed to determine the number of female and male eggs as a function of the sequence of their deposition. The overall sex ratio (% females in relation to total number of eggs : 74.6%) was used to determine the expected number of female eggs in each sequence (thick horizontal bars) if oviposition occurred randomly in relation to sex of egg. Observed numbers of females did not deviate significantly from expected numbers (χ^2 = 5.885; D.F. = 16; 0.99 > P > 0.975).

Variation of sex ratios with age of females. Sixteen C. bartletti females were allowed to mate between one and two days after emergence. They were then, in groups of four individuals, allowed to oviposit and host-feed for two days. For this purpose, individuals of each group were placed together on a single potato sprout containing an excess of suitable scale insects. A five-day 'rest' period followed during which honey was provided as the sole food source. It was found (Table 7.4) that wasps contained a maximum number of eggs if separated from hosts for between two and five days. After this the wasps were placed on another sprout for two days. This treatment continued until all wasps had died. Conditions during both phases of the experiment were 12 hours light (24 ± 1°C) and 12 hours dark (18 ± 1°C). Sprouts with parasitised hosts were held at room temperature. Once parasitoids reached the late larval or pupal stage they were dissected from the sprout (in the mummy) and sexed. Late larval and pupal mortality was therefore ignored. Ideally, hosts should have been dissected to establish the presence of ectoparasitic (male) and endoparasitic (female) eggs. This was impractical because of the large number of hosts presented to the wasps, which ensured a large ratio of unparasitised hosts to ovipositing wasps.

The results of this experiment are presented in Fig. 8.3. Adult females died regularly, and the oldest one survived for seven weeks (Fig. 8.3A). A few females escaped or were killed accidentally: they are recorded in the bar diagram as accidental deaths. Offspring production peaked during the second and third weeks of life (Fig. 8.3B). The wasps had not host-fed before the first week's exposure to hosts, so they probably contained few eggs then. The number of offspring produced in the fourth week is an underestimate, as two of the potato sprouts exposed during this period dried out before wasps completed development.

Sex ratios of offspring produced were variable from week-to-week (Fig. 8.3C). The only consistency occurred in the first two weeks, when females comprised about 64% of offspring. The percentage females dropped after that, to below 50% in the fourth week. During the fifth and sixth weeks sex ratios were near 80% and 64%, respectively. Only two males were produced in the final week.

These results show that there is probably no consistency in the ratio of male and female offspring produced by C. bartletti of different ages, at least in the laboratory. Nevertheless, wasps no older than three weeks in age were used, as in all previous experiments, for further observations.

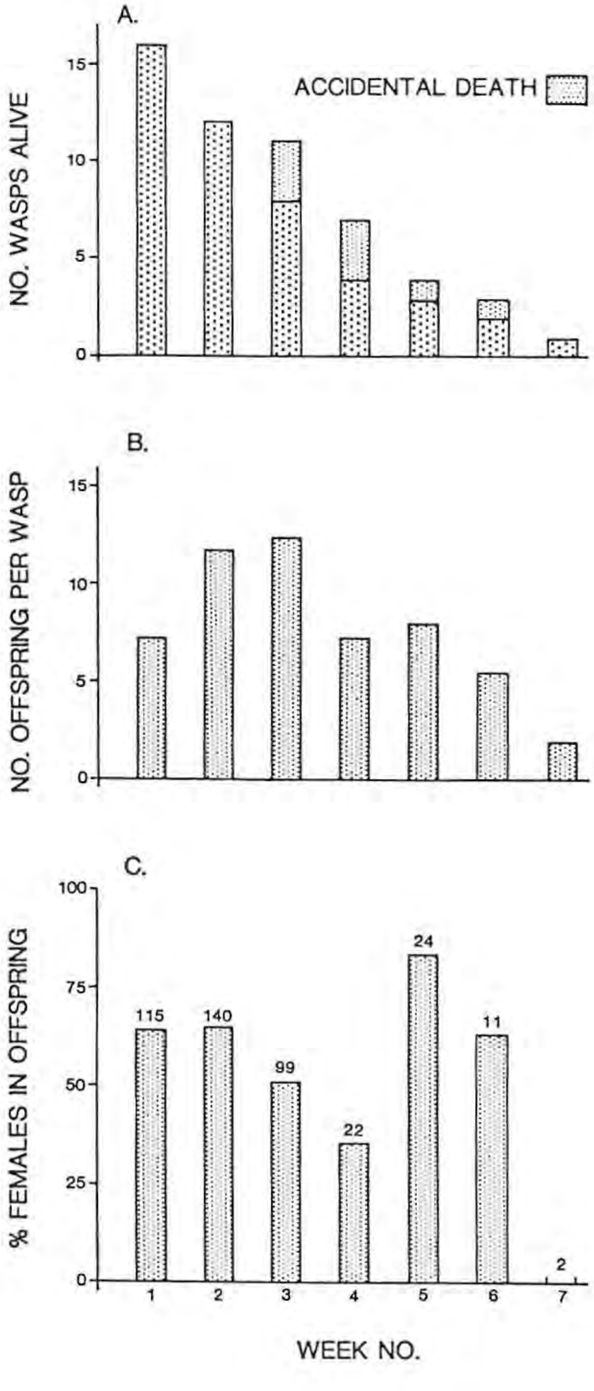

Fig. 8.3 : Variation of *Coccophagus bartletti* sex ratios with age of ovipositing females. Every week wasps (in groups of four) were exposed to an excess of hosts on potato sprouts for two days. A. Survival of adult female wasps represented by the number of wasps alive, and used in the experiment, each week. B. Average number of offspring produced per wasp per week. C. Sex ratio (% females) of offspring produced (number at top of bar) in each week.

Field sex ratios. Field samples were taken, at approximately 3-weekly intervals (see chapter 4) for just over a year (April, 1983 to May, 1984). Prior to enclosing the sample in an emergence box (Fig. 4.1), the scale insect hosts (Saissetia spp.) were classified into arbitrary size classes and counted. This was done because sex ratios of C.bartletti seemed to be male-biased early in scale insect generations, when the hosts were very small. As the scale insects grew larger, sex ratios of C. bartletti tended to become more female biased.

Four size classes for Saissetia were chosen:

(i) recently-settled crawlers and small insects up to 0.79 mm in length (referred to as stage 1 hosts),

(ii) those between 0.79 and 1.38 mm in length (stage 2), which represents the largest hosts acceptable to ovipositing C. bartletti,

(iii) those longer than 1.38 mm, but not yet in the 'humped' adult stage (stage 3), and

(iv) adult, stage 4, insects.

Width of scale insects was ignored in this classification because it is strongly correlated with scale insect length ($r = 0.9859$; D.F. = 148; $P \ll 0.001$).

Parasitoids that emerged into the glass vials of the emergence cages were labelled and stored. When emergence had ceased, the contents of the boxes were sifted and any remaining wasps were removed. All wasps were identified, at least to genus, and counted. Sexes of C. bartletti were counted separately. The number of C. bartletti found in each sample, the percentage of females, and the logarithm of the number of stages 1 and 2 scale insects is represented in Fig. 8.4. This figure shows that the number of C. bartletti per sample varied with the number of stage 1 and 2 scale insects in the sample. The sex ratio also varied, almost cyclically, and a G-test on the raw data indicated no homogeneity of sex ratios between sampling occasions ($G = 315.512$; D.F. = 19; $P \ll 0.005$).

Field sex ratios varied between 40% and 95% females, and samples were more often female-biased (13 of 20 samples). The mean sex ratio (± 1 S.E.) was 65 (± 4.26)%, and the overall ratio was 53% females. The

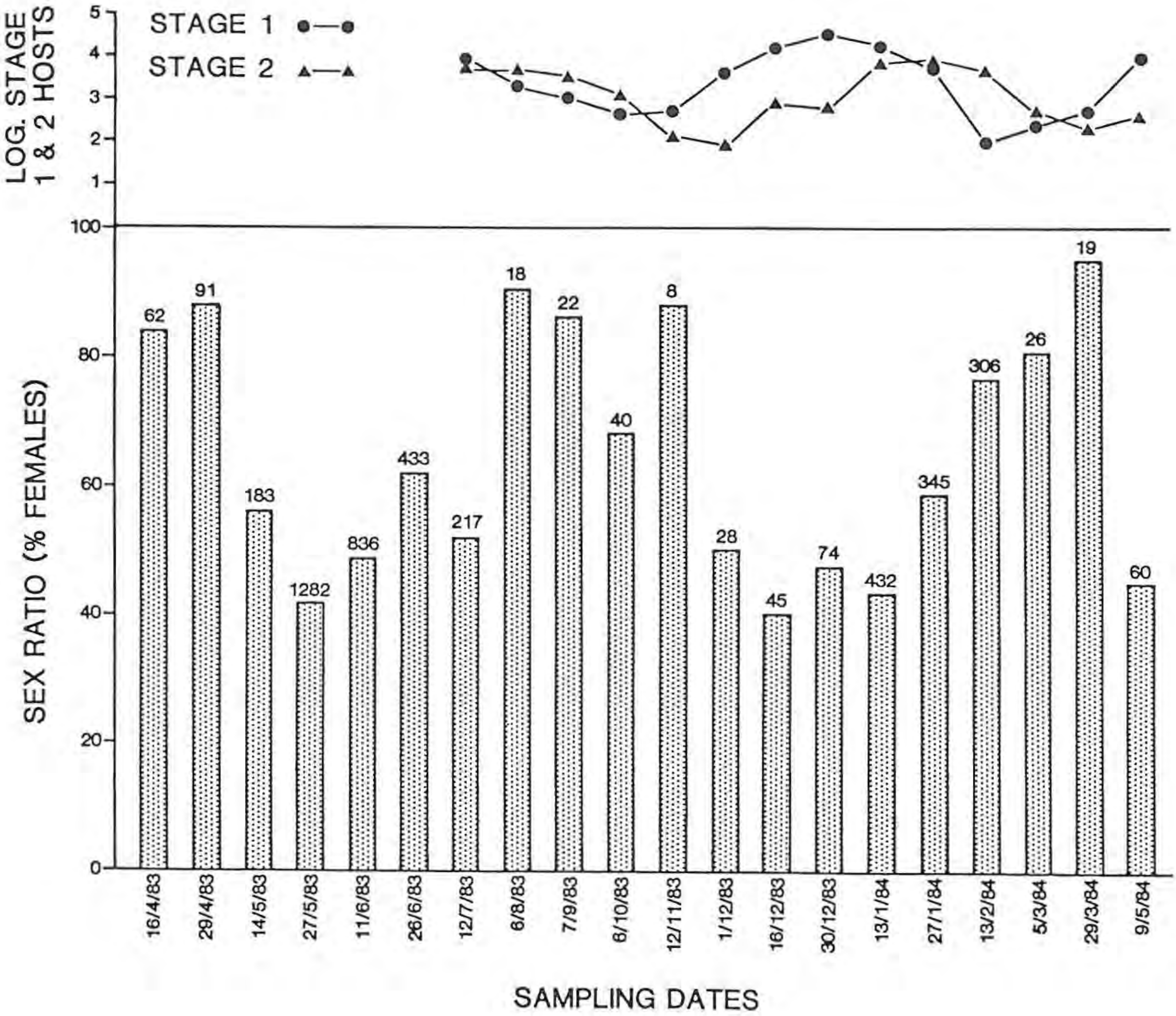

Fig. 8.4 : Sex ratios (% females) in samples of Coccophagus bartletti taken from the field. Sampling dates are given on the x-axis, and the number of wasps in each sample is given above each bar. The upper diagram represents the log number of stage 1 and 2 hosts in the sample. Stage 1 hosts have a maximum size of 0.79 mm and stage 2 hosts range between this length and 1.38 mm. Both are suitable for C. bartletti development.

female-biased sex ratios obtained in the laboratory (Fig. 8.2) are within the limits found in the field, with conditions indoors presumably approximating those outdoors during periods when sex ratios peaked (April, 1983; August to November, 1983; and January to March, 1984).

The cause of varying field sex ratios is unknown. A number of factors have been implicated in altering sex ratios of various parasitoid species (see reviews in Kochetova 1978 and Charnov 1982). They are:

(i) relative size of host insects available (eg Brunson 1937; Clausen 1939; Flanders 1946, 1965; van den Assem 1971; Charnov 1979; Sandlan 1979; Charnov et al. 1981),

(ii) relative density of host insects (eg Legner 1967; Sandlan 1979),

(iii) presence of conspecific females (eg Wylie 1966, 1976; Holmes 1972),

(iv) temperature (eg Hoelscher & Vinson 1971; Legner 1977),

(v) humidity (eg Legner 1977), and

(vi) photoperiod (eg Hoelscher & Vinson 1971; Werren & Charnov 1978).

The response of ovipositing wasps to host size is generally considered an adaptation, because female eggs are usually deposited in large hosts and male eggs in small hosts (Charnov 1982). It is possible that the size of hosts within a population may not greatly influence the sex ratio of parasitoids. The sex ratio may be 'set' at the optimum predicted by the Fisher (1930) and Hamilton (1967) model of sex ratio establishment, but with female larvae mostly being deposited in large hosts and males in small ones (see Charnov 1982; pp. 48-62). The effect of conspecifics, particularly in gregarious species, can also be directly related to the model of Fisher (1930) and Hamilton (1967). More equable sex ratios are expected when large numbers of conspecifics oviposit within a limited area because levels of local mate competition and inbreeding would be lower (see Hamilton 1967; Colwell 1981; Werren 1983). However, environmental influences on sex ratios are seen, by Charnov (1982), in a different light: they seem merely to interfere with sperm viability.

Tepedino & Torchio (1982) quantified field sex ratios in six generations of the univoltine non-social bee *Osmia lignaria propinqua* Cresson. The data show variation around a theoretical optimal sex ratio, and their favoured interpretation '...recognizes the role of environmental perturbations in deflecting the sex ratio away from equilibrium but also emphasizes that when such deflections occur, adult females that produce the sex in short supply will be favoured in the next generation...'. *C. bartletti* is multivoltine, with overlapping generations, and the field sex ratios plotted in Fig. 8.4 represent an estimated 5 or 6 generations of this species. The nature of the data is therefore akin to those of Tepedino & Torchio (1982), but their interpretation of fluctuating sex ratios is inappropriate for application to *C. bartletti* and perhaps even to *O. l. propinqua*. Selection for a particular sex ratio could presumably occur only at a very slow rate because offspring of parents that produce broods containing excessive numbers of males or females are not necessarily significantly disadvantaged. Some may never mate, but those that do will perpetuate genes for biased sex ratios. The adjustment of the population sex ratio towards equilibrium would therefore be gradual and probably take place over a few generations, and not vary greatly from generation to generation (*O. l. propinqua*) and even within generations (*C. bartletti*).

In the sampling programme, host density, host size and the number of other parasitoids (conspecifics and other species) were taken into account. However, temperature and humidity were excluded because developmental time of *C. bartletti*, under constant conditions in the laboratory (12h L at 24°C and 12h D at 18°C) varied between 38 and 60 days. So it was impossible to pinpoint a time interval when emergent parasitoids had been in the egg stage. This was complicated by a further unknown, the threshold age in the parasitoid life cycle at which immatures do not die with their hosts in the emergence cage.

Fig. 8.5 is a diagrammatic summary of how *C. bartletti* sex ratios were found to vary with those factors that were measured. The strongest relationship was between sex ratio and host density expressed as the logarithm of the number of suitable hosts per sample (Fig. 8.5A). At

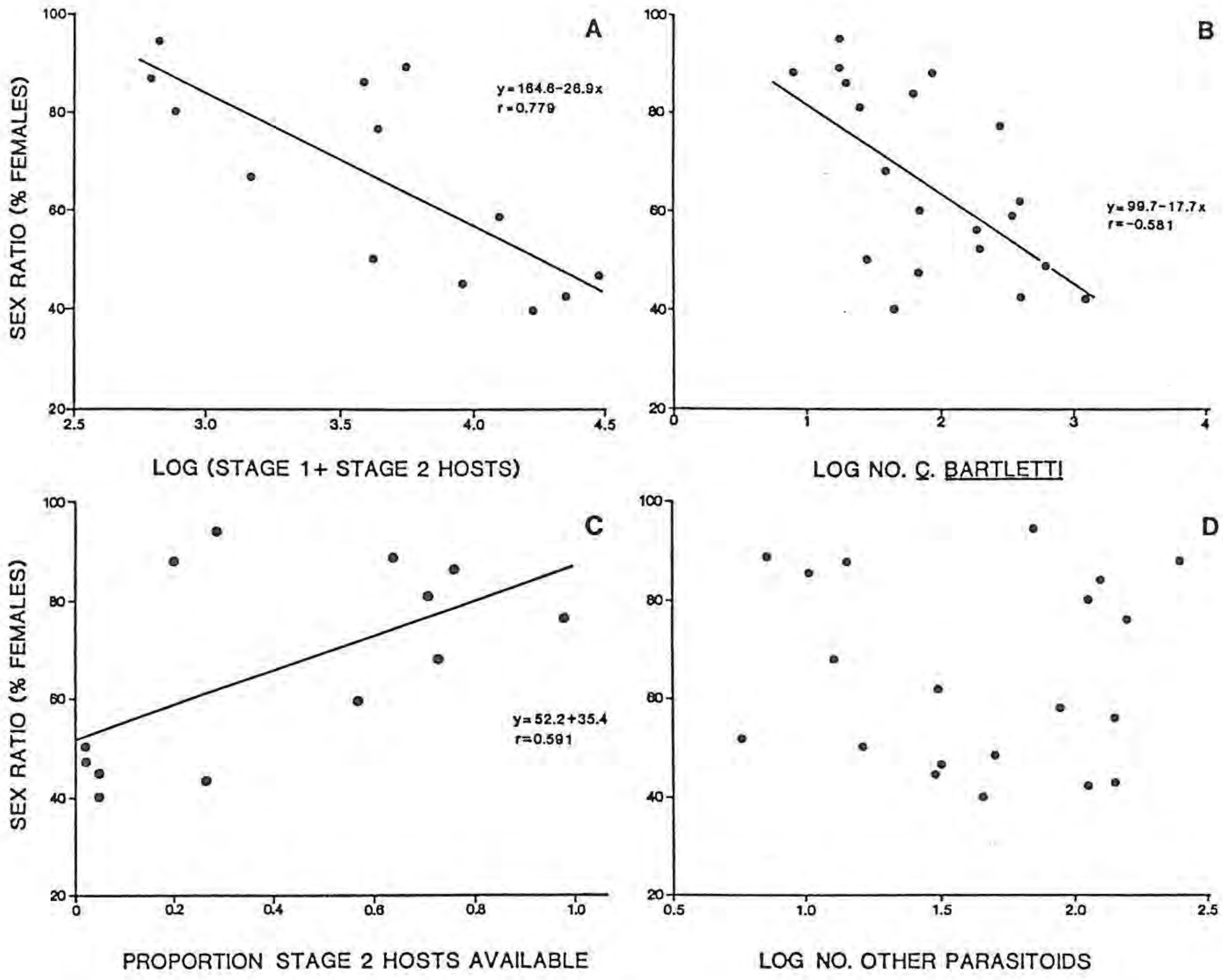

Fig. 8.5 : Field sex ratios (% females) of Coccophagus bartletti plotted against (A) the host density expressed as the logarithm of the number of suitable hosts per sample, (B) the logarithm of the number of C. bartletti individuals in the sample, (C) the proportion of stage 2 hosts in each sample and (D) the logarithm of the number of parasitoids of other species in the sample. In A to C the lines were fitted by linear regression and in all cases the slope was significantly different from 0. ($P < 0.05$). There is no correlation in D.

relatively lower host densities the sex ratio tended to be more female-biased. A similar, but weaker, trend emerged (Fig. 8.5B) when C.

bartletti sex ratios were plotted against the number of conspecifics that wasps may have encountered whilst ovipositing. Generally, the more wasps that emerged from the sample, the less female-biased was the sex ratio. This interpretation of Fig. 8.5B assumes the number of *C. bartletti* in the sample to be proportional to the number of ovipositing females at that time when the emergents were being deposited as eggs. Females may lay more male eggs in response to an increased number of contacts with conspecifics, either adult females, their pheromones or parasitised hosts. This may explain why sex ratios of offspring derived from one female are more female-biased (Figs 8.1 & 8.2) than sex ratios obtained from groups of females (Fig. 8.3). Alternatively, ovipositing wasps may respond to parasitised hosts by depositing haploid eggs on them, as postulated in the introduction.

More evidence, preferably of an experimental nature, is needed before the ultimate cause of varying *C. bartletti* sex ratios can be identified, because there is also a relationship between the two dependent variables in Figs 8.5A and B. That is, the number of *C. bartletti* specimens obtained from a sample is related to the number of scale insects present ($r = 0.645$; $F = 7.8$; D.F. = 1 & 11; $0.025 > P > 0.01$). In addition, the sex ratio of *C. bartletti* may be dependent upon the proportion of larger hosts within the acceptable host range (Fig. 8.5C). Proportionally more females emerge from samples that have more stage 2 than stage 1 hosts. Because hosts chosen for male egg deposition are not different in size from hosts for female eggs (Table 6.1), it is possible that female eggs deposited into very small hosts undergo delayed development (as do *Encarsia formosa* females: Nechols & Tauber 1977) until the size of the host is more suitable. Males, on the other hand, may develop immediately. This occurs in *Physcus seminotus*, a heteronomous hyperparasitoid with endoparasitic females and ectoparasitic males (Williams 1972). The emergent *C. bartletti* males are noticeably smaller than their females. Finally, no relationship existed between *C. bartletti* sex ratios and the number of parasitoids of other species (Fig. 8.5D), even when only potential competitors were included in the analysis.

Sex ratios obtained in the laboratory, from observations on individual females, were within the range of sex ratios found in the field. The behaviour of ovipositing females in the laboratory was therefore taken as representative of what occurs, at certain times, in the field.

Information about the biological attributes of C. bartletti reported in this and previous chapters laid the basis for answering the major question posed in the introduction about the evolution of heteronomous parasitism. 'Is male ectoparasitism an adaptation that allows adult female diphagous parasitoids to parasitise previously-parasitised hosts, and thus realise the hypothetical advantage proposed also for heteronomous hyperparasitoids in Fig. 1.1?' Of special importance in answering this question is the observation that, in the laboratory, there is no evidence for any fixed temporal pattern for deposition of male and female eggs. However, the overall sex ratio is approximately three females to one male. Therefore, if C. bartletti females are exposed to parasitised hosts, the observed pattern of deposition of male and female eggs and the resultant sex ratio can be compared with the normal response of ovipositing females to unparasitised hosts. The results of observations carried out to determine the response of C. bartletti females to previously-parasitised hosts are reported in the following chapter.

CHAPTER 9 : REACTION OF C. BARTLETTI FEMALES TO PARASITISED HOSTS

Smith (1916) drew the distinction between superparasitism and multiple parasitism. In both situations, more parasitoid eggs are deposited per host, after more than one oviposition, than can successfully develop. The resulting larvae must then compete for food (Salt 1961). Superparasitism is restricted to situations in which the competing larvae are of the same species. Multiple parasitism occurs when the larvae are of different species. Despite these distinctions, the end result of this competition is the same: the death of at least one larva. Ovipositing parasitoids of many species can recognise parasitised hosts and avoid ovipositing on them. Avoidance of superparasitism, a characteristic of numerous species, has been more commonly studied than multiple parasitism, and is the subject of a recent critical appraisal (van Lenteren 1981). On the other hand, with respect to multiple parasitism, Waage & Hassell (in press) state that '...parasitoid species rarely if ever recognize markers of other species (van Lenteren, personal communication), and parasitism of a host by more than one species... is not uncommon'. However, several species reputedly do have this ability (Fisher 1961; Force & Messenger 1965; Wylie 1970, 1971, 1972; van Lenteren & DeBach 1981; Browning & Oatman 1984). If diphagous parasitoids evolved male ectoparasitism as an aid to kill other parasitoid larvae, then C. bartletti may be expected to recognise markers of other species. Therefore the responses of C. bartletti to hosts parasitised by both conspecifics and by other species were investigated.

Recently-emerged parasitoids of at least some species cannot discriminate between parasitised and unparasitised hosts and therefore do oviposit in or on parasitised hosts (van Lenteren 1976, 1981). Experience of unparasitised hosts is necessary before discriminatory abilities develop. If young C. bartletti females were in this category, they would deposit eggs into or onto parasitised hosts, but because they had not previously identified the hosts' condition, oviposition would presumably be random in relation to sex of the eggs. To assess unambiguously whether mated C. bartletti females can detect a parasitoid egg or larva in a host and then preferentially deposit a male egg, experienced females should be used in experiments. For these reasons mated females that had oviposited at least one or two full batches of eggs were chosen for observations.

A situation can occur where parasitoids recognise that a host is parasitised, but nevertheless depart from their normal behaviour by ovipositing (Salt 1934, 1937; van Lenteren 1976, 1981; van Lenteren *et al.* 1978). This commonly occurs when females repeatedly encounter parasitised hosts. Experiments to determine the responses of *C. bartletti* females to parasitised hosts were designed to ensure that the ovipositing female repeatedly encountered parasitised hosts. The most reliable methods for detecting host discrimination are said to be observational (van Lenteren *et al.* 1978; van Lenteren 1981) and the other recommendations of these authors are incorporated in the following experimental design.

The methods used to make the observations described in this chapter were similar to those in chapter 8. Females were observed individually and relevant behavioural detail was recorded. In superparasitism trials, a single female was allowed to deposit a batch of eggs into or onto hosts on a leaf disc. Hosts that were probed for longer than 76 seconds were measured and given a unique mark. Unparasitised ones were removed, and after an appropriate time interval had lapsed, the remaining hosts were exposed to a second, experienced, individual female that had oviposited at least one or two full batches of eggs, and its behaviour recorded. The second wasp was removed after she had encountered all the hosts left on the disc, or after three hours. All hosts were measured and dissected to ascertain the sex of any eggs and which of the two parasitoids had oviposited. For the latter purpose, the interval between exposures had to be determined so that the older egg was visibly different from the younger one. In multiple parasitism trials this problem did not arise because eggs of the chosen species differed markedly in shape. Otherwise the methods used were similar to those in the superparasitism tests of this study.

Reaction to hosts parasitised by conspecifics. This experiment was done in two parts. Observations were conducted on females exposed to hosts 5 days after initial parasitisation. During the 5 day interval, hosts were held at 24°C (12h L) and 18°C (12h D). Most eggs had not hatched 5 days after oviposition, but their development had proceeded sufficiently to make them easily distinguishable from newly-laid eggs. Older eggs were transparent and appeared granular in texture. They had a white spot situated near their centre. Newly-laid eggs were white and granular, but with transparent ends. Exposure to hosts too soon after initial parasitisation would have introduced ambiguities in interpretation.

Secondly, the reaction of female *C. bartletti* to hosts containing larvae was examined. Hosts were used in this experiment 14 days after initial parasitisation, when they contained what appeared to be second- or third-instar *C. bartletti* larvae.

(i) Hosts containing *C. bartletti* eggs. Table 9.1 gives details of experimental methods. It also includes an analysis of the number of hosts left on the leaf discs, and their fate. A high percentage (78.2%) of scale insects left on the leaf discs were encountered (touched with the antennae, antennated or probed) by the second female on the discs. Unfortunately some (8.2%) were drilled for host-feeding and attempts to feed the parasitoids before the start of the experiment failed. Sixty-three (64.9%) hosts were probed with the ovipositor. Most of those not probed had either died and dried out, moved beneath the disc or left it entirely. *C. bartletti* females seem able to detect whether a host has been previously parasitised or not. When constrained on patches of parasitised hosts, they responded as would be predicted from the accounts of van Lenteren (1981, p.168) and van Lenteren *et al.* (1976). Numerous attempts to leave the patch of hosts took place (condensation on the glass surrounding the tube prevented this). On patches of healthy hosts, *C. bartletti* females left only after their bout of activity (chapter 7) had ended and they contained no more eggs.

Table 9.2 takes the analysis further. It shows that eggs were deposited within or upon 57% of the 63 hosts that were probed. However, not all of these had been parasitised by the first female to probe them: only

TABLE 9.1 : Number of scale insects and their state of parasitisation on leaf discs exposed to two mated *Coccophagus bartletti* females. The first female was observed for 3 hours and hosts that had been probed for longer than 76 seconds were given identifiable marks. Other hosts were removed. Five days later a second wasp was exposed to the marked hosts, and her responses were recorded. Details of oviposition are represented in Table 9.2. Wasps in the second group were removed after each had encountered all the hosts present on their particular disc, or after 3 hours. The hosts were then dissected for the presence of parasitoid eggs. Five *C. bartletti* wasps were used to prepare 10 leaf discs, and the response to parasitised hosts was judged on the basis of observations on six individuals. The number at the bottom of each vertical line is expressed in the next column as a percentage of the number at the top of the line.

AFTER EXPOSURE TO FIRST WASPS & REMOVAL OF UNPARASITISED HOSTS:		
No. left on discs	124	
No. parasitised	69	55.6%
No. with a female egg	56	81.2%
AFTER EXPOSURE TO SECOND WASPS:		
No. encountered by wasps	97	78.2%
No. used for host-feeding	8	8.2%
No. probed	63	64.9%

19 received two *C. bartletti* eggs, and 18 of those deposited into already-parasitised hosts were female eggs. The sex ratio of eggs deposited by the second group of females was not significantly

TABLE 9.2 : Details of egg deposition by mated *Coccophagus bartletti* females exposed, after 5 days, to previously-parasitised hosts. The number at the bottom of each vertical line is expressed in the next column as a percentage of the number at the top of the line.

NUMBER OF HOSTS:		
Probed	63	
NUMBER OF EGGS:		
Deposited	36	57.1%
In previously-parasitised hosts	19	52.8%
That were female	18	94.7%

different from that of the first group (Table 9.3).

Hosts increased significantly in size over the 5-day period of the experiment, from a mean (± 1 S.E.) of 0.52 (± 0.012) mm to 0.55 (± 0.013) mm (n = 105) (one-tailed paired 't' = 5.9015; D.F. = 104; $P \ll 0.0005$). However, this relatively small increase is unlikely to have affected the sex ratio of *C. bartletti*, especially since there seems to have been no preferential deposition of female eggs in larger hosts (Table 6.1). In addition, a similar test on only those hosts that were superparasitised indicated no significant increase in size due to normal growth (one-tailed paired 't'= 0.6448; D.F. = 17; $0.1 < P < 0.25$).

Two important conclusions can be drawn from the results of these experiments (Tables 9.1 to 9.3).

(a) The level of superparasitism observed (almost 53%: Table 9.2) is much higher than that seen previously when *C. bartletti* females were provided with an abundance of unparasitised hosts (2.9% of 272 eggs laid). The latter levels of superparasitism were low despite females regularly re-encountering parasitised hosts. Therefore, C. bartletti females do seem capable of discriminating between parasitised and unparasitised hosts, a faculty found in other aphelinids (Encarsia formosa: van Lenteren et al. 1976; Aphytis spp.: van Lenteren & DeBach 1981). However, if alternative hosts are not available, ovipositional restraint breaks down and C. bartletti females will superparasitise hosts.

(b) C. bartletti females do not preferentially deposit male eggs onto hosts that already contain a C. bartletti egg, as would be expected if male ectoparasitism evolved to allow exploitation of parasitised hosts.

TABLE 9.3 : Number of female and male offspring produced by Coccophagus bartletti females on unparasitised and parasitised hosts (see Table 9.1). Because of difficulties in predicting egg deposition when the ovipositor has been inserted (see Fig. 6.1), 47.2% of the 'parasitised' hosts did not contain a parasitoid egg (Table 9.2). There is no significant difference between the sex ratios (G = 1.0597 (after Williams' correction for a 2 x 2 table); $0.5 > P > 0.1$).

	FEMALE	MALE
Unparasitised hosts	56	13
Parasitised hosts	32	4

(ii) Hosts containing young C. bartletti larvae. Tables 9.4 and 9.5 give details of the experiments and the results. Although more than 37 hosts were left on the leaf discs originally, a considerable number died during the two-week period.

All hosts left on the discs were encountered by the second parasitoid, and of those not used for host-feeding, many (64.9%) were probed.

TABLE 9.4 : Number of scale insects and their state of parasitisation after consecutive exposure to two *Coccophagus bartletti* females. Results of an experiment similar to that reported in Table 9.1, but in which the second *C. bartletti* female was exposed to hosts 14 days after parasitisation (compared with 5 in the previous experiment). Four *C. bartletti* wasps were allowed to parasitise hosts on 5 leaf discs, and the responses of three individuals to parasitised hosts were tested. The number at the bottom of each vertical line is expressed in the next column as a percentage of the number at the top of the line.

AFTER EXPOSURE TO FIRST WASPS & REMOVAL OF UNPARASITISED HOSTS:		
No. left on discs	37	
No. parasitised	22	59.5%
No. with a female egg	18	81.8%
AFTER EXPOSURE TO SECOND WASPS:		
No. encountered by wasps	37	100.0%
No. used for host-feeding	7	18.9%
No. probed	24	64.9%

Compared with the previous experiment, however, proportionately fewer hosts that were probed (37.5% compared with 57.1%) received an egg from the second parasitoid, and this resulted in only 5 superparasitised hosts.

Three of these 5 ovipositions involved female eggs. In this experiment, hosts did increase considerably in size, from a mean (± 1 S.E.) of 0.52 (± 0.017) mm to 0.63 (± 0.022) mm (one-tailed paired 't' = 8.557; D.F. = 35; $P \ll 0.0005$).

The results of this experiment support the conclusions drawn from the results of the previous one, despite so few hosts being superparasitised. If diphagous host relationships evolved as a mechanism whereby parasitoid eggs or larvae, already in or on a host, could be destroyed to make the host available to a male diphagous parasitoid, one would expect *all* previously-parasitised hosts to receive male eggs.

TABLE 9.5 : Details of egg deposition by mated *Coccophagus bartletti* females exposed, after 14 days, to previously-parasitised hosts. The number at the bottom of each vertical line is expressed in the next column as a percentage of the number at the top of the line.

NUMBER OF HOSTS:		
Probed	24	
NUMBER OF EGGS:		
Deposited	9	37.5%
In previously-parasitised hosts	5	
That were female	3	

During these two experiments, probe durations of the second group of females in the first experiment were noticed to differ from those in the second experiment. Table 9.6 shows that whether hosts were parasitised or not, probe duration by these females is substantially longer and also more variable in the second series of experiments. Whether this was due to some of the hosts containing parasitoid larvae or to the greater age of the scale insects is unknown. The latter seems more likely as probes into non-parasitised hosts were also significantly longer in this group of females.

TABLE 9.6 : Duration of ovipositor probes by *Coccophagus bartletti* females into parasitised and non-parasitised scale insect hosts. Means (± 1 S.E.) and sample sizes are given. Wilcoxon two-sample tests were done on groups of data within each column. Data, in each column, followed by the same letter are not significantly different at the 5% level.

PROBE DURATION (secs)

INTERVAL BETWEEN EXPOSURE	FEMALE	WITH OVIPOSITION*	n	PARASITISED HOSTS	n	NON-PARASITISED HOSTS	n
5 days	1	160 (±7.40)^{a}	75				
	2	156 (±8.10)^{a}	32	124 (±11.52)^{a}	74	104 (±11.45)^{a}	44
14 days	1	147 (±12.12)^{a}	20				
	2	249 (±32.29)^{b}	9	159 (±31.25)^{a}	18	204 (±37.83)^{b}	9

* Includes the first probe of haploid egg deposition, which is regarded as a test probe (chapter 6).

Reaction to hosts parasitised by Metaphycus species. Similar experiments to the previous ones were run, but with the first group of *C. bartletti* replaced by *Metaphycus saissetiae* Annecke & Mynhardt or *M. africanus* Compere. Females of these two species are indistinguishable from one another (Annecke & Mynhardt 1981). *M. saissetiae/africanus* was chosen because it is fairly numerous in field samples, oviposits in hosts that are of a size acceptable to *C. bartletti*, and *Metaphycus* species are known to leave an egg stalk protruding from the host (Maple 1947), thus leaving parasitised hosts readily identifiable. Parasitised hosts could therefore be exposed to *C. bartletti* females on the day of parasitisation. Again, only experienced females were used, and were exposed to a maximum of 2 or 3 parasitised hosts at a time. (The *Metaphycus* females used appear to lay only 2-3 eggs per day). The response of the *C. bartletti* females to the hosts is recorded in Table 9.7. As before most of the eggs (8 out of 9) deposited by *C. bartletti* into hosts parasitised by individuals of another species were female ones.

TABLE 9.7 : Responses of mated *Coccophagus bartletti* females to hosts parasitised by *Metaphycus saissetiae* or *M. africanus*. Six *C. bartletti* individuals were used in this experiment.

No. *Metaphycus*-parasitised hosts	22
No. encountered by *C. bartletti*	20
No. parasitised by *C. bartletti*	9
No. female eggs laid by *C. bartletti*	8
No. hosts used for host-feeding	5
No. probed, no egg deposited	1
No. antennated, no further response	5

In total, about 84% of *C. bartletti* eggs deposited into already-parasitised hosts were females (n=43). So this species appears not to practise a strategy of depositing male eggs into parasitised hosts, regardless of whether parasitised by conspecifics or other species.

In superparasitised hosts there was a strong tendency for eggs of the same sex to be associated (Table 9.8). That is, superparasitised hosts that received male parasitoid eggs first, tended to be parasitised the second time also with male eggs. Those that had female eggs first were parasitised again with female eggs. Some hosts therefore seem acceptable (and are presumably more suitable) for male offspring, and others for female offspring. This could, possibly, be detected in the laboratory at the chemical level. It would be useful to know to what extent sex ratios of this species are governed by characteristics of the host, as the implications for sex-ratio theory are considerable. It is conceivable that the cyclical nature of *C. bartletti* sex ratios in the field may follow a changing ratio of hosts suitable for females and hosts suitable for males.

TABLE 9.8 : Number of occasions on which the possible combinations of sexes occurred in hosts superparasitised by *Coccophagus bartletti*. Data from same sources as that reported in Tables 9.1-9.5, plus 11 additional superparasitisms observed during other observations on *C. bartletti*.

		FIRST EGG	
		MALE	FEMALE
SECOND EGG	MALE	6	1
	FEMALE	2	26

In summary, *C. bartletti*, like numerous other parasitoid species seems to have a mechanism whereby superparasitism is avoided. Whether parasitism by other species can also be detected is uncertain: data presented here are too meagre even to speculate. However, under certain conditions, and particularly when parasitoids are confined with parasitised hosts, the protective mechanism fails and superparasitism occurs. Contrary to expectations of male eggs being preferentially deposited on parasitised hosts, there is no pattern to superparasitism in *C. bartletti* other than the second egg usually being of the same sex as the first egg (Table 9.8). *C. bartletti* females therefore seem to class hosts into one of three categories, either suitable for female offspring, suitable for males, or entirely unsuitable. In addition, *C. bartletti* deposited mainly female eggs into hosts that already contained a *Metaphycus* egg. The implications of these findings in relation to the evolution of heteronomous parasitoids is discussed in the following chapter.

CHAPTER 10 : DISCUSSION

Diphagous parasitism, in which female larvae are endoparasitic and males are ectoparasitic, seems to be the most primitive of heteronomous host relationships. The hypothesis tested in this thesis (chapter 1) is that diphagous parasitism evolved to allow females of diphagous species to deposit ectoparasitic, and therefore strongly competitive, males on parasitised hosts. Thus, the host range of each diphagous species would be increased, to the detriment of competing individuals (Fig. 1.1). Predictions arising from this hypothesis would be that mated diphagous parasitoid females (i) can discriminate parasitised from unparasitised hosts, (ii) readily parasitise hosts that already contain a parasitoid egg or young parasitoid larva, and (iii) deposit male eggs in previously-parasitised hosts.

Before experiments could be designed to test these predictions, information on certain aspects of the biology of C. bartletti, the species chosen for study, was required and is presented in chapters 5 to 8. The following interpretation is therefore based as much on a knowledge of the behaviour and biology of C. bartletti as on the experiments described in chapter 9.

The first prediction is upheld: C. bartletti females seem quite able to discriminate previous parasitism, an ability common to many parasitoid species. However, contrary to prediction (ii), they parasitise already-parasitised hosts only with reluctance. Finally, C. bartletti females were found not to superparasitise hosts preferentially with male eggs (prediction (iii)).

Before the acquisition of heteronomous host relationships is further discussed, the distinction between the evolution of characteristics that occur species-wide and those that are found only in local populations of a species is drawn. This is necessary because, in evolutionary terms, these are qualitatively quite distinct phenomena, and local adaptations are of no concern in this discussion. Species-wide characteristics could be acquired only during speciation, when all individuals of the small isolate (see Mayr 1963; Paterson 1978, 1981; Futuyma & Mayer 1980) are under the same directional selection (Paterson in press). Once the species has become widespread, selection pressures exerted on different populations are unlikely to be identical. Gene frequencies may vary considerably between such populations (eg Skibinski et al. 1983) and substantial geographic variation may be maintained by selection (Charlesworth et al. 1982). Nevertheless, certain characters of species remain constant through both time and space (eg Henderson & Lambert 1982; Angus 1983; Paterson in press). Host relationships of aphelinids are constant, species-specific, characters and therefore any changes in host relationships must have occurred at speciation.

Three possible interpretations may account for the findings reported in chapter 9.

(i) Diphagous host relationships may have evolved as a mechanism to enhance the competitive ability of aphelinids, but have since been lost in the case of C. bartletti.

(ii) A central tenet underlying the design of experiments presented in this thesis presumes diphagous parasitoids to be the most primitive of heteronomous aphelinids. If this proposition is incorrect, this could also explain the results presented in chapter 9. For example, diphagous parasitism may be derived from heteronomous hyperparasitism, so the predictions listed above would not all hold.

(iii) Diphagous host relationships may have evolved in response to selection pressures not connected with competition generated by other wasp species.

These three interpretations will be discussed in turn.

Loss of competitive ability in C. bartletti. If diphagous host relationships did evolve in response to pressure from competitors, it does seem unlikely that the adaptive behaviour associated with these intricate host relationships would be lost, since it is very unlikely that diphagous species commonly find themselves in the absence of competitors. Both conspecifics and parasitoids of other species can be very common in scale insect infestations. Species-wide, adaptive, characters may be lost by animal species, but such loss does seem to be associated with speciation events, and with the absence of selection pressures that may oppose loss of the character. Flightless land birds on isolated oceanic islands illustrate this point well. Both Snow (1966) and Olson (1973) believe that flight would not have been lost had terrestrial predators been present on the islands, despite positive selection for flightlessness.

Sequence of evolution in heteronomous aphelinids. There are three possible sequences along which the various groups of heteronomous parasitoids may have evolved. The feasibility of each will be discussed separately, with conclusions drawn at the end of this section.

(i) Walter (1983a). This interpretation of the evolutionary sequence in heteronomous aphelinids is based on host-searching behaviour. The sequence leads from diphagous parasitism, in which is found the simplest set of behaviours (same host species; different oviposition sites), to heteronomous hyperparasitoids, which have more complex behaviours (males have hosts different from those of females, although there are features common to the coccoid host and parasitoid host within a coccoid). This more complex system is thought to have then led to the evolution of heterotrophic parasitoids, which probably have the most complex behaviour (males in hosts quite different from those of females). Zinna (1962) proposed a similar evolutionary sequence, but gave no reasons for his viewpoint.

(ii) Flanders (1967). Flanders suggested that indirect heteronomous hyperparasitoids (see Smith 1912; Flanders 1943, 1963; Walter 1983a, b) are the most primitive of heteronomous aphelinids. These species deposit male eggs into coccoid hosts regardless of whether they are parasitised or not. Males then develop further only if a second larva happens to have been deposited before the arrival of the male, or after it, and then grows to a size suitable for the male's development. Hatching of these males is inhibited until a future host consumes all the body fluids of the scale insect (Cendaña 1937; Bess 1939; Walter 1983b).

Direct heteronomous hyperparasitoids (male egg laid into or onto a wasp host) are said to have evolved from indirect heteronomous hyperparasitoids, through a state represented by Coccophagus gurneyi which deposits male eggs either indirectly or directly (Flanders 1964; but see Walter 1983b). According to this theory, diphagous and heterotrophic parasitism evolved from heteronomous hyperparasitoids (Flanders 1967).

It seems unlikely that indirect heteronomous hyperparasitoids evolved, without intermediate steps, from a parasitoid with conventional host relationships. If a male egg is always deposited into a scale insect, upon which it primitively fed and developed, there is no selective advantage to its evolving a 'waiting period' so that it can develop at the expense of parasitoids that may or may not appear. In this scenario, waiting for a parasitoid is detrimental: the probability of mortality is increased.

(iii) Unpublished alternative. Heteronomous hyperparasitoids may have evolved from species that are facultative hyperparasitoids. If this is correct, diphagous parasitism would then be a more recent development, or still be primitive, but representing an alternative evolutionary route amongst a polyphyletic assemblage.

A number of objections can be raised against this latter view of aphelinid evolution.

(a) Facultative hyperparasitoids enjoy more advantages than heteronomous hyperparasitoids. In particular, they suffer no constraints as to the sex of the egg they deposit, in either unparasitised or parasitised hosts. Neither do facultative hyperparasitoids need separate behavioural mechanisms to aid identification of hosts suitable only for males and hosts suitable only for female offspring. There therefore seems no reason to relinquish facultative hyperparasitism in the adoption of new host relationships that are specific to sex.

(b) The vast majority of facultative hyperparasitoids recorded in the literature are ectoparasitic (see Muesebeck & Dohanian 1927; Gahan 1933; Force 1974; Askew 1975; Dahms 1984 for examples), probably because ectoparasitoids do not encounter the immune responses of hosts. If the pre-heteronomous aphelinid had been an ectoparasitic facultative hyperparasitoid, then, in addition to the male becoming an obligate hyperparasitoid, the female would simultaneously have to become endoparasitic.

(c) There are very few records of scale insect parasitoids being facultative hyperparasitoids. Chumakova and Goryunova (1963) described it as uncommon in *Aphytis proclia* Walker. *Aphytis* is ectoparasitic, as is *Moranila californica* (Howard), which has also, but on only one occasion, been observed developing hyperparasitically (Timberlake 1913). In addition, the endoparasitoid *Aspidiotiphagus citrinus* (Craw) is supposedly hyperparasitic upon occasion, but both Benassy's (1956) and Zinna's (1962) accounts are confusing, and this species may be a heteronomous hyperparasitoid.

Together these points weaken the argument for acceptance of this pathway in the evolution of heteronomy in aphelinids. However, more detailed information on the host relationships of *Aspidiotiphagus citrinus* may alter this viewpoint.

Alternative (iii) is the only viable alternative to Walter's (1983a) suggested route, although there are a number of objections to its acceptance. One of the objections raised (point a) is particularly strong because it describes a theoretical disadvantage to facultative hyperparasitoids becoming heteronomous hyperparasitoids. If this were not true, presumably others of the many facultative hyperparasitoid species present would have evolved heteronomous host relationships. When diphagous parasitoids evolved, they suffered no disadvantage in terms of the sex of egg they would deposit in or on a host. Only differential adaptation in each sex occurred.

There may also be recent support for accepting Walter's (1983a) sequence. Clark (1984) studied host-searching behaviour of a facultative autoparasitoid, *Coccophagus atratus*. Male larvae of this species require, as hosts, hymenopterous pupae within scale insects. However, both mated and virgin females are attracted more strongly by unparasitised scale insects. Hosts suitable for male offspring are only slightly attractive to the wasps, and the discovery of 'male' hosts seems largely incidental to the search for 'female' hosts. In addition, the cues, from parasitised hosts, to which they do respond, are the same cues they use to find female hosts. Differentiation between hosts for males and hosts for females takes place only upon contact by the wasp. If heteronomous hyperparasitoids evolved from facultative hyperparasitoids, equal attraction from both host types may be expected in the primitive and derived states. Otherwise, facultative hyperparasitism would not, perhaps, have been sufficiently common for selection to have favoured hyperparasitic males. On the other hand, if heteronomous hyperparasitoids evolved from diphagous parasitoids, it is not suprising that host-searching behaviour remains similar to this behaviour in the primitive state. That is, females search primarily for unparasitised scale insects. An investigation of host-searching behaviour by facultative hyperparasitoids may alter the impact of this evidence, but the most likely evolutionary sequence for heteronomous aphelinids remains that postulated in Walter (1983a).

Evolution of diphagous host relationships. The different developmental pathways of male and female diphagous parasitoids were postulated, in the introduction, to be adaptations that reduced intersexual competition for food by males feeding on parasitised scale insects and females on unparasitised ones. This 'intersexual resource partitioning' has often been invoked to explain the evolution of differences in size, feeding structures and foraging behaviour between the sexes of bird and lizard species (Schoener 1965, 1967; Selander 1966, 1972; Hooper & Lennartz 1981), other vertebrates (Keast 1977), insects (Price 1975) and planktonic invertebrates (Gilbert & Williamson 1983). However, application of this theory to diphagous aphelinids was demonstrated (Tables 9.1 to 9.7) to be an untenable interpretation, and an alternative explanation is required.

The only observations that may shed light on this problem are: (a) the tendency of the ectoparasitic non-heteronomous aphelinid, Aphytis melinus DeBach, to deposit male eggs ventrally on its diaspidid hosts and female eggs dorsally on the scale insect's body, but beneath the 'scale' cover (Abdelrahman 1974; Luck *et al*. 1982), and (b) the tendency of different *C. bartletti* females attacking the same host to deposit eggs of the same sex into the host (Table 9.8), even if the attacks were separated by as much as 14 days.

Two assumptions about the pre-heteronomous aphelinid may be made; that placement differentiation occurred in the deposition of male and female eggs in the ancestral form, with male eggs beneath the host and female ones above, as in *A. melinus*, and that the hosts attacked had diaspidid-like scale coverings under which female eggs could be laid.

If, during a speciation event, the pre-heteronomous aphelinid was forced by chance circumstances to change hosts from a diaspidid-like one to a coccid-like one, female eggs could not be deposited ectoparasitically beneath a scale covering, and if deposited dorsally on the scale insect they would be entirely unprotected. The vulnerability would presumably result in selection pressures for female eggs to be deposited endoparasitically. Because males are protected beneath the scale insect, there would not necessarily be similar selection pressures for their endoparasitism. Diphagous aphelinids seem, therefore, to exemplify that view of evolution advocated by Jacob (1977): 'Evolution does not produce novelties from scratch. It works on what already exists, either transforming a system to give it new functions or combining several systems to produce a more elaborate one.'

Whatever led to differential deposition of male and female eggs in the pre-aphelinids, stochastic effects or specific selection pressures, must have resulted in the potential for an independent evolutionary pathway for each sex. In Slatkin's (1984) view 'It should be no surprise that different selection acting on members of the two sexes produces differences between the sexes.' Choice of particular host types for offspring of each sex, as in *C. bartletti*, is possibly one of the early manifestations of this 'principle' in heteronomous aphelinids.

This view of diphagous parasitism raises three points. Firstly, independent evolutionary pathways for members of each sex (besides the usual sexual differences) in a species is well known in insects, but not common. Perhaps the best known examples are fig wasps (Agaonidae), stylopids (Strepsiptera) and glow worms (Lampyridae), all insects in which neotony seems to have played a role in the evolution of one of the sexes. Secondly, the possible importance of stochastic events in speciation should be investigated, because it seems unlikely that differential egg placement, as found in A. melinus, is adaptive. The importance of stochastic events at speciation is emphasised, too, by Paterson (in press). Finally, if diphagous parasitoids evolved as postulated, then the sexes did not adapt to exploit 'dimorphic' or 'bimodal' niches (discussed by Slatkin 1984).

Is heteronomous hyperparasitism an adaptation? Diphagous parasitism seems to have evolved in response to a number of sequential contingencies, amongst which fixation of stochastic genetic effects and change in host type seem likely possibilities, but not selective pressures arising from either intra- or interspecific competition (chapter 9). How does this affect our view of heteronomous hyperparasitoids, which appear to have evolved from diphagous parasitoids? They are theoretically (Fig. 1.1), and perhaps in the field (Williams 1977), strong competitors that appear to have evolved this adaptation in response to selection pressures from competitors.

It is an easily envisaged step for male diphagous parasitoids, which are ectoparasitic, to become hyperparasitic on mature hymenopterous larvae or pupae: facultative hyperparasitoids are usually ectoparasitic. Two exceptional observations reported in the literature provide examples of species that illustrate the sorts of evolutionary steps that may link diphagous parasitoids and heteronomous hyperparasitoids. Males of C. ochraceus, a diphagous parasitoid, occasionally develop hyperparasitically (Cendaña 1937), and males of Euxanthellus philippiae Silvestri, a heteronomous hyperparasitoid, may sometimes develop as primary ectoparasitoids (Valentine 1964). Two questions need be answered to explain the evolution of heteronomous hyperparasitism. What selection pressures resulted in male hyperparasitism, and why did the relationship become obligatory, and not remain facultative? The latter is important because, in discussing evolutionary sequences in heteronomous aphelinids, it was suggested that the step from facultative hyperparasitism to heteronomous hyperparasitism is unlikely because, amongst other reasons, there are fewer advantages to the obligatory relationship.

During speciation, when diphagous parasitoids evolved into direct heteronomous hyperparasitoids with ectoparasitic males, wasps ovipositing beneath the host could conceivably have laid haploid eggs on the parasitoid larvae or pupae by penetrating the sternites from below: the sternites of scale insect mummies seem so fragile that they are probably easily penetrated by the probing ovipositor. (When depositing male eggs, C. bartletti females often jab with the ovipositor whilst it is beneath the host). Survival chances of these ectoparasitic hyperparasitoids may be good because, as has been mentioned, they are not exposed to any immune responses. Evidence of this step should be sought in the oviposition behaviour of those heteronomous hyperparasitoids that have ectoparasitic males. There may be some species that deposit male eggs ventrally on the pupa within a mummy.

One of two causes may have been responsible, at speciation, for male diphagous parasitoids becoming hyperparasitic.

(i) A shortage of unparasitised scale insects relative to parasitised ones may have caused mated females to deposit female eggs in the few unparasitised hosts, and male eggs on old primary parasitoid larvae or pupae. The ectoparasitoid Aphytis proclia is known to hyperparasitise hosts in the field when a high proportion of parasitised hosts is present (Chumakova & Goryunova 1963). All that remains during speciation is for the behaviour to be refined. If it is, the change in hosts for males, coupled with the change in deposition site for male eggs, may cause the hyperparasitic habit to be obligatory. In this view the important selection pressure is competition from other parasitoids.

(ii) If the habitual hosts of a diphagous species remained unavailable during an isolation event, selective premiums would exist for adaptation to a new host species. Assume the oviposition habits of this species resembled those of C. bartletti, with certain hosts acceptable for male eggs and others for females (Table 9.8). Possibly the sensory information derived from the new host most closely resembled that of the original host species, but only ones in which female eggs would be deposited. On the other hand, cues from parasitised scale insects, of this or other species, may have attracted male egg deposition. Obligatory male hyperparasitism would then evolve.

Of these alternatives, the first is probably more appealing intuitively, but the second may be the more likely one because the selection pressures involved are quite possibly stronger and more consistent in time (see Connell 1980). Schoener (1982, 1983) argued that competition in the field is often intense and sustained. Connell's (1983) conclusions from a similar analysis contradict, to a large extent, those of Schoener. Theoretically, strong directional selection is an important prerequisite before speciation will occur (Paterson in press), and interspecific competition, because of its intermittent nature and patchy geographical occurrence (Andrewartha & Birch 1954; Wiens 1977), may not generate sufficiently strong and consistent selective pressures to produce such an effect (Connell 1980; Walter et al. 1984: Appendix 3, which also contains a reply by Schoener (1984), expressing his viewpoint).

Unfortunately, unless an aphelinid species was found with characteristics suitable for testing between these two theories, their validity is probably impossible to establish. No doubt further advances in our understanding of evolutionary biology would aid interpretation of heteronomous hyperparasitoid evolution. For example, how small need an isolate be before speciation will occur, and for how long are selection pressures necessary before speciation becomes irreversible? Even the duration of speciation is unknown (see Stanley 1979, pp. 42-43). If information on these points could be gathered, the efficacy of interspecific competition as a selective force in this situation could be assessed.

Once ectoparasitic male hyperparasitoids had evolved in aphelinids, then endoparasitism and indirect hyperparasitism could develop easily in subsequent speciations. A possible selection pressure in these cases was the absence of mature parasitoid larvae or pupae within scale insects. Male larvae could 'wait' in the egg (inhibited hatching) for young larvae to mature, as do indirect hyperparasitoids, or the male egg could be deposited into the young larva and have its larval development synchronised with that of the host (see Salt 1941; Askew 1971).

The importance of competition at speciation. The discussion on the evolution of heteronomous parasitism in its various forms raises a number of points about competition theory.

(i) Intersexual competition. Although male heteronomous hyperparasitoids have a diet quite different from their conspecific females, this was not referred to in this thesis as 'intersexual resource partitioning'. The latter is a term that connotes a specific function, prevention of competition between the sexes (Keast 1977). However, restriction of the hyperparasitic habit to males may largely have been due to various coincidences during the evolutionary development of heteronomous host relationships. So although the sexes have mutually exclusive diets, they probably did not evolve in this way to 'partition resources'.

It is likely that the other cases of this phenomenon reported in the literature (examples in Selander 1966; Price 1975; Keast 1977; Pianka 1978) should be interpreted in similar fashion. Alternative explanations should be sought and tested, as Johnson (1982), Wheeler & Greenwood (1983) and Price (1984) have done for sexually dimorphic brentid weevils, birds of prey and Darwin's finches respectively. Selander (1966) and Morrison (1982) also suggested an alternative hypothesis: that males of indigo buntings and certain warbler species may forage at a higher level than their females because they sing from high perches but females spend most of their time near the lowly-placed nest. Information on whether this behaviour is genetic or not would be useful. In addition, fixation of random genetic processes or pleiotropic effects may also be more likely explanations in certain cases, but would be very difficult to confirm.

'Intersexual resource partitioning' is said to evolve in response to increased levels of intraspecific competition (Keast 1977), perhaps associated with less interspecific competition (Selander 1966). However, in proposing the evolution of 'intersexual resource partitioning' in heteronomous aphelinids, I postulated that its development was related to increased competition from other parasitoids generally (chapter 1). This is not inconsistent with Keast's (1977) views because increased intraspecific competition may follow if resources are limiting. Although the first step in the evolution of heteronomous host relationships cannot be viewed as an evolutionary response to increased levels of intra- or interspecific competition, the selection pressures in the evolution of heteronomous hyperparasitism from diphagous parasitism may have arisen from the presence of competing parasitoids. This is discussed in the following section.

(ii) Interspecific competition. Interspecific competition, and even the lack of it, is seen as a powerful evolutionary force that frequently results in the formation of new species (eg Mayr 1963, 1982; Stebbins 1977, 1982; Rosenzweig 1978; Charlesworth et al. 1982), although this viewpoint is being increasingly questioned (Stanley 1979; Paterson in press; Walter et al. 1984). The results presented in this thesis, and their interpretation, show that interspecific competition need not be invoked to explain the evolutionary origins of diphagous parasitism. However, in the derivation of heteronomous hyperparasitism from diphagous parasitism, individuals of other species had of necessity to be present, and therefore did affect the outcome of speciation. Whether or not this presence resulted in selection pressures that ultimately caused males to become hyperparasitic remains debatable.

Good examples do exist of species that evolved, if not in direct response to interspecific competition, then under conditions in which interspecific competition may have resulted in selection pressures of sufficient strength to change species characteristics. Limpets of certain species may protect, for their grazing requirements, a small territory by actively dislodging intruders (Branch 1981). Walter et al. (1984) considered the possibility that such a characteristic evolved because space, and therefore algal food, was limiting at speciation. Pentalitomastix sp. is a polyembryonic encyrtid parasitoid, which, in each brood produces a small proportion of sterile larvae that protect their sexual siblings from larvae of other species (Cruz 1981). In both cases, food must have been limiting at speciation. Moreover, this limitation is compounded by the relatively sedentary existence of adult limpets to leave and seek food elsewhere, and the inability of Pentalitomastix larvae to leave their resource 'package'. Selection pressures generated by food limitation would be reinforced if movement was restricted like this. Salt (1961) illustrated the range of mandibular weapons found in first-instar parasitoids. These, too, may have evolved in response to the presence of competing larvae. However, alternative hypotheses also need examination. Selection pressures for such defensive or offensive structures in parasitoid larvae may not have been the ultimate factor causing speciation. In fact, during the throes of speciation, intraspecific competition could result in selection pressures for stronger mandibles.

Other examples, especially those in which the resources are not 'packaged' around the organism for most of its life, are not clear at all. For instance, it is difficult to envisage food resources of seed-eating birds being limited in such a way, by competitors, that selection pressures generated by the competitors could lead to the species permanently feeding on different-sized seeds. This viewpoint is contrary, in part, to that of Grant's (1984) interpretation of evolution in Darwin's finches on the Galapagos Islands. However, here the birds are restricted in geographical distribution to small islands, so food may become limiting over the whole range of an island population. There is an additional complictation in this example. The size of the bill, which has been used to interpret the role of interspecific competition in the evolution of these birds, is one of a suite of characters important in recognition of potential mates (Lack 1983; Ratcliffe & Boag 1983; Grant 1984). Again, information on the duration of speciation and the geographical range occupied by the small isolate would aid interpretation of the role of competition at speciation.

The interpretation, in this thesis, of the evolutionary effects of interspecific competition implies that competition should probably not be invoked to explain the differences between species, as is so often done, under the title of resource partitioning (eg Diamond 1978; Schoener 1982; Roughgarden 1983). These differences could be the result of many diverse selection pressures operating at the time of speciation (eg pressures from the physical environment, or predators). Even in those cases where interspecific competition has been important in generating differences between species at speciation, these selection pressures were functional in changing characters of the species only at speciation. The resultant differences between species should, nevertheless, not be referred to as resource partitioning, for the following reasons. Later in the species' history when it becomes more widespread it could, in different areas, come into contact with vastly different faunas and even species that resemble it almost precisely in resource requirements. Even though differences occur between the species, they are not there for partitioning of resources.

In addition, resource partitioning in the post-speciation history of a species is unlikely because it requires an improbable set of conditions (Walter et al. 1984), and even examples of local effects due to competition, called character displacement, are not satisfactory (Grant 1972, 1975; and see Arthur 1982; Levinton 1982). Therefore, interspecific competition should not be seen as a universal selection pressure to account for differences between species. Instead, it should be regarded only as one of a large number of possible selection pressures that may have an affect at speciation.

To summarise, this study has demonstrated that it is probably unnecessary to invoke intra- or interspecific competition as a causative agent in the evolution of diphagous parasitism, the most primitive of heteronomous host relationships. Instead, a combination of preadaptation and chance provides the most likely explanation for their origins. Preadaptation is represented by the presumed differential oviposition behaviour for male and for female eggs, and chance possibly had a number of influences (eg formation of the geographical isolate, the host species available for parasitisation, and genetic composition of wasps in the isolate). Both Mayr (1963, p. 462) and Price (1980, p. 37) have suggested that adaptation to a new host type may be important in speciation of parasitic organisms. An interpretation of the selective advantages to heteronomous hyperparasitism, which is thought to have evolved from diphagous parasitism, is more complex. Interspecific competition may have been involved. Alternatively, the sensory cues associated with host selection seem to be unique to offspring of each sex in *C. bartletti*, a diphagous parasitoid. If, during speciation, hosts containing parasitoid larvae or pupae resembled, in sensory terms, the hosts usually chosen for male eggs, male hyperparasitism could have evolved. The latter alternative is favoured because interspecific competition is generally inadequate to generate consistently strong selection pressures.

A phylogenetic analysis of heteronomous aphelinids would probably prove useful in interpreting the sequence in which the various host relationships evolved (see Wanntorp 1983) but as yet data are too meagre, particularly on the host relationships of species in the genera *Aspidiotiphagus*, *Azotus*, *Ablerus* and perhaps *Encarsia*. Species in unstudied genera may also yield useful information. A species worth investigating from this point of view would be *Encarsia partenopea* because Mazzone (1983) recently described primary endoparasitism in both males and females. Mazzone (1983) was surprised that the host relationships of this species should be primitive in comparison with adult morphology. However, *E. partenopea* may have evolved from a species with males that were endoparasitic hyperparasitoids. So the host relationships of *E. partenopea* may be advanced rather that primitive. Unfortunately the higher taxonomy proposed for aphelinids (eg Yasnosh 1976) has no pattern that may be of use in interpreting the evolution of heteronomous host relationships.

Finally, my analysis is seen as a first step in unravelling the evolutionary complexities of aphelinid host relationships.

CHAPTER 11 : SUMMARY

1. Aphelinid species in about 8 genera are unique among parasitic organisms because males are consistently different in their host relationships from conspecific females, a phenomenon called heteronomous parasitism. Females are ALL endoparasitoids within scale insects or white flies. Males fall into one of three groups, depending on their host relationships. They may be primary ectoparasitoids of scale insects or white flies, hyperparasitoids, or primary endoparasitoids in lepidopterous eggs. Species in these groups are called DIPHAGOUS PARASITOIDS, HETERONOMOUS HYPERPARASITOIDS and HETEROTROPHIC PARASITOIDS, respectively. This terminology is developed in chapter 2 and the biology of heteronomous aphelinids is reviewed in chapter 3.

2. Diphagous parasitism is thought to be the most primitive of heteronomous host relationships, followed by heteronomous hyperparasitism and then heterotrophic parasitism. This postulated evolutionary sequence is based upon the increasingly complex host-searching behaviour of adult females.

3. Observations were carried out to test the hypothesis that diphagous parasitism evolved in response to competition from other parasitoids. Male ectoparasitism was thought to be a mechanism evolved to enable male larvae to kill other larval parasitoids already in a host. This interpretation was based on the general observation that ectoparasitic Hymenoptera usually destroy endoparasitic ones in larval competition. Predictions generated by these propositions were that female diphagous parasitoids should (i) be able to discriminate parasitised from unparasitised hosts, (ii) readily oviposit in recently-parasitised hosts, and (iii) preferentially deposit male eggs on parasitised hosts.

4. *Coccophagus bartletti* was chosen as the subject species. Although it is one of a few cryptic species within the *ochraceus*-group of *Coccophagus*, living specimens can be identified on the basis of

mating behaviour. The consistent differences between the mating behaviour of *C. bartletti* and *C. lutescens* are described and illustrated.

5. *C. bartletti* deposits female eggs endoparasitically and male eggs ectoparasitically. Oviposition behaviour therefore allows the sex of the deposited egg to be predicted with certainty. However, not all probes of the ovipositor into the host resulted in deposition of a female egg. Probes shorter than 76 seconds seldom resulted in egg deposition, and only about 60% of longer probes yielded an egg. Dissection of hosts was therefore necessary to confirm that oviposition had occurred.

6. The activity of mated females, when presented with suitable hosts, followed a definite pattern. Oviposition usually proceeded immediately hosts were available and most ovipositions for that day (about 84%) occurred within the first three hours. Thereafter, only sporadic oviposition punctuated long periods of inactivity. Experiments demonstrated that only one such peak of oviposition per day was usual and that eggs were seldom deposited at night.

7. Ovipositional activity was governed by the availability of eggs in the oviducts. A maximum of 17 eggs was deposited per bout of activity and the most found in the oviducts, by dissection, was 19. Egg replenishment was dependent upon regular host-feeding.

8. Twenty-six bouts of activity (of at least 3 hours duration) yielded no temporal pattern for deposition of male and female eggs. Sex ratios determined by observation in the laboratory were generally female biased, with the average about 78% females. There was no pattern of progeny sex ratio derived from females of different ages. Regular samples of wasps taken from the field showed that sex ratios varied cyclically. There was no seasonal basis to the cyclicity, which remains unexplained. Sex ratios were, however, correlated with the number of suitably-sized hosts available, the number of parasitoids in the sample, and the proportion of relatively large hosts in the sample.

9. Observational experiments were designed to test the three predictions (point 3, above) of the proposed hypothesis. Mated females on unparasitised scale insects seldom superparasitised hosts (about 3% of 272 eggs laid). When provided with parasitised hosts (conspecifics and other species) they did oviposit, but reluctantly. They also tried persistently to leave the arena. Therefore, discrimination of parasitised hosts occurs as proposed in prediction (i). However, the other predictions, a readiness to superparasitise hosts and to preferentially deposit male eggs on parasitised hosts, were not supported by experimental evidence. The only significant pattern found in these experiments was that the superparasitising female usually deposited an egg of the same sex as the first female. Therefore C. bartletti females seem, in some way, to identify hosts as suitable either for female offspring or for male offspring.

10. It is unlikely that intra- or interspecific competition was important in the evolution of diphagous parasitism. This interpretation is used as a basis for discussing the evolution of heteronomous hyperparasitoids, as well as the postulated evolutionary role of competition in generating 'intersexual resource partitioning'. The possible role of competition at speciation, generally, is also considered.

11. It is concluded that the evolution of diphagous host relationships, as in C. bartletti, probably involved a combination of preadaptation (represented by a presumed differential oviposition behaviour for male and female eggs), and chance, especially in the availability of host species to which the pre-heteronomous aphelinid could adapt.

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APPENDIX 1 : APHELINID GENERA WITH SPECIES KNOWN TO HAVE HETERONOMOUS HOST RELATIONSHIPS

Walter (1983a) listed 9 genera known to contain species with heteronomous host relationships (*Aneristus* Howard, *Coccophagoides* Girault, *Coccophagus* Westwood, *Encarsia* Foerster, *Euxanthellus* Silvestri, *Lounsburyia* Compere & Annecke, *Physcus* Howard, *Prococcophagus* Silvestri and *Prospaltella* Ashmead). *Aleurodiphilus* DeBach & Rose, *Bardylis* Howard and *Trichaporus* Foerster should also have been included (Valentine 1964; DeBach & Rose 1981).

Recent adjustments to the supraspecific classification of aphelinids have been incorporated into two recent keys to the genera of this family (Hayat 1983; Yasnosh 1983) and they affect the above list.

1) *Aneristus* has been synonymised with *Coccophagus* (Hayat 1983).
2) *Physcus* is evidently a synonym of *Coccobius* Ratzeburg. However, both Graham (1976) and Yasnosh (1983) gave reasons for preferring the former name. Hayat (1983) nevertheless formalised the synonomy. *Physcus* is better known and is used in this thesis.
3) *Encarsia* now encompasses several genera, including *Prospaltella* (Viggiani & Mazzone 1979; DeBach & La Salle 1981), *Trichaporus* (Viggiani & Mazzone 1979; DeBach & La Salle 1981), and *Aleurodiphilus* (Hayat 1983). These changes are accepted here but, as Yasnosh (1983) warned, *Prospaltella* species attack diaspidids whereas *Encarsia* species parasitise white flies, so *Prospaltella* may form a useful taxonomic division.

Therefore, depending on the author followed, 8 (Hayat 1983) or 10 (Yasnosh 1983) genera contain species with heteronomous host relationships. This list will no doubt be lengthened as biological information on species in related genera becomes available.

APPENDIX 2 : SEX RATIOS IN A SPECIES OF SOLITARY PARASITOID

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Sex ratios of *Spalangia endius* (Hymenoptera: Pteromalidae), in relation to current theory

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ABSTRACT. 1. *Spalangia endius* Walker is a solitary parasitoid of house fly puparia.

2. The sex and size of *S.endius* was not related to host size.

3. In the laboratory the mean sex ratio of all offspring of nine groups, each comprising twenty females, was consistently female-biased ($\bar{x} = 83.5\%$, range 79–87%). The sex ratio in the field was less female-biased and showed greater fluctuation (61–75%). This may be a consequence of females laying male eggs before mating, some females remaining unmated, possible shorter adult life expectancy in the field than in the laboratory, and, perhaps, the presence of conspecific females.

4. The sex ratio of offspring of individual females varied from 66% to 100% females, and males were deposited early in the oviposition sequence.

5. Although a large number of fly puparia died before adult flies or parasitoids emerged (64.5%; $n = 5874$), there was no differential mortality of either sex.

6. Our results fit no general sex ratio hypothesis and we conclude that (i) the genetic nature of sex ratios in these insects needs careful examination, and (ii) the prevalence of female-biased sex ratios in solitary parasitoids needs investigation.

Key words. *Spalangia,* house flies, parasitoids, sex ratios.

Introduction

The mode of sex determination in most Hymenoptera is that of arrhenotokous parthenogenesis (Crozier, 1975), i.e. males originate from unfertilized eggs, and females from fertilized ones. Since inseminated females can determine the sex of each offspring, there has been considerable speculation about the factors that govern sex ratios in Hymenoptera (see Charnov, 1982).

Fisher (1930, p. 141) regarded the sex ratio of a species as an adaptation and argued that generally there should be selection for a 50:50 sex ratio: deviation from this would result in selection for individuals that produce offspring of the rarer sex. However, many haplodiploid animals, including certain Hymenoptera, regularly produce more females than males. Hamilton (1967) proposed that these ratios evolved in response to local mate competition (see Colwell,

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1981) in those species where inbreeding (sibmating) is a normal feature of mating behaviour, a condition evident in gregarious hymenopteran parasitoids. In solitary, outbreeding, hymenopteran species equal numbers of male and female offspring are expected.

However, environmental factors also affect sex ratios of Hymenoptera. The most commonly reported for parasitoids is host size (e.g. Brunson, 1937; Flanders, 1946, 1965; van den Assem, 1971; Sandlan, 1979; Charnov, 1979). Large hosts of certain parasitoid species receive female eggs and small hosts male ones. Although host size does affect sex ratios, Charnov (1982) considers that these shifts occur within the framework of Hamilton's theory. In other words, unless large or small hosts are excessively abundant, the sex ratio should remain at a predicted equilibrium, but with female eggs being deposited mainly in large hosts. Waage (1982a) predicts this to occur in species that attack non-growing stages (e.g. eggs, pupae) of their host species.

The general predictive value of these views of sex ratios were tested with *Spalangia endius* Walker (Hymenoptera: Pteromalidae) and its hosts, house fly puparia. We chose these species for the following reasons. (a) *S.endius* is a solitary parasitoid, and often occurs in large numbers in areas of suitable habitat. Because many foundresses are expected, local mate competition is unlikely to result in the evolution of female-biased sex ratios (see Colwell, 1981). (b) House flies constitute the majority of *S.endius* hosts in the area sampled. In addition, puparia can be cultured in different sizes, a condition regularly observed in the field (both observations from P. E. Hulley, pers. comm.).

It is possible that the cumulative sex ratio of offspring may change with the age of the ovipositing female (e.g. Mackauer, 1976), a factor not taken into account in most sex ratio studies. We therefore determined the sex ratio of the entire brood produced by individual *S.endius* females that were subjected to various experimental treatments. The sequence of male and female emergence was also recorded. Because a large number of hosts in these experiments gave rise to neither flies nor adult parasitoids, we investigated, too, whether fly mortality or parasitoid mortality was mainly responsible for this, and also whether the extent of male parasitoid mortality was different from that of females. To aid the interpretation of the sex ratios observed, field samples of this species were regularly collected.

Materials and Methods

House flies and *Spalangia* wasps were obtained from puparia collected from a poultry farm near Southwell (33°34'S; 26°43'E) and were used to establish laboratory cultures.

All puparia used in experiments were less than 48 h old, because older ones become unsuitable for oviposition by *S.endius* females (Morgan, 1965). Small puparia (3.3–4.3 mm in length) were obtained by maintaining larvae at high densities (100 larvae/100 ml of culture medium) whereas large puparia (6.0–7.0 mm in length) were obtained by rearing larvae at lower densities (100 larvae/300 ml).

Parasitized puparia taken from *Spalangia* cultures were isolated until adult wasps emerged. The females were then mated, confined with a puparium for 24 h to allow the parasitoid to host-feed (see Legner & Gerling, 1967), and were then used in experiments.

The effect of host size and host clumping (to test whether an 'alternative sex ratio strategy' exists in this species: see below) on the sex ratio of the offspring of *S.endius* females was examined by means of an experiment designed for a two-way analysis of variance and which consisted of nine treatments (see Table 1A). Twenty replicates of each treatment were run in a constant environment room at 23±2°C and 70±5% r.h. Experiments were continued daily until each female died ($\bar{x} = 5.5$ days; SE = 0.13; $n = 180$). If a female died before she had been exposed to ten puparia, the replicate was discarded and repeated.

If host size is important in the determination of the sex of *S.endius* offspring then sex ratios obtained in this experiment should match the predictions in Table 1B. Predicted sex ratios for outbreeding species are listed in Table 1C. Adult females in the field may encounter hosts in small, isolated clumps. Under these conditions there may be only one foundress and therefore increased local mate competition between male siblings. We therefore expected to detect, under conditions of host-clumping in the laboratory, whether *S.endius* practises an 'alternative sex ratio strategy' (Waage, 1982b): hence the

TABLE 1. (A) Design of an experiment to determine whether host size and the degree of inbreeding are important in the determination of *Spalangia endius* sex ratios. (B) Predicted sex ratios if host size is important. (C) Predicted sex ratios if the extent of inbreeding is important. See text for details.

Host size	Host clumping		
	One host	Two hosts	Five hosts
A. Small (3.3–4.3 mm)	Treatment 1	4	7
Large (6.0–7.0 mm)	2	5	8
Random (small and large)	3	6	9
B. Small	♂ bias	♂ bias	♂ bias
Large	♀ bias	♀ bias	♀ bias
Random	50:50	50:50	50:50
C. Small	50:50	50:50	50:50/♀ bias
Large	50:50	50:50	50:50/♀ bias
Random	50:50	50:50	50:50/♀ bias

alternative predictions in the right-hand column of Table 1C.

In treatments 1, 2 and 3 (Table 1A) a single female was placed in a gelatin capsule with a single host for 1.5 h (observations showed that at 23°C most females oviposited only once within this period). The female was then removed and placed with another puparium for the same period. They were given five hosts per day for the duration of their life-span. The female was transferred at each change rather than the puparium, because the previous presence of conspecific females is known to affect the sex ratios of other parasitoids, e.g. *Nasonia vitripennis* (Walker) (Wylie, 1966; Werren, 1983). After each change the old puparium was catalogued so any pattern in oviposition could be monitored.

In treatments 4, 5 and 6 (Table 1A) a single female was placed in a glass vial (23 mm diameter × 15 mm height) with two puparia. She was kept in the arena for 3 h, enough time to parasitize both puparia, and was then removed and placed with two new hosts. Each female was presented with six hosts per day.

For treatments 7, 8 and 9 (Table 1A) each female was placed in an arena (23 mm diameter × 15 mm height) with five puparia for 7.5 h (an allotted 1.5 h per puparium) per day.

In addition to laboratory experiments, samples of puparia collected from the poultry farm were also analysed. Single samples, each comprising 18 litres of chicken manure (see Hulley, 1983), were collected at approximately 3 week intervals from 8 December 1980 to 18 January 1982. Puparia were floated off the manure with water and placed individually in gelatin capsules until flies or wasps emerged.

Results and Discussion

Host size and clumping

Host size and host clumping (clumps of one, two or five hosts) had no effect on the sex ratio of *S. endius* (analysis of variance, $P > 0.2$), which was consistently female-biased (Table 2). This species therefore differs from parasitoids such as *Coccygomimus turionella* (Linnaeus) (Sandlan, 1979) and *Lariophagus distinguendus* (Foerster)

TABLE 2. Results of an experiment to determine the effect of host size and host clumping on the sex ratio of *Spalangia endius*. The mean sex ratios (% females) for twenty replicates are given with standard errors. Overall mean = 83.5% females ($n = 2243$).

Host size	Host clumping		
	One host (n)	Two hosts (n)	Five hosts (n)
Small (3.3–4.3 mm)	86 ± 2.54 (214)	82 ± 2.37 (177)	79 ± 2.66 (236)
Large (6.0–7.0 mm)	87 ± 2.66 (286)	85 ± 2.57 (303)	82 ± 2.54 (273)
Random (large and small)	86 ± 2.17 (241)	84 ± 2.58 (263)	81 ± 2.27 (250)

(van den Assem, 1971) in which male eggs are more often oviposited in smaller hosts and females in larger hosts. In treatments 3, 6 and 9 (Table 1A) in which both large and small hosts were offered to *S.endius* females, 48.3% of female eggs were deposited in small puparia, 51.6% in large puparia ($n = 649$). Therefore *S.endius* does not discriminate between large and small hosts, even when the relative sizes of hosts can be compared in rapid succession by the parasitoid (as found necessary in some species: see van den Assem (1971)).

Charnov *et al.* (1981) reasoned that female eggs should be deposited in relatively bigger hosts because larger females develop. They demonstrated that *Lariophagus distinguendus* and *Heterospilus prosopidis* Viereck females from large hosts were larger than those from smaller hosts and laid more eggs than them. The head capsule width of fifty *S.endius* females from large hosts (0.57 mm; SE = 0.004) did not differ from that of females from small hosts (0.56 mm; SE = 0.007). Since *S.endius* females apparently attain their maximum size even in small puparia there would be no selective advantage for the preferential deposition of female eggs into larger puparia. This is the likely reason why *S.endius* does not fall into Waage's (1982a) predicted pattern of sex ratios being governed by host size in parasitoids that attack a non-growing host stage.

Differential mortality

Sex ratios obtained in these experiments refer only to emerged adults. Because a large proportion of puparia (64.5%, $n = 5874$; see also Legner, 1977; Morgan *et al.*, 1976) yielded neither parasitoids nor flies, there is a possibility of the sex ratio being affected by differential mortality. We therefore determined whether parasitoid mortality was similar for each sex. This experiment was based on Sandlan's (1979) idea of comparing the emergence of adults from two groups of hosts: one parasitized by virgin parasitoids, the other by mated females. We also included control groups of unparasitized puparia. Of the experimental females, thirteen unmated and seventeen mated ones produced offspring. The others were excluded from the analysis because we were uncertain whether they had been inactive and not parasitized hosts, or whether all their offspring had died. Our results indicate that the former was more likely (see below).

The results of this experiment are presented in Table 3. Data for fly emergence and death of puparia (including those successfully parasitized) are given. A greater proportion of puparia died after exposure to mated females (66%) than after exposure to virgin females (57%) (χ^2 on raw data = 13.7, $P < 0.001$). Total fly mortality comprises the following:

Adult parasitoids that emerge +
Natural death of flies +
Death of juvenile parasitoids +
Death due to probing by parasitoids

Of these categories, adult parasitoid emergence is known, and natural death of flies can be estimated from the twenty controls of unparasitized puparia (Table 3). In these controls, mortality was remarkably consistent ($\bar{x} = 32.1\%$; 95% confidence limits after arcsine transformation = 29.4% to 34.8%). A 32% natural fly mortality was therefore subtracted

TABLE 3. Fate of house fly puparia presented to virgin and mated *Spalangia endius* females for oviposition. Twenty virgin and twenty mated *Spalangia* wasps were given five puparia per day for the duration of their lives. Of these, thirteen of the former and seventeen of the latter produced offspring and were included in the analysis. On each day of the experiment five unparasitized puparia were added to each of twenty controls, which eventually comprised sixty-three puparia (only three added on the last day) drawn from the same source as those used in the experiment.

	Virgin females	Mated females	Control puparia
Flies emerged	318 (43%)	346 (34%)	852 (68%)
Dead puparia (incl. parasitoids)	422 (57%)	669 (66%)	408 (32%)
Total puparia	740	1015	1260

TABLE 4. Fate of house fly puparia presented to virgin and mated *Spalangia endius* females for oviposition. Data revised from Table 3. The number of healthy puparia presented was determined by subtracting natural fly mortality (from controls) from the total number of puparia in each treatment (Table 3). Parasitism and fly emergence is listed as is the mortality of puparia due to factors other than natural death of flies, i.e. probing by the adult parasitoid, or death of parasitoid immature stages. χ^2 statistics calculated on the raw data are also listed.

	Virgin females	Mated females	χ^2	Significance
No. healthy puparia presented	500	686	-	-
Fly emergence	318 (63.6%)	346 (50.5%)	-	-
Parasitism	74 (14.8%)	145 (21.1%)	7.45	$P<0.01$*
Mortality (other than natural death of flies)	108 (21.6%)	195 (28.4%)	7.27	$P<0.01$*

from the total number of puparia presented to the female wasps. This assumes that dead or dying puparia are avoided by the wasps, and if not, that virgin and mated wasps have the same response to these unhealthy puparia. The adjusted totals are presented in Table 4, together with fly emergence, parasitism and mortality of puparia other than those that died naturally.

A significantly smaller proportion of hosts was successfully parasitized by virgin females than by mated females (Table 4). This could have been due either to greater mortality of juvenile males, or to ovipositing virgin females probing fewer hosts than mated ones (see above equation). If the former was responsible then mortality (Table 4) should have been higher for offspring of virgin females than for those of mated ones. However, mortality was lower for unmated females by almost the same proportion as successful parasitism was lower. This suggests that the difference in parasitism was due to a difference in activity between mated and unmated females, and not a difference in mortality of immature males and females. This interpretation would have been aided by an understanding of the daily activity patterns and associated changes in the ovaries of mated and unmated *S. endius* females.

Oviposition sequence

Host size and host clumping did not affect the overall sex ratio of the progeny of a group of *S. endius* individuals. Neither, it seems, did differential mortality. The mean sex ratio of offspring of nine groups of twenty randomly-chosen wasps was 83.5% females. The range was fairly narrow (79–87%), so it is possible that these ratios are a consequence of male and

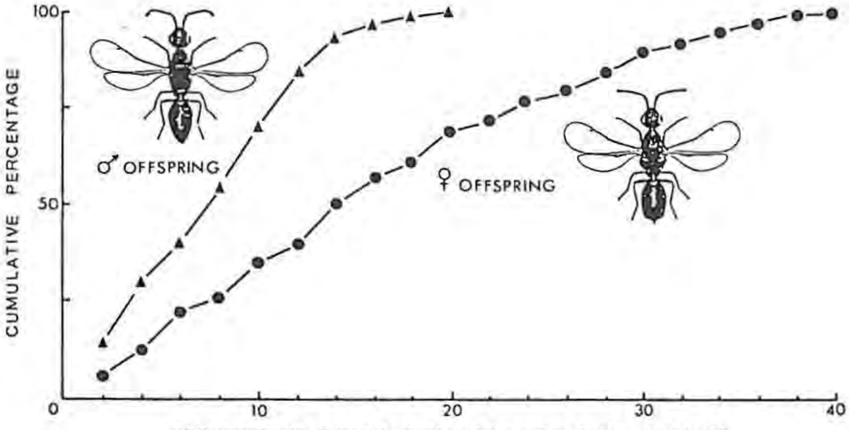

FIG. 1. Cumulative percentages of males and females that emerged from housefly puparia parasitized by *Spalangia endius*. The combined results of 120 females each ovipositing in a sequence of forty housefly puparia are included.

female eggs being deposited in a particular sequence by each female (as found for other parasitoids by Waage (1982a, b)). Our analysis showed that the sex ratios of all offspring from individual females ranged from 66% females to 100% females ($\bar{x}$ number of offspring per female (± 1 SE) = 12.4 (± 0.34); $n = 180$). The variation in progeny sex ratio was not significantly different from the binomial distribution ($P > 0.05$; $n = 180$), despite twenty-eight of the females producing entirely female broods. No female produced more than four male offspring, and seventy-three of 180 females analysed produced two males. All males that survived to the adult stage were deposited before the twentieth puparium encountered by females (Fig. 1). So it appears that *Spalangia* females may lay, in a definite sequence, a fixed proportion of males to females, but this needs further examination.

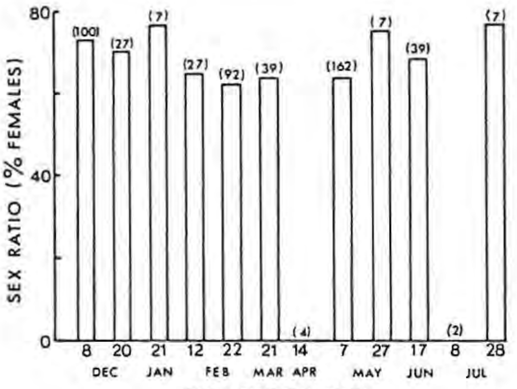

FIG. 2. The sex ratios (% females) of *Spalangia endius* collected from December 1980 to July 1981. (Sampling was continued for a further 6 months but wasp numbers during this period were too low to be included.) Figures in parentheses represent total numbers of *S. endius* collected per sampling occasion.

Field sex ratios

In the field, populations of *S. endius* sampled at different times of the year had sex ratios of 61–75% females (Fig. 2). Compared to sex ratios obtained in the laboratory, these were (a) 8.5–22.5% lower, and (b) there was considerably more variation in the sex ratio between sampling occasions than between the progeny sex ratio of groups of females in the laboratory. There are a number of possible causes that could explain these observations.

(i) Females in the field may lay eggs before insemination and therefore produce relatively more male eggs. If females remained unmated this would contribute substantially to fluctuating sex ratios (see Hamilton, 1967).

(ii) The presence of conspecific females nearby may influence females to lay more male eggs (e.g. see Wylie, 1966; Werren, 1983).

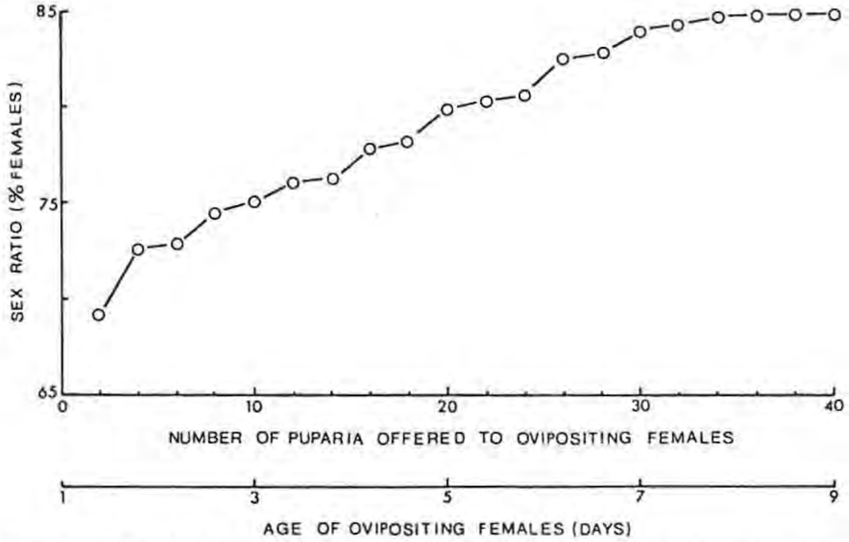

FIG. 3. The effect of female longevity, and therefore number of hosts attacked, on the sex ratio (% females) of *Spalangia endius*. All offspring of 120 females are included, and puparia were presented singly or in twos.

(iii) Adult lifespan may be longer in the laboratory than in the field and because males seem to be oviposited early in the oviposition sequence (Fig. 1), extended longevity would increase the proportion of females (Fig. 3).

(iv) Variation in temperature and humidity may also partly account for the fluctuations (Legner, 1977).

Conclusions

The sex ratios of *S.endius* in nine experimental groups each comprising twenty females were always close to a mean value of 83.5% (range 79–87%). This constancy occurred despite the variation in the male–female ratio in broods of individual females (66–100% females), but this is very likely just a consequence of a large sample in each group and all broods containing more than 66% females. Therefore the sex ratio produced by individual females should be further examined to determine whether they definitely lay male and female eggs in a given, genetically-determined, ratio. Both Fisher's (1930) and Hamilton's (1967) theories are based on the sex ratio trait being genetic, but, as far as we know, this has not been determined for hymenopterous parasitoids. Investigation of the genetic basis for sex ratios in parasitic Hymenoptera is particularly necessary in those species where 'alternate sex ratio strategies' are practised by individuals (e.g. *Nasonia vitripennis*; Werren, 1980) and in those where males are more common than females (e.g. *Campoletis perdistinctus* (Viereck); Hoelscher & Vinson, 1971). If the sex ratio trait is genetic, two further aspects require investigation: (i) the ease with which the sex ratio trait can be altered by natural selection, and (ii) the incidence and the nature of pleiotropic effects associated with the sex ratio trait.

The sex ratios of *S.endius* in laboratory experiments and field samples (see also Legner, 1976, 1977) cast doubt on the ability of current theory to predict the proportion of males in populations of solitary parasitoids. We suggest two approaches to test this conclusion. The possible effect of other habitual host species on *S.endius* sex ratios should be determined in the field and laboratory, perhaps under a variety of experimental and environmental conditions. Secondly, other solitary parasitoids that consistently have female-biased sex ratios (e.g. *Hungariella peregrina* Compere; Wysoki, 1977) should be investigated more thoroughly.

Acknowledgments

We are especially grateful to P. E. Hulley who kindly placed his field samples of *S.endius* at our disposal. We thank P. E. Hulley and J. K. Waage for discussing our ideas. M. M. Clark, J. H. Hoffmann, P. E. Hulley, V. C. Moran, S. Neser and H. G. Robertson kindly improved on the manuscript, and we thank G. J. Walter for typing it. We appreciate the finance made available by the C.S.I.R. and the Council of Rhodes University.

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Ed.

Speciation, adaptation and interspecific competition

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Summary. The species concept and current ideas on speciation are relevant to the debate on the significance of competition theory in community ecology. The speciation event and the subsequent post-speciation history of a species must be distinguished when applying competition theory. For post-speciation events we draw the important distinction between local and species-wide adaptations attributed to the action of competition. These ideas have the following consequences for community ecology. (1) Competition may have a limited evolutionary role at speciation. It is one of the possible factors that influence adaptation in the small isolated populations that are the major, or only, source of new species. This would occur where competition is sustained because of a consistent limiting resource such as space for sessile animals. (2) Once a new species comes under stabilising selection and expands, competition can produce only local and relatively minor adaptations. The acquisition of species-wide characteristics because of competition requires an improbable set of conditions. (3) The concept of an "organised" community, particularly if it carries evolutionary connotations, is unrealistic.

Introduction

The evolutionary importance of interspecific competition in natural populations of animals is at present in dispute. Wiens (1977), Connell (1980), Taylor (1980), Lawton and Strong (1981), Simberloff (1982) and others have criticised aspects of competition theory, while a recent defence is given by Schoener (1982). We draw attention to some neglected evolutionary points, in particular the distinction between local and species-wide characteristics and the vital role of the species concept and speciation. We acknowledge our indebtedness to H. E. H. Paterson from whose ideas stem many of the consequences that we deal with here.

A species could acquire its characteristics at two different stages of its history, either during the speciation event or through subsequent post-speciation adaptive changes. We begin by considering the nature of species and speciation, then we consider the possible effects of competition at speciation and during the post-speciation history of the species. The implications for community ecology are discussed.

Species and speciation

The species has been defined by Mayr (1970:12) as "groups of interbreeding natural populations that are reproductively isolated from other such groups". This and similar definitions relate to the biological concept of species in which species are defined in relation to one another. The emphasis is on reproductive isolation. An alternative species concept is the Recognition Concept proposed by Paterson (1978, 1980, 1981, 1982), which emphasises that the achievement of intraspecific fertilization is of far greater evolutionary consequence than the prevention of interspecific matings.

According to Paterson (1982) "... the essential features of the Recognition Concept can be stated as follows: The members of a species share a common specific-mate recognition system. The raison d'être of an SMRS is to ensure effective syngamy within a population of organisms occupying their preferred habitat. The characters of the SMRS are adapted to function efficiently in this preferred habitat. Hence, it follows that a new species will have arisen when all members of a small, isolated, subpopulation of a parental species have acquired a new SMRS, which facilitates the achievement of syngamy under the new conditions, and which, quite fortuitously, makes effective signalling impossible between members of the daughter and parental populations". Speciation is thus an incidental effect (sensu Williams 1966:8) of general adaptive changes in small isolated populations. Species-specific characteristics are laid down during a relatively short period of adaptation to the prevailing conditions. The newly-established SMRS will be subject to strong stabilising selection (Paterson 1982, Henderson and Lambert

1982, and see Angus 1983). Speciation is at an end once a new SMRS has been acquired, and adaptive characters return from directional selection to stabilizing selection. We do not consider sympatric speciation since, despite many recent theories (summarised by White 1978), these remain hypothetical models.

The effect of competition at speciation

Interspecific competition at the time of speciation could influence the acquisition of species-wide characteristics, since the entire population would be affected. At this time a small isolated population in a state of genetic flux, due to strong directional selection, could be affected by competitors, predators and other aspects of the biotic as well as the physical environment. Because interspecific competition is normally intermittent (Wiens 1977) its impact is likely to be weak compared with the effect of predation and other, more constant factors, such as the nature of the vegetation and the physical environment. Where a resource, such as space for sessile animals (Branch 1981), is limiting the effect of competition would be strengthened. This is probably the only way in which species-wide characteristics could be acquired under the influence of competition. The interference behaviour described in the limpet *Patella longicosta* by Branch (1981) could have originated in this way.

Post-speciation adaptation: local versus species-wide phenomena

One way in which communities might be structured by interspecific competition is through modification of the member species to lessen competition (resource partitioning, e.g. Schoener 1982). We discuss this evolutionary explanation for community structure here. Local genetically-based changes could be the result of competition-induced resource partitioning, although such character displacement has not been convincingly demonstrated (Grant 1972, 1975, and see Arthur 1982, Levinton 1982). However, many of the adaptations of species attributed to competition are species-wide (e.g. Heinrich 1979), or at least not demonstrated to be local features (e.g. Heatwole and Davis 1965). Such adaptations give no selective advantage in areas where members of a species are not sympatric with their competitor and there is no reason for the adaptation to spread to all populations of the species. In addition, Wright (1982) has emphasised that pleiotropic effects are commonplace and probably universal. Some of the pleiotropic effects accompanying a genetic change would be deleterious. They might be outweighed by the selective advantage of these changes in the presence of the particular competitor, but would prevent the spread of the alleles concerned in the competitor's absence. Even in the presence of a competitor, pleiotropy could hinder the establishment of all but strongly advantageous adaptations. There is therefore no reason to expect adaptation as axiomatic each time a potential advantage is perceived by an observer (Williams 1966).

Local genetically-based change due to competition is unlikely for two reasons: (1) pleiotropic effects and (2) the restricted occurrence in space and time of resource limitation and therefore of strong competition (Wiens 1977). The latter view has been criticised by Schoener (1982) on the grounds that there is experimental evidence from field studies for sustained and intense competition, or of sufficiently frequent periods of resource limitation for effective selection. However, even Schoener's chosen example (Brown et al. 1979) does not, on critical examination, convincingly demonstrate sustained or intense competition.

Populations would not necessarily adapt to successive periods of intense competition in a cumulative fashion. The individuals which produce the most offspring will be those that cope best under all the different conditions, disadvantageous or advantageous, which prevailed prior to and during reproduction. If an animal, due to its short generation time should show an increment of adaptation during an episode of intense competition, this could equally be lost between episodes, on the reestablishment of stabilising selection.

Implications for community ecology

Speciation, according to the Recognition Concept, is an event of relatively short duration which is complete once a new SMRS is established. In those isolates in which adaptation to local conditions has been successful and adaptive characters have returned to stabilising selection, the population will subsequently expand through areas of suitable habitat. Any further major species-wide changes become improbable. This focusses attention on the factors operating at the time of speciation i.e. the local conditions under which speciation occurred, and the nature of the variability in the population, which depends upon the evolutionary history of the species. Thus, although speciation may be a random event, the products of speciation are themselves not random. Therefore the explanation of differences between groups of closely-related species in communities lies not in the post-speciation workings of character displacement or resource partitioning (e.g. see Pianka 1978:189) but in the nature of speciation. Species descended from the same stock may often differ only in minor ways due to differences in the habitat in which speciation occurred, or possibly to stochastic events such as the founder effect. The differences between unrelated species are adequately accounted for by differences in their origins, without invoking changes induced by competition (Andrewartha and Birch 1954:462).

These views on speciation, and an appreciation of the different evolutionary implications in local and species-wide characteristics, have the following consequences for community ecology. Speciation and adaptation do not mould species to fit them into any larger "entity" such as a community. In those cases where an animal species has an obligate association with another animal or plant (e.g. hosts and parasites, specific pollinators), the former very likely speciated in close association with its host or prey. The latter associations are perhaps the closest we come to seeing "organised" communities in ecology. More loosely-knit communities are aggregations of species that share the same habitat preferences.

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Counters to the claims of Walter et al. on the evolutionary significance of competition

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Walter et al. (1984) advocate Paterson's "recognition concept" mechanism of speciation and argue that this mechanism implies little importance for interspecific competition as a selective agent. Without commenting at all on the appropriateness of the speciation mechanism, I would like to correct what I see as misconceptions about interspecific competition. I will make six points, somewhat repetitious of those I have made elsewhere.

1) Walter et al. write that "interspecific competition is normally intermittent ..." and therefore "its impact is likely to be weak compared with the effect of predation and other, more constant factors such as the nature of the vegetation and the physical environment". They

write farther on that I have criticized Wiens's view "on the grounds that there is experimental evidence from field studies for sustained and intense competition ...". As I reviewed (Schoener 1982, 1983), some experimental field studies demonstrate intermittent competition and about as many demonstrate continuous competition; the majority cannot be evaluated (see also Connell 1983). Hence, at present, we do not have enough information to be certain on average just how intermittent competition is. Certainly we do not have evidence that competition is more intermittent than predation or physical factors, and indeed, this would largely in principle seem to be untrue. Intermittency of competition is ascribed mainly to intermittency of climatic factors, a subclass of physical factors (Wiens 1977, Schoener 1982). And if competitors are predators (sensu lato), then intermittent intense competition at one trophic level implies intermittent intense predation at another (the logic of Hairston et al. 1960). Finally, physical catastrophes, such as hurricanes and volcanic eruptions, are highly episodic. Moreover, intermittency need not imply evolutionary impotence; I have tried to argue (Schoener 1982) that seasonal or annual patterns in ecological overlap suggest that intermittent competition is a major selective force. My criticism of Wiens's views is not based solely or even largely on field-experimental results, but rather on these observations of overlap and on logical considerations.

2) Walter et al. write "even Schoener's chosen example (Brown et al. 1979) does not, on critical examination, convincingly demonstrate sustained or intense competiton". They do not say why not, nor how they performed the "critical examination". However, my statement that there is "experimental evidence from field studies for sustained or intense competition" is not based on a single study, as detailed above. Moreover, there are no experimental demonstrations of highly intermittent competition, i.e., one year in 10 or less, though of course no continuously monitored studies have lasted that long, so we do not know anything about the really long term, one way or the other.

3) Walter et al. write "such character displacement [based on competition-induced resource partitioning] has not been convincingly demonstrated". Four references are given. I would like to give cases which, in my opinion, convincingly demonstrate ecologically based character displacement (in the broad, static sense of Brown and Wilson [1956], which includes Grant's [1972] "character release").

Huey and Pianka (1974) showed that two species of burrowing lizards (*Typhlosaurus*) have different body sizes and food sizes in sympatry, whereas that species also occurring in allopatry has body and food sizes intermediate between the two species in sympatry.

Fenchel (1975) showed that two species of particle-ingesting gastropods (*Hydrobia*) differ in shell size in sympatry but not in allopatry; numerous localities were studied. These body-size differences are associated with particle-size differences. Levinton (1982), whom Walter et al. cite, has questioned the ecological interpretation of this displacement but not the fact of the displacement; his failure to show in the laboratory for a North American species of *Hydrobia* that growth rate varies between two particle-size treatments may indicate that particle size is not the basis for the displacement or that his laboratory did not duplicate field conditions.

Ficken et al. (1968) showed that a species of warbler (*Dendroica*) has a longer, more attenuated bill in the presence but not absence of another; this bill enables prey to be probed from loblolly pine cones. This situation has been argued by Grant (1972) as possibly being a secondary result of clinal variation having nothing to do with competition (e.g., James 1970), but his argument here is much less convincing than for the Asian nuthatch (*Sitta*) case, for which he was unable to find a consistent ecological consequence of bill-size differences (Grant 1975). Parallel clinal variation in general can be an alternative explanation for displacement data in some cases, though it is conceivable that competitively driven selection can, with migration, result in clinal variation. In one case of displacement, that between two *Anolis* lizards, one species falls in the middle of the range of the other, so parallel clinal variation is impossible (Fig. 1). In another set of cases the tables are turned: ecoclimatic rules in certain mammals have been re-interpreted in terms of ecological character displacement (McNab 1971). In a masterful multivariate study, Dunham et al. (1979) pitted geographical variables against characteristics of co-occurring species as predictors of character change in catostomid fishes. The latter were found much more important than the former.

In an extensive, statistically very powerful survey of grebes (Podicipedidae), at least one species displaces in bill morphology in each situation of geographical overlap examined (Fjeldså 1983). Such morphological differences are shown correlated with reduced food overlap in several cases.

A number of cases of displacement are known from Galápagos finches (*Geospiza*), and their ecological significance understood, although quite a few cases of

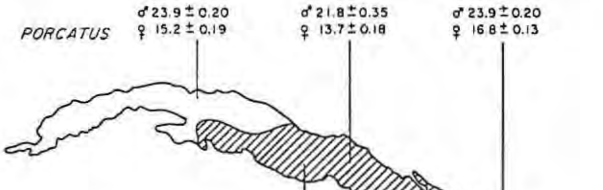

Fig. 1. Character displacement in *Anolis porcatus* of Cuba. Where overlapping the larger *A. allisoni* in central Cuba, *porcatus* decreases in size. Numbers are head lengths with standard error. (From Schoener 1977. With permission from Gans, C. and Tinkle, D. W. (eds.), Biology of the Reptilia. Copyright: Academic Press Inc. (London) Ltd.).

sympatry in these species have apparently not resulted in displacement (Grant and Schluter 1984, Schluter et al. unpubl.).

Numerous cases of character change, some involving displacement, are known in *Anolis* lizards, a genus for which, virtually without exception, where investigated interspecific differences in morphology are accompanied by differences in structural habitat and/or prey size (Schoener 1977). Statistically significant structural habitat shift in the presence of similar species is sometimes accompanied by striking changes in color and pattern (Schoener 1975). Cases of divergence and convergence in size (Schoener 1970) are consistent with a model (Schoener 1969) of optimal size based on feeding considerations. Net convergences are also possible using co-evolutionary models of resource competition (Slatkin 1980, Roughgarden et al. 1983).

In summary, cases of character displacement are more common than Walter et al. imply. Nonetheless, only a few studies have systematically searched for such cases among all possible opportunities for a given group (e.g., Schoener 1970, Dunham et al. 1979, Fjeldså 1983, Grant and Schluter 1984), or have carefully evaluated alternative explanations such as geographical variables (e.g., Dunham et al. 1979) or variability in resource availability (e.g., Schoener 1975, Schluter et al. unpubl.). More of each kind of study are badly needed.

4) Walter et al. write that "many of the adaptations of species attributed to competition are species-wide" and go on to argue that "such adaptations give no selective advantage in areas where members of a species are not sympatric with their competitor ...". I am unaware that anyone has argued that adaptations that reduce competition with a specific other species should be widespread where that other species does not occur, except, as suggested above, where migration is strong. Furthermore, in a large fraction of cases reported under (3), one species' range is contained entirely within another's, so that species-wide adaptations can be consistent with competitive adjustment to another species.

5) Walter et al. write "even in the presence of a competitor, pleiotropy could hinder the establishment of all but strongly advantageous adaptations". But this argument applies to any kind of adaptation, not just those that reduce competition. Moreover, pleiotropy can facilitate adaptive change by providing a genetic basis for a functionally advantageous correlation between traits (e.g., Cheverud 1982).

6) Walter et al. write "if an animal ... should show an increment of adaptation during an episode of intense competition, this could equally be lost between episodes, on the reestablishment of stabilising selection". I do not see how stabilizing selection during resource-rich periods can counter periods of directional selection, on average. For that you need periods of directional selection in the opposite direction. And what would cause such directional selection? Not *intraspecific* competition, because by assumption during non-competitive times resources are superabundant. In other words, intensity of inter- and intraspecific competition will be temporally correlated.

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