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SEX RATIOS OF COCCOPHAGUS ATRATUS COMPERE
(HYMENOPTERA: APHELINIDAE) IN RELATION
TO HOST AVAILABILITY.

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requirements for the degree of

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by

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FRONTISPIECE



A Coccophagus atratus female examining a scale insect host prior to depositing a female egg into it. The actual size of the wasp is indicated by the black spot in the lower left hand corner of the photograph. (Photograph from a painting by the author).

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RÉSUMÉ

The mode of sex determination in most Hymenoptera is that of arrhenotokous parthenogenesis, i.e. females originate from fertilised eggs and males develop parthenogenetically from unfertilised ones. Females can therefore determine the sex of each of their offspring by controlling fertilisation. This has led to much speculation about the factors that govern hymenopteran sex ratios.

Many sex ratio theories predict that solitary Hymenoptera should produce male and female offspring in a ratio of 1:1. The limited data available on sex ratios in solitary Hymenoptera does not support this prediction. However, for various reasons, data may not be representative and few detailed studies have been done. The purpose of this study was, therefore, to examine sex ratios of Coccophagus atratus, a solitary hymenopteran parasitoid, to determine whether sex ratios in this species approach 1:1. C. atratus is a suitable species for sex ratio studies because male and female eggs are deposited in different hosts. The sex of deposited eggs can, therefore, be determined at oviposition. This eliminates any possible effects of differential mortality.

Laboratory experiments were designed to determine whether C. atratus females deposit male and female eggs in fixed proportions or in a set sequence. In addition, samples of hosts were collected from the field to assess C. atratus sex ratios under natural conditions.

Results show that C. atratus females do not produce sex ratios that approach 1:1 and male and female eggs are not deposited in a set sequence. Sex ratios are variable and appear to be related to the relative abundance of hosts suitable for female development and for male development. These results are discussed in relation to current sex ratio theories.

CHAPTER 1

INTRODUCTION

The mode of sex determination in most Hymenoptera is that of arrhenotokous parthenogenesis (Crozier, 1975), i.e. females originate from fertilised eggs and males develop parthenogenetically from unfertilised ones. A mated female can therefore determine the sex of each of her offspring by controlling fertilisation. This unusual ability has led to much speculation about the factors that govern sex ratios in Hymenoptera (see Charnov, 1982).

Most theories formulated to explain sex ratios in general (Fisher, 1930; Shaw & Mohler, 1953; Bodmer & Edwards, 1960; Kolman, 1960; Verner, 1965; Charnov, 1982) consider sex ratios to be adaptive, i.e. that the proportion of males to females within a brood of offspring influences the genetic contribution of parents to the next generation. Although these theories of adaptive sex ratio usually apply to systems where diploidy and genetic control of sex ratios are assumed, it is generally believed that these theories also apply to arrhenotokous organisms (Hamilton, 1967; Hartl & Brown, 1970; Charnov, 1982). Most of the theories of adaptive sex ratios conclude that selection should favour equal investment in male and female offspring. Therefore, where the sexes are equally expensive to produce, a 1:1 sex ratio should be selected. This conclusion was first reached by Fisher (1930) and is based on the premise that deviation from a 1:1 ratio should lead to a mating advantage for the rarer sex and consequently selection for those parents that produce the rarer sex.

With few exceptions (e.g. Verner, 1965; Maynard Smith, 1978; Taylor & Sauer, 1980), theories of adaptive sex ratios examine only the total investment in sons and daughters by all parents in the population. The brood sex ratios of individual parents are not considered. A 1:1 ratio could therefore be obtained from many different combinations of brood sex ratios produced by individual females. For example, all parents could produce equal numbers of male and female offspring or, in contrast, half the parents could produce only males and the other half only

females. However, for an equilibrium ratio of 1:1 to be maintained in a population where individual females produce different brood sex ratios, a stable frequency of females that produce different sex ratios would have to exist in the population. In other words there must be a balance between the number of females that produce female-biased sex ratios and those that produce male-biased ratios. Such a population structure is unlikely to occur in nature (Charnov, 1982, p.31). Also, if such an equilibrium was disturbed, as it could be in any finite population (Verner, 1965; Green, 1980), those parents producing sex ratios biased in favour of the more abundant sex after perturbation would be at a disadvantage. Verner (1965) argued that the decrease in fitness suffered by females producing sex ratios biased in favour of the more abundant sex would be greater than the corresponding increase in fitness gained by parents producing the rarer sex. Thus, recurring perturbations that caused random excesses of either sex would result in a net decrease in fitness for parents that produced either male- or female-biased sex ratios. Theoretically, females that produce brood sex ratios of 1:1 are least likely to be detrimentally affected by changes in the population sex ratio. Verner (1965) therefore proposed that where the population equilibrium sex ratio is 1:1, individual parents should produce brood sex ratios of 1:1. Similar conclusions have been reached by Maynard Smith (1978) and Taylor & Sauer (1980), but with different arguments.

Some Hymenoptera do produce sex ratios close to unity, e.g. Aphidius platensis Brèthes (Aphidiidae) (Hofsvang & Hågvar, 1975a) and certain Apanteles species (Braconidae) (Ford, 1943). However, despite the fact that males and females are probably equally expensive to produce, the sex ratios of many Hymenoptera do not seem to satisfy the prediction of a 1:1 sex ratio. The sex ratios of many species are female-biased (e.g. Hofsvang & Hågvar, 1975b; Wysoki, 1977; Werren, 1980; Green et. al., 1982), some are male-biased (e.g. Askew, 1975; Cloutier et. al., 1981) and some are variable (e.g. Brunson, 1934, 1937, 1938).

Hamilton (1967) proposed that female-biased sex ratios should evolve where local mate competition and sib-mating are regular features of mating behaviour. Under these conditions females should produce only sufficient males to fertilise all their female offspring. This hypothesis

is supported by data for gregarious parasitic Hymenoptera (Green et. al., 1982; Werren, 1983) and some solitary egg parasitoids (Waage, 1982) where clusters of host eggs are likely to be parasitised by a single parasitoid female. However, for most solitary parasitoids, random mating probably occurs and parent females would therefore be expected to deposit equal numbers of male and female eggs. Even if mating was not random, but occurred between the offspring of more than one female, a 1:1 sex ratio would be expected unless group selection is implicated (Colwell, 1981; Taylor, 1981; Wilson & Colwell, 1981).

Charnov (1979) presented a model to explain variable sex ratios in certain Hymenoptera. This model is restricted to those Hymenoptera where the sex of eggs deposited is related to the size of the host; females usually being deposited in large hosts, males in small ones (e.g. Brunson, 1937; van den Assem, 1971; Charnov et. al., 1981). Charnov (1979) proposed that in these species the sex ratio should fluctuate according to the abundance of large and small hosts. Wasps without size-related sex determination would therefore still be expected to produce a 1:1 sex ratio.

Even with the exclusion of gregarious Hymenoptera and those with size-related sex determination, the sex ratios of many Hymenoptera do not approximate 1:1. However, sex ratios quoted in the literature have often been obtained from only one or a few field samples and may therefore not be representative, especially since hymenopteran sex ratios are affected by environmental factors such as temperature (Kfir & Luck, 1979; Kochetova, 1978), humidity (Legner, 1977) and photoperiod (Hoelscher & Vinson, 1971). Also, these field sex ratios reveal nothing about the brood sex ratios produced by individual females. Where brood sex ratios have been examined, there has been a tendency to concentrate on gregarious species (e.g. Walker, 1967; Werren, 1980; Green et.al., 1982). Consequently there is little detailed information about brood sex ratios in solitary Hymenoptera and it is difficult to relate observed sex ratios to theoretical predictions. Studies on brood sex ratios of Spalangia endius Walker (Pteromalidae) (Donaldson & Walter, 1984: Appendix), a solitary parasitoid of fly puparia, showed that in this species sex ratios did not satisfy the predictions of a 1:1 sex ratio. However,

Donaldson & Walter (1984) found that because the sex of offspring could be determined only at the adult stage, high pre-imaginal mortality hampered interpretation of the results. For instance, any patterns in the deposition of male and female eggs, such as those found in gregarious parasitoids producing precise brood sex ratios (Mertins, 1980; Waage & Ming, 1984) were possibly obscured by the high mortality. Therefore, to determine whether solitary parasitoids produce male and female offspring in a given proportion and a given sequence, a species of wasp should be used where the sex of an egg can be determined at oviposition. An observer could then determine both the sex ratio and any temporal pattern in the deposition of male and female eggs without waiting for adult parasitoids to emerge.

Heteronomous hyperparasitoids, which belong exclusively to the family Aphelinidae (Walter, 1983a&b), seem ideal for such a study. The females develop as primary parasitoids of coccoid scale insects and Aleyrodidae and males develop as hyperparasitoids of their own and other hymenopteran species (Flanders, 1936a, 1952a, 1959; reviewed in Walter, 1983a). Because the hosts for the sexes are so different in these heteronomous hyperparasitoids, the sex of an egg can be determined at oviposition.

Coccophagus atratus Compere (Hymenoptera: Aphelinidae) is a heteronomous hyperparasitoid found commonly in the southern regions of South Africa (Anneck & Insley, 1974; Clark, 1984). Specimens, collected near Grahamstown ($33^{\circ} 23' S$; $26^{\circ} 29' E$), were used to assess the suitability of this species for sex ratio studies. These studies on the biology are recorded in Chapter 2. This background information was then used to develop methods for culturing the parasitoid and its scale insect host and to develop certain experimental techniques (Chapter 3). The daily ovipositional activity of female C. atratus was then studied to ascertain the most suitable time for observations and to assess the effect of egg availability on ovipositional behaviour (Chapter 4). Knowledge of ovipositional activity was also needed to distinguish females that had ceased ovipositing due to a lack of mature eggs from those that had ceased ovipositing due to a change in their requirements for hosts. The brood sex ratios deposited by individual females throughout their entire lifespan was then examined to see whether it conformed to the

expectations of a 1:1 sex ratio (Chapter 5). The results of field sampling, designed to examine the population sex ratio, are presented in Chapter 6. Finally, in Chapter 7, the adaptive significance of sex ratios in C. atratus is discussed and sex ratios in this species are compared with those found in other Hymenoptera. Also, data collected on C. atratus has implications for theories relating to the evolution of heteronomous hyperparasitism, and this is discussed in Chapter 7.

CHAPTER 2

BIOLOGY OF C. ATRATUS AND ITS SCALE INSECT HOSTBiology of C. atratus

C. atratus is a minute parasitoid measuring approximately 1.5 mm in length. It has a black body and mustard-coloured face (see frontispiece). Nothing has yet been published about the biology of C. atratus and preliminary studies were therefore necessary so that culture techniques and experimental methods could be developed. These studies focussed on five aspects of the wasp's biology. 1) The range of host species habitually parasitised by C. atratus was assessed so that the most common habitual host could be used for both laboratory and field studies. 2) Oviposition was observed to determine the stage of host insect preferred for deposition of both male and female eggs so that hosts suitable for parasitoid development could be cultured. Studies on oviposition were also necessary to differentiate behaviour associated with egg deposition from that associated with inconsequential probes. 3) Many experiments required mated females and inclusion of unmated females would have biased results. Mating behaviour was therefore observed to determine when parasitoids were most likely to mate, how often they mated, and what constituted a successful mating. 4) Adult females of many parasitoid species need to feed on host haemolymph to obtain nutrients for egg development (Bartlett, 1964), so adult feeding behaviour was observed to assess the requirements of C. atratus females. 5) Studies on developmental time were included to facilitate efficient culture of parasitoids and to aid in the interpretation of sex ratio data obtained in the field.

Habitual Host Species in the Field

Female C. atratus larvae are polyphagous endoparasitoids of coccoid scale insects. In the Grahamstown area they were always associated, in large numbers, with Filippia gemina De Lotto scale insects (Homoptera: Coccidae) on Chrysanthemoides monolifera (Linnaeus) Norlindh (Compositae) and Cliffortia strobilifera Mettenius (Rosaceae). They were also collected, but in low numbers, from waxy scale insects (Coccidae: Gascardia spp.) on Acacia karroo Hayne (Leguminosae) and Tecomaria

capensis (Thunberg) Spach (Bignoniaceae) and from black scale insects (Coccidae: Saissetia spp.) on Trichilia emetica Vahl (Meliaceae).

Male C. atratus are hyperparasitic ectoparasitoids of late larval, prepupal and occasionally pupal stages of their own species as well as other hymenopteran parasitoids of scale insects. Males were collected most commonly from F. gemina scale insects parasitised by conspecific females and by Metaphycus sp. (Hymenoptera: Encyrtidae). They were also collected, in low numbers, as hyperparasitoids of unidentified parasitoids that attacked Saissetia species on Trichilia emetica. Clark (1984) gives a more comprehensive list of hosts suitable for both male and female C. atratus.

Because of these host preferences, unparasitised F. gemina scale insects were used as hosts for C. atratus females during experiments and conspecific females were used as hosts for males. Metaphycus parasitoids were used as hosts for males during observations on oviposition behaviour.

Oviposition

Mated females, when presented with all developmental stages of F. gemina, preferred to lay female eggs in second-instar scale insects that ranged in size from 1.1×0.6 mm to 2.8×1.4 mm (mean ± 1S.E. = 1.7 ± 0.04 mm x 0.8 ± 0.02 mm, n = 130). The egg was inserted into either the scale insect's midgut or the adjacent haemolymph. Similar behaviour has been observed in Coccophagus semicircularis (Foerster) (Jarraya, 1975) and Coccophagus caridei (Brèthes) (Flanders, 1959), although the reason for placing eggs in the alimentary canal is unknown. In C. caridei it may have been accidental as only a few eggs laid in the alimentary canal gave rise to larvae, but this seems unlikely for C. atratus and C. semicircularis because eggs in these species were frequently deposited in the alimentary canal. Oviposition, from insertion to withdrawal of the ovipositor, lasted between 3 and 10 secs (mean ± 1S.E. = 5.9 ± 0.16 secs, n = 100). Such rapid egg deposition has also been observed in other aphelinids e.g. Coccophagus basalis Compere (Flanders et. al., 1961), Coccophagus capensis Compere and Lounsburyia trifasciata (Compere) (Cendaña, 1937). This short oviposition time made it extremely difficult to judge whether

an egg had been deposited or whether the probe had been inconsequential. Hosts that had been probed therefore had to be dissected to determine if an egg had been deposited. With practice, 76% of ovipositions could be predicted ($n=200$ ovipositions). The criterion used was that, when a parasitoid initially probed a host, its abdomen was positioned at approximately 60° to the horizontal. If oviposition followed, the abdomen usually flattened out to about 25° to the horizontal (see Fig.2.1). Oviposition was preceded by antennal tapping on the body of the scale insect especially in the region of the anal plates, a behaviour similar to that described for another heteronomous hyperparasitoid, Coccophagoides similis (Masi) (Zinna, 1962).

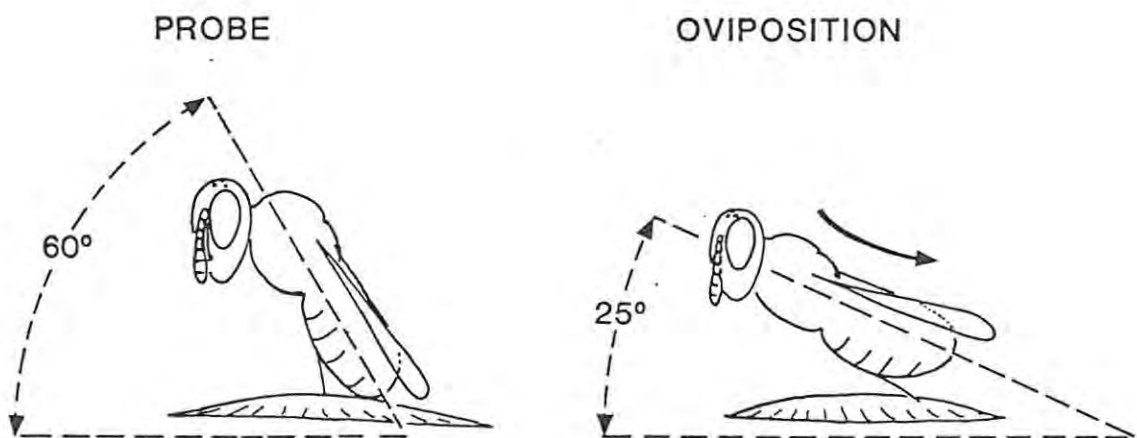


Fig.2.1. Posture of a Coccophagus atratus female while probing a host suitable for deposition of a female egg and the subsequent change in posture during oviposition. Legs have, for clarity, been omitted from the diagram

Deposition of male eggs was observed using both C. atratus and Metaphycus hosts. The latter were useful because the scale insect mummy was transparent and all details of egg deposition could be observed. In contrast, C. atratus mummies were black and heavily sclerotised. When depositing a male egg, a female mounted the scale insect mummy and drilled through the sclerotised shell. Once the mummy had been penetrated, the female probed with her ovipositor to locate the host, she then extruded a single egg and attached it to the host. There appeared to

be no preferred site of attachment. Oviposition time for male eggs ranged between 12 secs and 13 mins (mean \pm 1S.E. = 63 ± 11.3 secs, n = 100). The great variation was due mainly to differences in times for drilling through the integument of the scale insect mummy and probing to locate the parasitoid within the scale insect. Deposition of male eggs could be accurately predicted (98%, n = 100) because the change in the female's posture during oviposition was similar to that described for deposition of female eggs (Fig.2.1), but lasted for a longer time.

Feeding by Adult C. atratus

Adult females fed almost exclusively on honeydew. They were often seen stroking the anal plates of scale insects with their antennae, a behaviour that elicited the release of a drop of honeydew that was then consumed by the parasitoid. Females were also seen feeding on plant juices. Feeding on host haemolymph was observed only twice in over 300 hours observation of more than 60 wasps. In both instances, parasitoid females drank haemolymph that had seeped from a wound in the host caused by oviposition. Host-feeding, where hosts are specifically drilled to obtain haemolymph (Bartlett, 1964), was never observed.

Although host-feeding occurs commonly in the Aphelinidae (Bartlett, 1964), including the genus Coccophagus (see Cendaña, 1937; Walter, 1984), C. atratus is by no means exceptional in not doing so. Coccophagus gossypariae Gahan (Ceianu, 1968) and Coccophagus semicircularis (Cendaña, 1937; Jarraya, 1975) show behaviour similar to that of C. atratus in obtaining honeydew from their hosts and they too do not host-feed. Also, Coccophagus gurneyi Compere and Coccophagus capensis feed exclusively on droplets of honeydew found mainly on the plant surface (Cendaña, 1937). These species must therefore gain sufficient nutrients during the larval stage and from feeding on honeydew to produce their eggs.

Mating Behaviour

Mating was observed after a single male and female had been released into a glass vial. The female would almost inevitably remain still while the male would become very active and search in a zig-zag fashion, apparently attracted to the female by a pheromone. The attraction of males to pheromones emitted by conspecific females is a common feature of mating

behaviour in aphelinids (see Walter, 1984). Once he had located the female, the male approached her from the front. He then moved round the side of the female, whilst continually facing her body and keeping his antennae pointing forwards. The male then mounted the female from the rear. Copulation followed immediately and lasted only a few seconds (mean \pm 1S.E.= 2.8 ± 0.17 secs, n= 50). The male dismounted as soon as the aedeagus was withdrawn from the female. Similarly short times for mating have been observed in another aphelinid Encarsia pergandiella Howard (Viggiani, 1984). Although males often attempted to copulate a second time, females, once inseminated, would jump away from approaching males and were never seen to copulate more than once. Males, on the other hand, were polygynous and copulated successfully as many as 16 times over a period of days (mean \pm 1S.E.= 3.1 ± 0.7 copulations, n= 25); successful copulation was taken as the ability of the inseminated female to lay diploid eggs. Males appeared to need time to replenish sperm supplies as they would seldom copulate with more than three females consecutively. A break of 12 to 24 hours was then necessary before the males would again respond to females. This differs from Aphytis lingnanensis Compere (Aphelinidae) and some other Hymenoptera (reviewed in Gordh & DeBach, 1976) where the male's willingness to inseminate the female does not depend on the amount of sperm available. In the latter species males will copulate consecutively with many females, depositing diminishing amounts of sperm.

Developmental Duration

Studies on the developmental time of male and female wasps were carried out by allowing ten inseminated females to lay approximately ten female and five male eggs each. The parasitised hosts were then kept in a controlled environment (C.E.) room under specified conditions (light, 15 hours, $26 \pm 2^{\circ}\text{C}$, $75 \pm 5\%$ RH; dark, 9 hours, $18 \pm 2^{\circ}\text{C}$, $85 \pm 5\%$ RH) until adult parasitoids emerged. If parasitoids failed to emerge after sixty days the hosts were dissected to determine whether the parasitoids were dead or not. Figures for pre-imaginal mortality of male and female wasps were thus incidentally obtained.

The developmental duration for males and females (from oviposition to emergence) is presented in Fig.2.2. This bar diagram shows that male

developmental time is shorter and less variable than that of females, a phenomenon also observed in other heteronomous hyperparasitoids such as *Coccophagus caridei* (Flanders, 1939a), *Coccophagus gurneyi* (Compere & Smith, 1932) and *Coccophagoides utilis* Doutt (Broodryk & Doutt, 1966). Developmental time for females was extremely variable (25-43 days). Variability in female developmental time has been shown to depend on host size in *Pteroptrix smithi* (Compere) (Aphelinidae) (Gerling & Bar, 1971), whereas Broodryk & Doutt (1966) found that in *C. utilis* females, there were intrinsic differences that were independent of both host age and host size. The influence of host size on female development was not studied in *C. atratus*, but hosts offered to females were of similar size (range= 2.3x 1.1- 2.8x 1.4 mm), so any differences in developmental time were probably intrinsic.

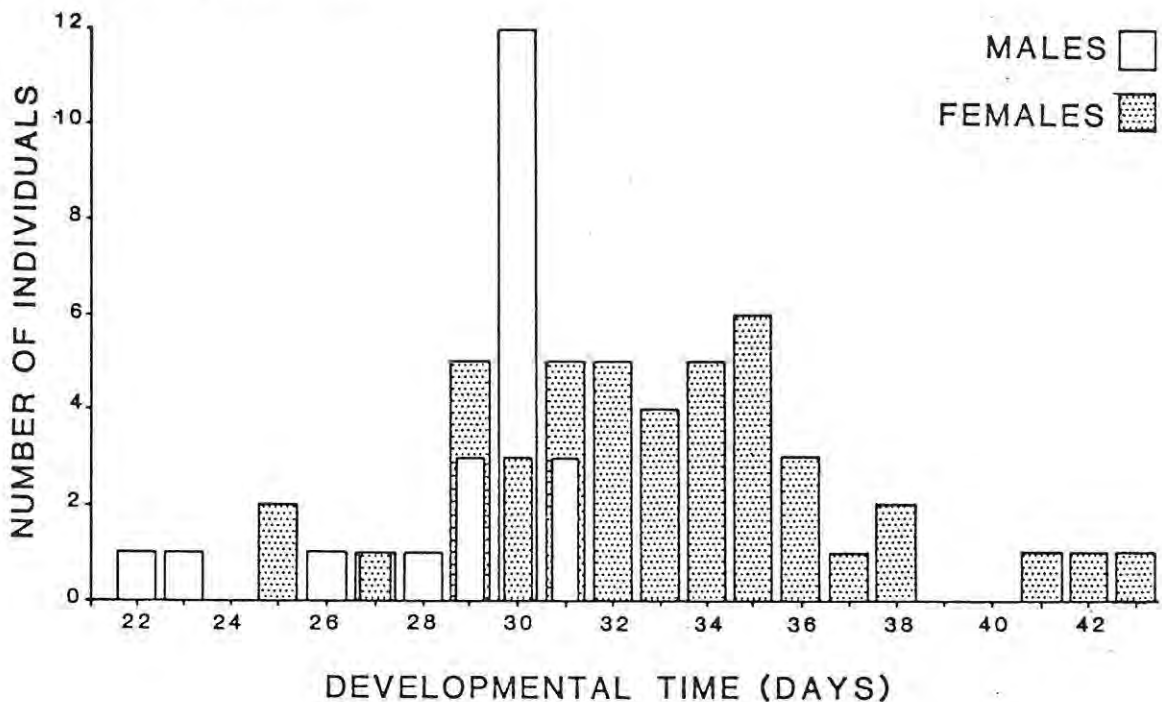


Fig.2.2. Duration of larval development in 22 male and 45 female *Coccophagus atratus*. Developmental time was measured as the period from the day an egg was deposited to the day an adult parasitoid emerged. Conditions were: day, 15 hours, 26 ± 2 °C, 65 ± 5 % RH; night, 9 hours, 18 ± 2 °C, 80 ± 5 % RH.

The observed pre-imaginal mortality was corrected to compensate for mistaken predictions of egg deposition (24% for females and 2% for males:

see above). The resultant mortality for males was 39% and for females, 34%. A χ^2 analysis on the raw data showed that there was no significant difference in the mortality of larvae and pupae between males and females ($P > 0.05$).

Biology of the Host Insect, *Filippia gemina*

F. gemina was described by De Lotto (1974) and specimens in this study were identified by M.M. Clark (confirmed by J.R. Williams at the British Museum of Natural History). No biological studies have been done on this species and no detailed observations were carried out during this study. However, those aspects of the scale insect's biology necessary for the development of culture methods and the interpretation of field work were studied and are recorded here.

The female scale insect almost certainly has three instars of which the third instar has a pregravid and an egg packet stage. The male has five easily-identifiable stages. Two nymphal instars are followed by a prepupal and then a pupal stage. The winged adult emerges from a transparent cover produced by the pupa. Male and female scale insects appear almost identical in the second instar, after which the male scale insect becomes humped prior to pupation. Females grow larger than males and retain a flat oval shape until they reach reproductive age when wax is secreted over the entire dorsal surface to form an egg packet, a characteristic of the genus *Filippia* (see Brain, 1924). Prior to this stage, the female usually migrates to a leaf that harbours no other scale insects. This may be a means of providing the crawlers with a suitably uninfested substrate, because crawlers tend to settle in large numbers around the egg packet after they hatch.

F. gemina has two discrete generations per year, one from January/February to June and a second from July/August to December (see Chapter 6).

The information on the biology of *C. atratus* and *F. gemina* was used to develop the culture techniques and experimental methods discussed in the following chapter.

CHAPTER 3

MATERIALS AND METHODS

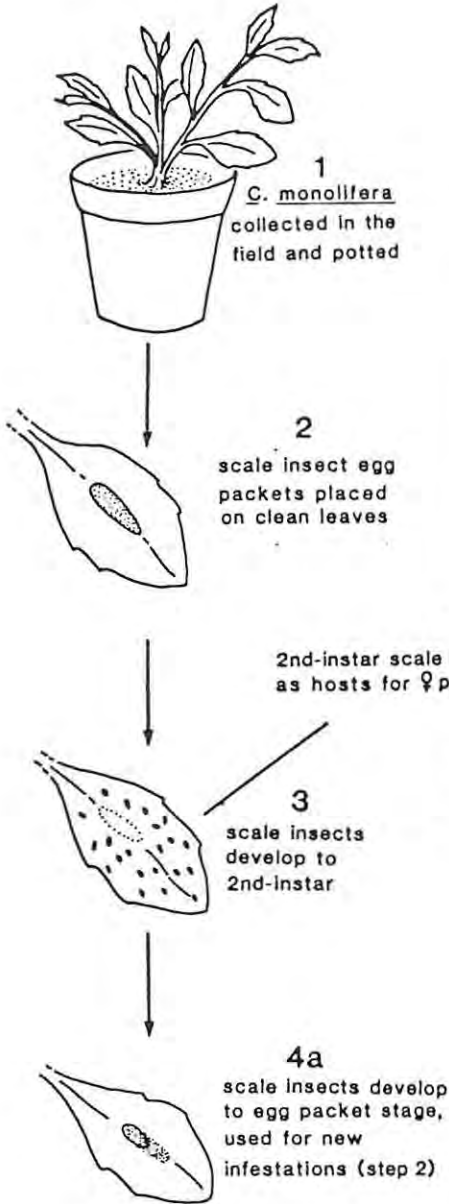
Techniques for the culture of scale insects and parasitoids are dealt with in this chapter. Also included is the design of an arena in which parasitoids were exposed to hosts whilst observational experiments were carried out. Procedures are described for the removal of the female reproductive system, so that mature eggs could be counted, and for the detection of sperm in the spermatheca. Precise details of experimental methods are given in the appropriate chapters.

Culture of Scale Insects and Parasitoids

An outline of the procedures for culturing both scale insects and parasitoids is presented in Fig.3.1. F. gemina scale insects were cultured by placing egg packets onto the clean leaves of C. monolifera plants (Fig.3.1, steps 1 & 2) that were held in a C.E. room under conditions best suited for the survival of scale insects. These conditions were found to be: light, 15 hours, 26 ± 2 °C, $65 \pm 5\%$ RH; dark, 9 hours, 18 ± 2 °C, $85 \pm 5\%$ RH). C. monolifera plants, although cumbersome and difficult to handle in the laboratory, were used because the scale insects failed to mature on more manageable plants such as potato sprouts and squashes, which are often used as substrates for the culture of scale insects (Finney & Fisher, 1964). Cultures were maintained (Fig.3.1, steps 3 & 4a) so that scale insects in all stages of development were present simultaneously.

The high humidity (60-90% RH) required for the scale insects encouraged the growth of fungus on the honeydew exuded by them. This resulted in death of scale insects and deterioration in the health of the plant. Excess honeydew was therefore removed by spraying the plants with water every two weeks. A subsequent application of Benlate fungicide proved effective in controlling the fungus without harming the scale insects.

CULTURE OF SCALE INSECTS



CULTURE OF PARASITOIDS

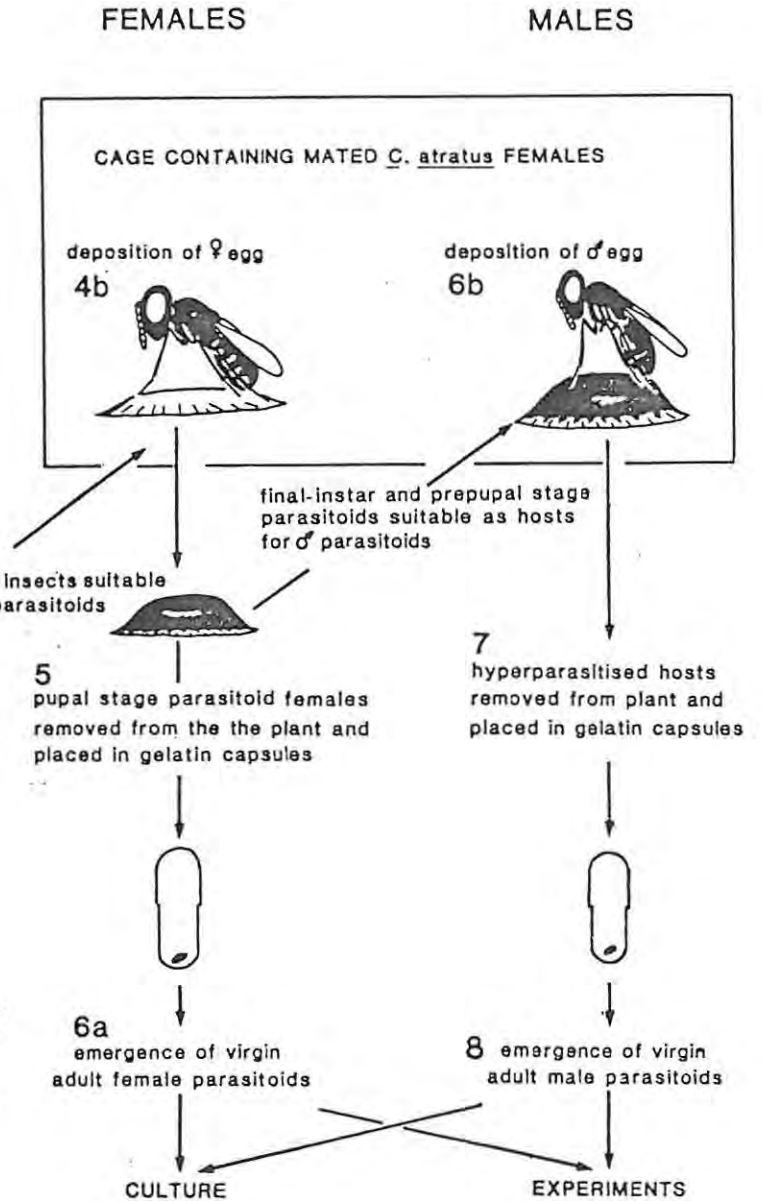


Fig.3.1. Diagrammatic representation of methods for culturing *Filippia gemina* scale insects and *Coccophagus atratus* parasitoids. Further details of culture methods are given in the text.

Female parasitoids, required for experiments, were obtained by removing plants infested with second-instar scale insects from the scale insect culture, and placing them, for two days, in a muslin-lined cage that contained mated C. atratus females (Fig.3.1, step 4b). This cage was kept in a second C.E. room that maintained the same conditions as the one housing the scale insect cultures. After removal of the scale insect-infested plants from the cage, parasitised scale insects were left on the plant until the parasitoid larvae had voided their meconia, characterised by the host scale insect turning black (Fig.3.1, step 5). They were then either removed from the host plant and placed individually in gelatin capsules to obtain virgin adult female parasitoids (Fig.3.1, step 6a), or maintained on the leaf as hosts for male parasitoids (Fig.3.1, step 6b).

Adult male parasitoids were obtained by returning blackened parasitised scale insects to the cage containing C. atratus females so that the parasitoid larvae could be hyperparasitised (Fig.3.1, step 6b). Hyperparasitised hosts were then removed from the plant and placed individually in gelatin capsules where they were left until adult males emerged (Fig.3.1, steps 7 & 8).

The parasitoid culture was periodically augmented by the introduction of C. atratus adults collected in the field.

Experimental Arenas

All experiments that required observation of ovipositing wasps were initially done by enclosing scale insect-infested leaves in a modified petri dish, whilst the leaves were still attached to the plant. This method proved unsatisfactory for two reasons. 1) It was difficult to manipulate leaves under the microscope. 2) Leaves were seldom the same size, so it was difficult to present females with uniform densities of hosts. This method was therefore discarded in favour of an alternative one in which leaf discs were used (see Walter, 1984). These discs were easily manoeuvred under the microscope and had a uniform size (diameter= 25 mm). Discs were obtained by removing scale insect-infested leaves from plants in culture, cutting the leaves into circular discs and sealing the cut edges with paraffin wax. Wax proved to be less damaging and less messy than other sealants such as petroleum jelly and a bitumen sealer

used for treating plant wounds. For observation a leaf disc was enclosed in a cylindrical glass arena of the same diameter as the disc, 25mm in height and sealed from above with a glass plate (Fig.3.2). The leaf disc and glass cylinder are referred to as an 'arena' in the remainder of the text.

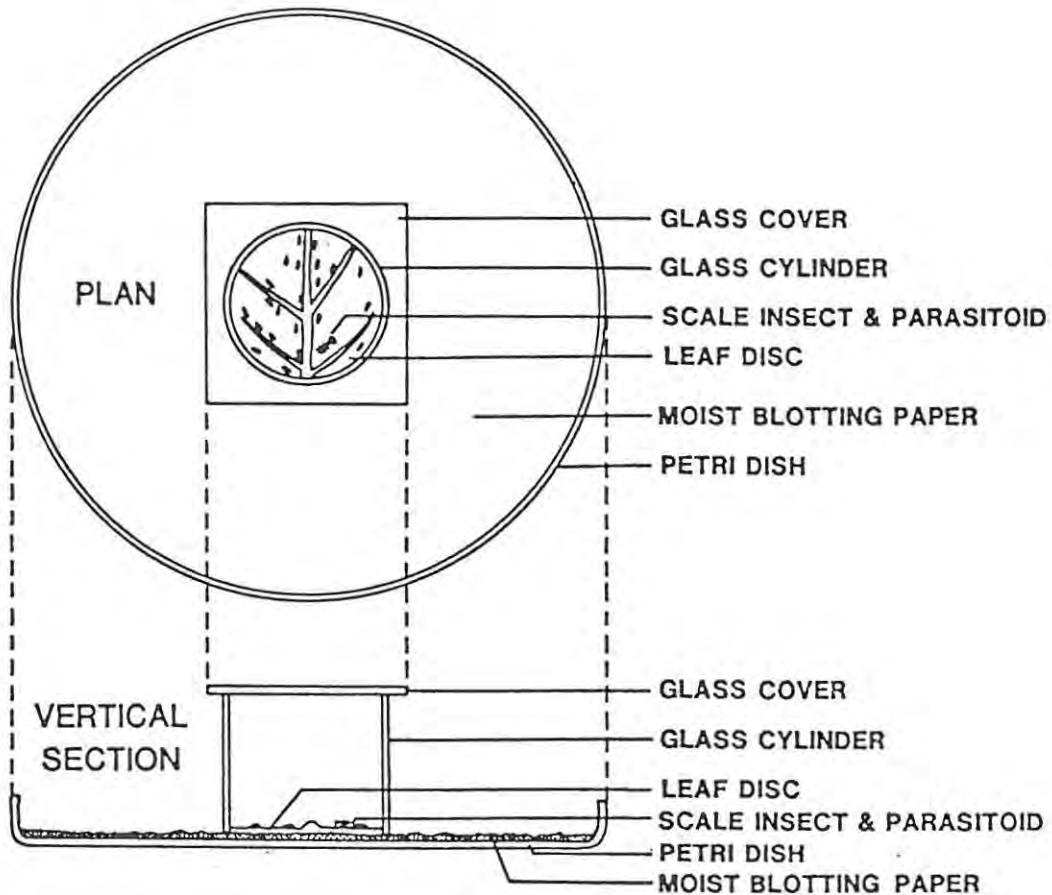


Fig.3.2. For observation, Coccophagus atratus females needed to be confined in an arena that was easy to manipulate under the microscope. The design of such an arena, taken from Walter (1984), is shown above.

During observations, the arena was kept on moist filter paper, but between observations leaf discs were separated from the rest of the arena and were floated in a specially-designed water bath (Fig.3.3), based on the design of Walter (1984).

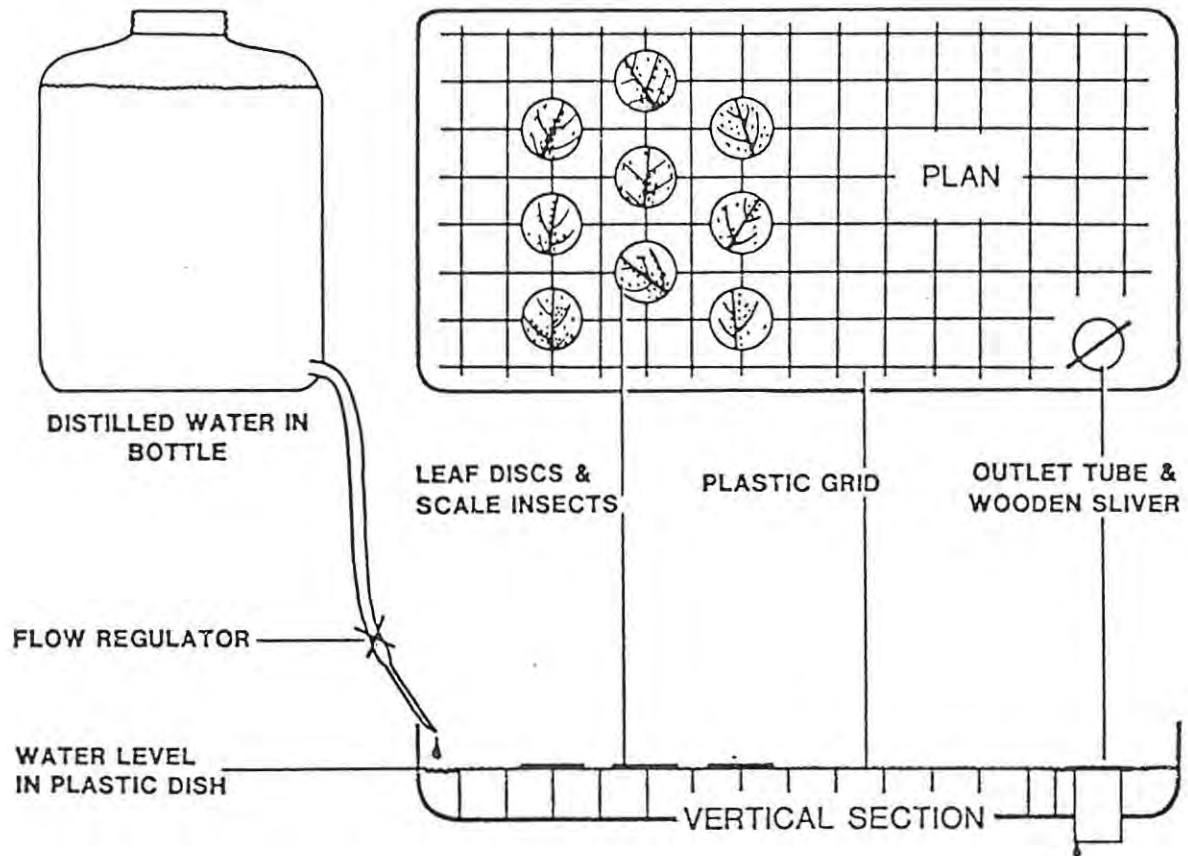


Fig.3.3. Design of a constant depth water bath in which leaf discs infested with scale insects could be floated. The plastic grid prevented leaf discs from sinking into the water. Water was maintained at a constant depth by a regulated inflow from the water bottle and an outlet tube to drain off excess water. Fertiliser was added to the distilled water in the inlet bottle to provide nutrients for the leaf discs. (Design and drawing from Walter, 1984).

Dissection of the Female Reproductive System

To extract the reproductive organs from the extremely small female wasps, the following procedure was used. 1) Wasps were submerged in 30% ethanol in an excavated block placed above a piece of glossy blue plastic. Twin fibre optic lights were then adjusted to give the greatest contrast when

looking through a dissecting microscope. Wasps killed by submersion in alcohol were used in preference to ones killed with ethyl acetate because the ovaries of the latter tended to disintegrate on contact with alcohol. 2) Using a microprobe with the point bent at 90° to the shaft, the wasp's abdomen was severed from the thorax. 3) A sharp probe was used to hold the posterior abdomen and a second probe was used to remove the anterior tergites, thus enlarging the gap created when the thorax was severed. 4) A bent probe was then used to ease the contents of the abdomen into the alcohol medium.

This method of dissection ensured that the whole reproductive system was removed intact. Also, the white eggs contrasted vividly with the blue background and were therefore easily counted (see Chapter 4).

Detection of Sperm in the Spermathecae of Females

To detect whether females had been inseminated or not, the presence of sperm in the female spermatheca was determined in the following way. The whole reproductive system was removed, as explained above, and placed in an excavated slide. It was then submerged in haematoxylin stain for 1-5 mins, depending on the age of the stain. Excess stain was then washed away with 70% ethanol and the reproductive system was transferred to a drop of Berlese mounting fluid on a microscope slide. The spermatheca was then separated from the remainder of the reproductive organs, the latter removed from the slide and the spermatheca covered with a coverslip. The spermatheca could then be observed under a compound microscope. If sperm was present, individual cells were usually detected with ease. If, however, no sperm was visible, the slide was examined under a phase contrast microscope to confirm the absence of sperm. Sperm cells were more easily detected under phase contrast microscopy (Fig.3.4). The spermatheca was considered empty only if no sperm appeared under phase contrast microscopy.

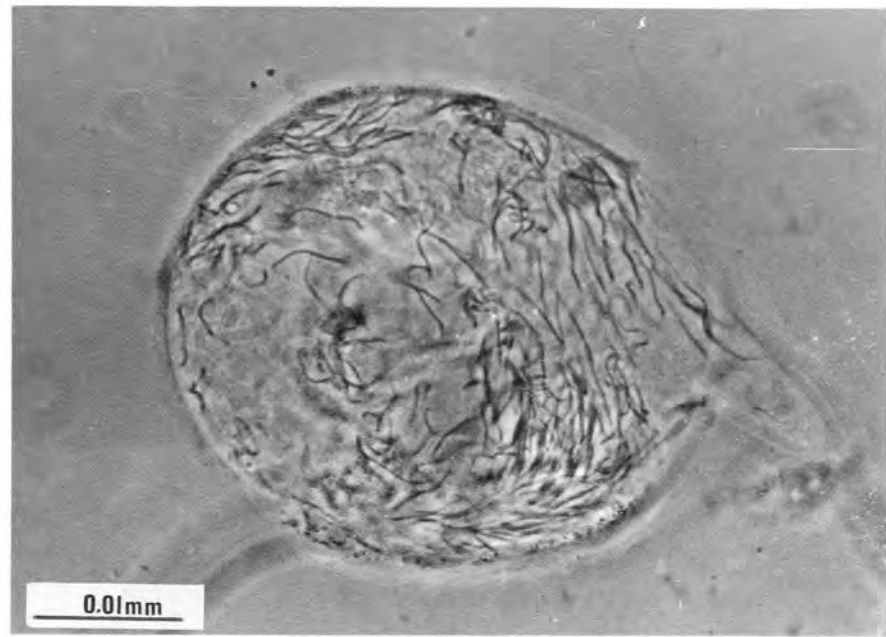


Fig.3.4. Spermatheca of a Coccophagus atratus female viewed under a phase contrast microscope to reveal the presence of sperm. (x 1850).

Only the general materials and methods have been mentioned in this section because precise details vary from experiment to experiment. The specific methods used in each experiment are described in the appropriate chapters.

CHAPTER 4

DAILY PATTERNS OF OVIPOSITIONAL ACTIVITY AND OVARIOLE DEVELOPMENT

The observational experiments anticipated for C. atratus made it necessary to study their daily patterns of ovipositional activity to determine at what time of day females were most active and for how long they remained so. The optimum duration and timing of observations could then be worked out. Also, a previous study on Spalangia endius (Donaldson & Walter, 1984) suggested that a knowledge of ovipositional behaviour coupled with information on ovary development would aid interpretation of brood sex ratios produced by individual wasps.

Daily Ovipositional Activity

To determine daily patterns of ovipositional activity, ten female wasps were allowed to mate immediately after emergence. They were then placed in an arena with a large number of hosts, approximately half of them suitable for development of female larvae and half suitable for development of males. The adult parasitoids were observed individually for six hours per day from emergence until they died (approximately 40 hours observation per wasp). Between observations the wasps were placed individually in vials that contained a drop of honey.

The number of eggs laid per half hour of the six hour observation period is presented in Fig.4.1. This figure shows that in each six hour bout, most of the eggs (83%) were deposited in the first half hour. During this time the parasitoid was very active, searching for hosts and frequently probing or ovipositing in them. Towards the end of the first half hour, the female's levels of activity began to decrease. This decrease in activity was associated with an increase in the amount of time spent drinking honeydew and preening. A negligible number of eggs were deposited in the remaining five and one half hours, when the wasps tended to remain stationary, either preening or sitting on the glass of the arena. Inactivity, after the initial burst of egg-laying, was attributed to the female having oviposited all her available eggs. This is dealt with in the subsequent section.

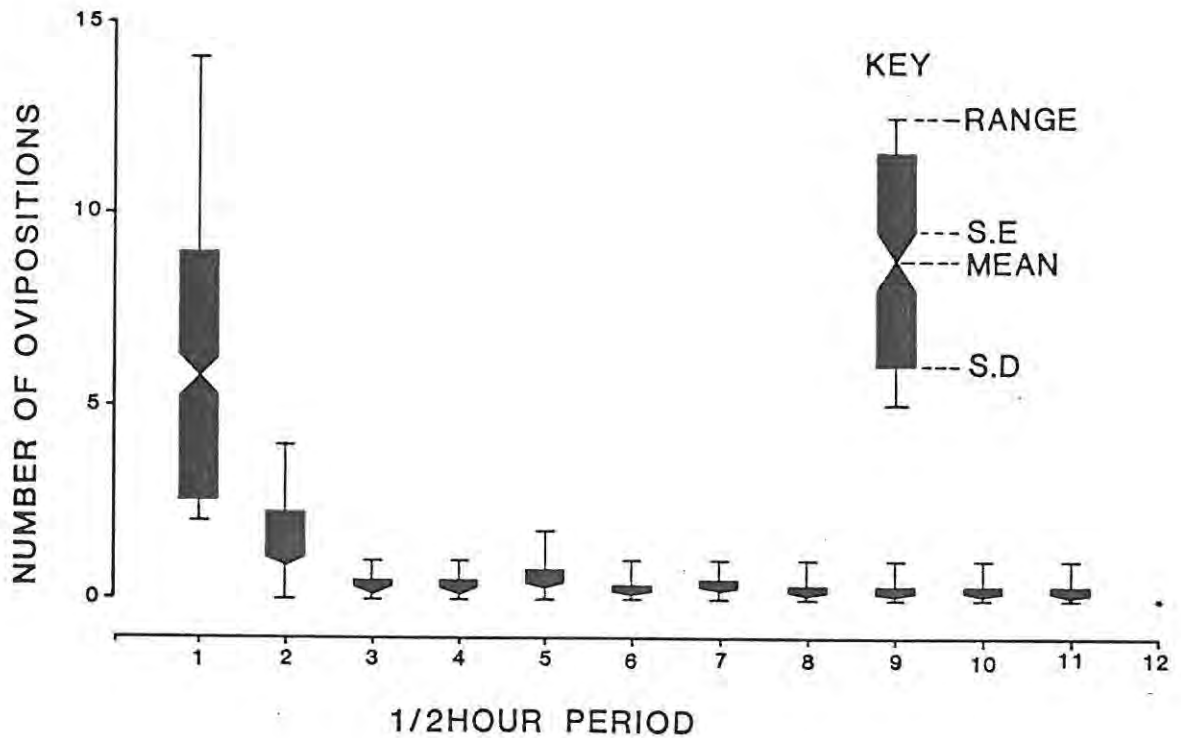


Fig.4.1. Number of eggs laid per half hour by mated *Coccophagus atratus* females exposed to hosts for six hours per day from the day of emergence until the females' death. Females were presented with hosts suitable for deposition of both male and female eggs. The results represent the mean, standard error, standard deviation and range for ten females, each exposed to hosts on at least four occasions (mean= six exposures per female).

The pattern of oviposition from day to day was characterised by distinct peaks of egg-laying activity on some days with intermittent troughs on other days when few eggs were laid (Fig.4.2). The incidence of the peaks and troughs was highly consistent between individual wasps (see ranges, Fig.4.2). This suggested that the number of eggs deposited on any particular day was not governed by the suitability of the hosts provided, but rather by some intrinsic factor such as the availability of mature eggs.

It was crucial to establish the reasons why females were reluctant to oviposit on certain days. Any uncertainty in this regard would have hampered interpretation of behavioural data obtained in later

experiments. Therefore, experiments were performed to determine the effect of egg availability on ovipositional activity.

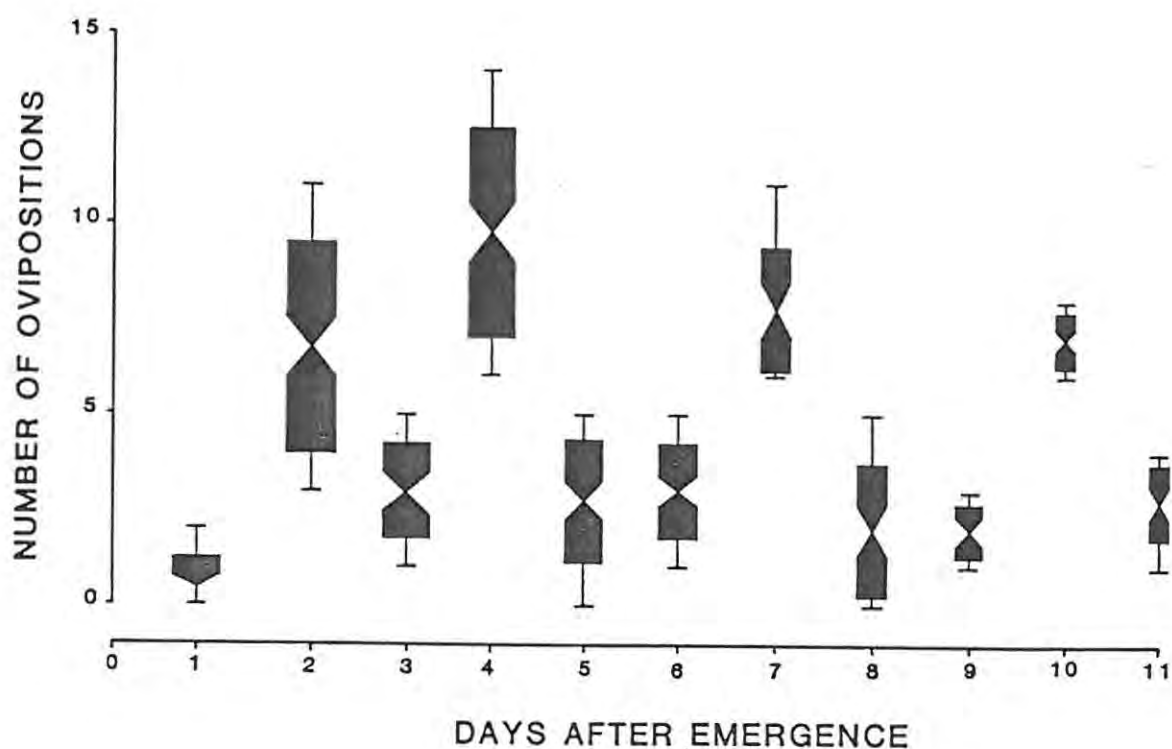


Fig.4.2. Number of eggs laid per day by mated Coccophagus atratus females exposed to hosts for six hours each day from the day they emerged until the day they died. Hosts suitable for both male and female offspring were provided. Initially ten females were used, but after the fourth day mortality progressively reduced the number of experimental females until only three were still alive on the eleventh day of the experiment. Data points represent the mean, standard error, standard deviation and range for all surviving females. For explanation of the kite formation see Fig.4.1. Width of the kite denotes the number of replicates.

Effects of Egg Availability on Patterns of Ovipositional Activity

C. atratus females were inactive on the day of emergence as well as immediately after a bout of oviposition and on days following a bout of oviposition (Figs 4.1 & 4.2). To determine whether these periods of low egg-laying activity were related to the availability of mature eggs in the ovaries, three questions had to be answered. 1) How soon after

emergence are mature eggs available for oviposition? 2) Do C. atratus females deposit all their available eggs in a single half hour bout of oviposition? 3) How rapidly are eggs replenished after oviposition? These three questions are dealt with separately.

Egg Production After Emergence

Egg production after emergence was determined in the following way. Seventy C. atratus females were allowed to mate as soon as they issued from their hosts and were then maintained on a diet of honey. They were then dissected in batches of ten at 1, 3, 6, 12, 24, 48 and 72 hour intervals after emergence. All mature eggs in the ovaries were counted. The ovaries of C. atratus (Fig.4.3) are similar to those of Encarsia tricolor (Foerster) (Arzone, 1976), Coccophagoides utilis (Zinna, 1962) and various other aphelinids (Copland, 1976). Each ovary comprises three ovarioles. The largest eggs accumulated in the proximal section of the ovarioles, at the junction with the lateral oviduct (Fig.4.3). These eggs were the same size as oviposited diploid eggs (mean \pm 1S.E.= 0.19 ± 0.018 mm, range= 0.17- 0.22 mm, n= 100) and were therefore considered to be mature. Similar conclusions were reached by Cendana (1937) and Copland (1976).

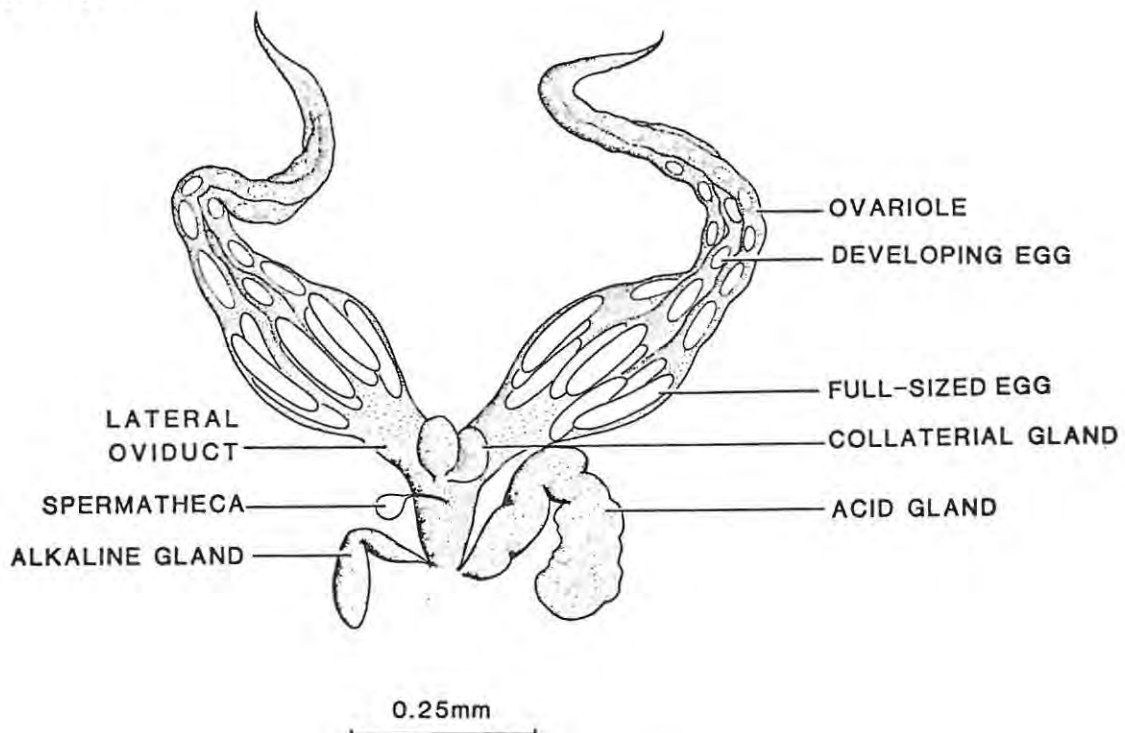


Fig.4.3. Structure of the female reproductive system of Coccophagus atratus showing the development of eggs in the six ovarioles and the accumulation of full-sized eggs in the proximal section of each ovariole.

The results obtained from the dissections discussed above show that C. atratus females emerged with few or no mature eggs in their ovaries (Fig.4.4). Eggs matured rapidly within the first 12 hours after emergence and the number of eggs produced reached a plateau after 24 hours (mean= 18 eggs) (Fig.4.4). Copland (1976) found that an Aphelinus species produced a maximum of four eggs per ovariole (total in ovary= 24 eggs) and stated that he had found comparable maximum numbers of eggs in other aphelinid species. Flanders (1943) also reported that the number of mature eggs reached a plateau in "many Hymenoptera" when females were withheld from hosts for a few days. Flanders (1943) proposed that egg production was inhibited when females did not encounter hosts. This may also be the case for C. atratus. Another proposal of Flanders' (1943), that the maximum number of eggs was maintained by resorption of mature eggs, seems improbable in C. atratus because no partially resorbed eggs were ever observed in any of the dissections. Egg resorption could possibly occur at a later stage. Lack of nutrients for further egg production also seems an unlikely cause of the halt in egg production because C. atratus females never host-fed and females in this experiment were supplied with honey as a substitute for honeydew.

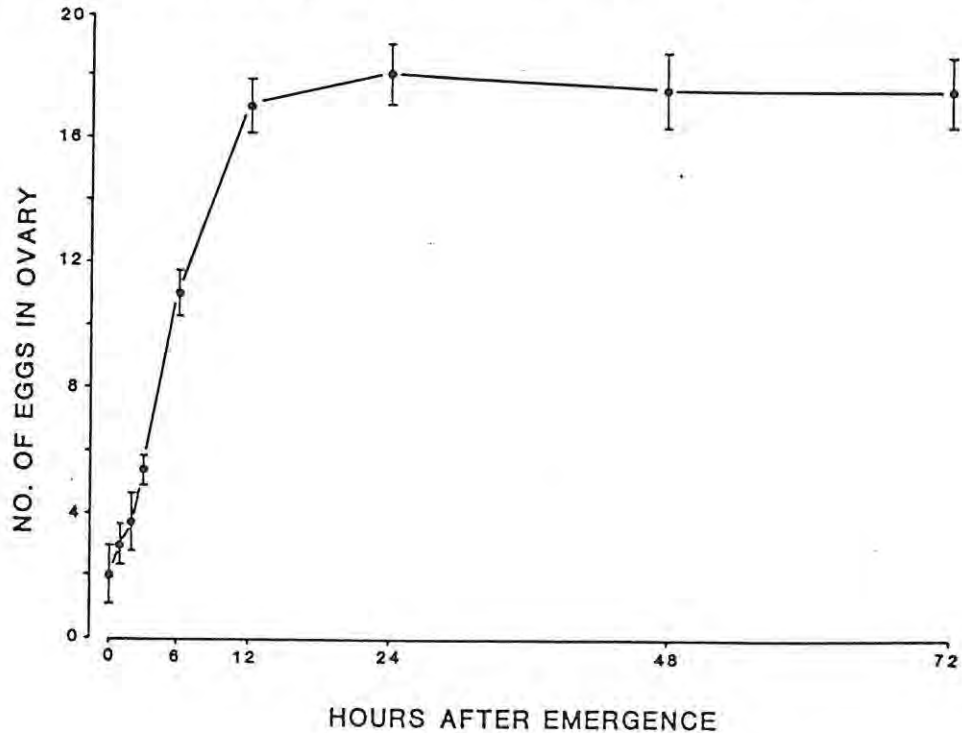


Fig.4.4. Number of full-sized eggs present in the ovaries of Coccophagus atratus females from 0-72 hours after emergence. Each point represents the mean and standard error for ten females. Prior to dissection, females were confined in glass vials and fed only honey.

Therefore, in summary, the ovipositional restraint of C. atratus females on the day of emergence (see Fig.4.2) coincided with a low number of mature eggs in the ovaries for the first few hours after emergence. Also, the peak in egg-laying activity on the second day coincided with a peak in the number of eggs available for deposition. Therefore, egg-laying activity in the first two days after emergence does seem to be related to the availability of mature eggs.

Depletion of Eggs During Bouts of Oviposition

To assess whether periods of inactivity that followed immediately after bouts of oviposition (Fig.4.1) were due to a lack of mature eggs, the following experiment was performed. Four groups of mated C. atratus females, each comprising of ten individuals were allowed to deposit either one, two, three or four batches of eggs. Females were then dissected immediately after their final bout of oviposition and the number of mature eggs present in their ovaries was counted.

The number of eggs (mean \pm 1S.E.) remaining in the ovaries of females after consecutive bouts of oviposition were: after one bout, 8 ± 1.8 ; after two bouts, 4 ± 0.9 ; after three bouts, 2.1 ± 0.9 and after four bouts, 3.5 ± 0.7 . Therefore, although the number of mature eggs was depleted after each bout of oviposition, eggs were never completely expended. Thus, either full-sized eggs were not necessarily mature, or periods of inactivity after bouts of oviposition were not due to a shortage of mature eggs. To test whether all full-sized eggs were mature, it was reasoned that since females from 12 to 72 hours after emergence had similar numbers of full-sized eggs in their ovaries (Fig.4.4), they should lay similar numbers of eggs. If however, eggs need time to mature, older females should lay more eggs than younger ones. Mated females 12, 24, 48 and 72 hours old were observed while ovipositing for half an hour. They were then dissected and the number of full-sized eggs remaining in their ovaries was counted.

Fig.4.5 shows that older females did lay a higher percentage of the full-sized eggs present in their ovaries. Egg size, therefore, does not seem to be a reliable measure of egg maturity. This result suggests that eggs remaining in the ovaries of females that had completed a bout of

oviposition, were not yet fully mature, even though they had reached their full size. All mature eggs were probably deposited. Therefore periods of inactivity immediately after oviposition were most probably due to a shortage of mature eggs.

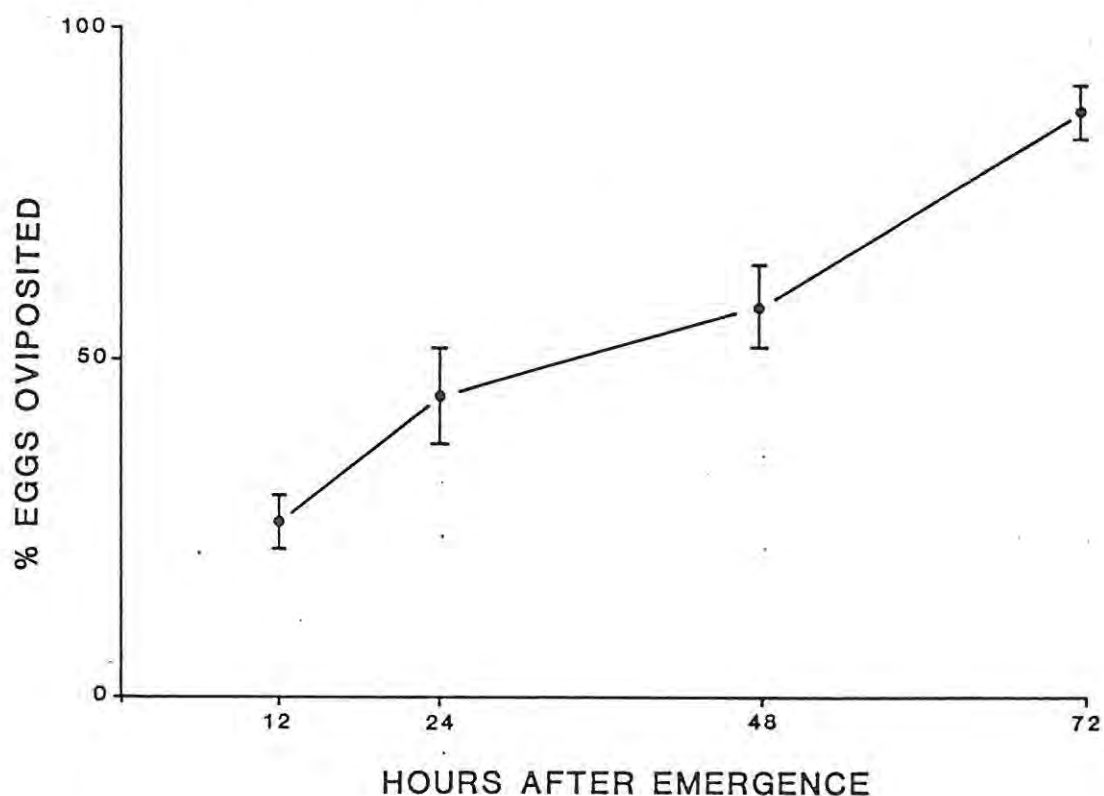


Fig.4.5. Percentage of full-sized eggs deposited during a half hour bout of oviposition by Coccophagus atratus females between 12 and 72 hours of age. Each female was allowed to deposit only one batch of eggs at either 12, 24, 48 or 72 hours after emergence. Following oviposition, the females were dissected and all the full-sized eggs remaining in the ovaries were counted. The percentage of eggs oviposited was then calculated as:

$$\frac{\text{no. of eggs oviposited}}{\text{no. of eggs oviposited} + \text{no. of eggs remaining in ovary}} \times 100.$$

Each point on the graph represents the mean and standard error for ten females. Prior to oviposition females were mated and fed on a diet of honey.

Full-sized eggs have been regarded as mature in other aphelinids such as Coccophagus semicircularis (Cendaña, 1937) and various other species

(Copland, 1976). It is possible that egg size is also an unreliable measure of egg maturity in these species, especially in C. semicircularis. In this species females refrain from ovipositing in the first 24 hours after emergence (Jarraya, 1975) and in some cases even longer (Cendaña, 1937), even though they have many full-sized eggs in their ovaries.

Replenishment of Eggs

C. atratus females were reluctant to oviposit on days following a bout of oviposition. Females that had deposited one batch of eggs were reluctant to oviposit again in the following 48 hours (Fig.4.2). Females that had deposited two, three, or four batches of eggs, were inactive for much longer periods of time, usually about 72 hours (Fig.4.2). To determine whether these periods of low ovipositional activity were related to the rate of egg replenishment, the following experiment was performed. Four groups of mated C. atratus females, each comprising approximately twenty wasps were allowed to deposit either one, two, three or four batches of eggs. Equal proportions of the surviving wasps in each group were then dissected at intervals of 24, 48 and 72 hours after their final bout of oviposition. The number of full-sized eggs in the ovaries was counted. This experiment complemented that reported in the previous section where wasps were dissected immediately after completing a bout of oviposition. The results were therefore combined and are presented graphically in Fig.4.6.

The results of the above experiment show that after each bout of oviposition, eggs were replenished. However the rate of egg replenishment varied. It was most rapid in those females that had deposited only one batch of eggs and slowed down after each consecutive bout of egg-laying (Fig.4.6). This decreased rate of egg replacement corresponded to increases in the period of inactivity between bouts of oviposition (Fig.4.2). The increased periods of inactivity were therefore probably caused by the decreased rate of egg replenishment.

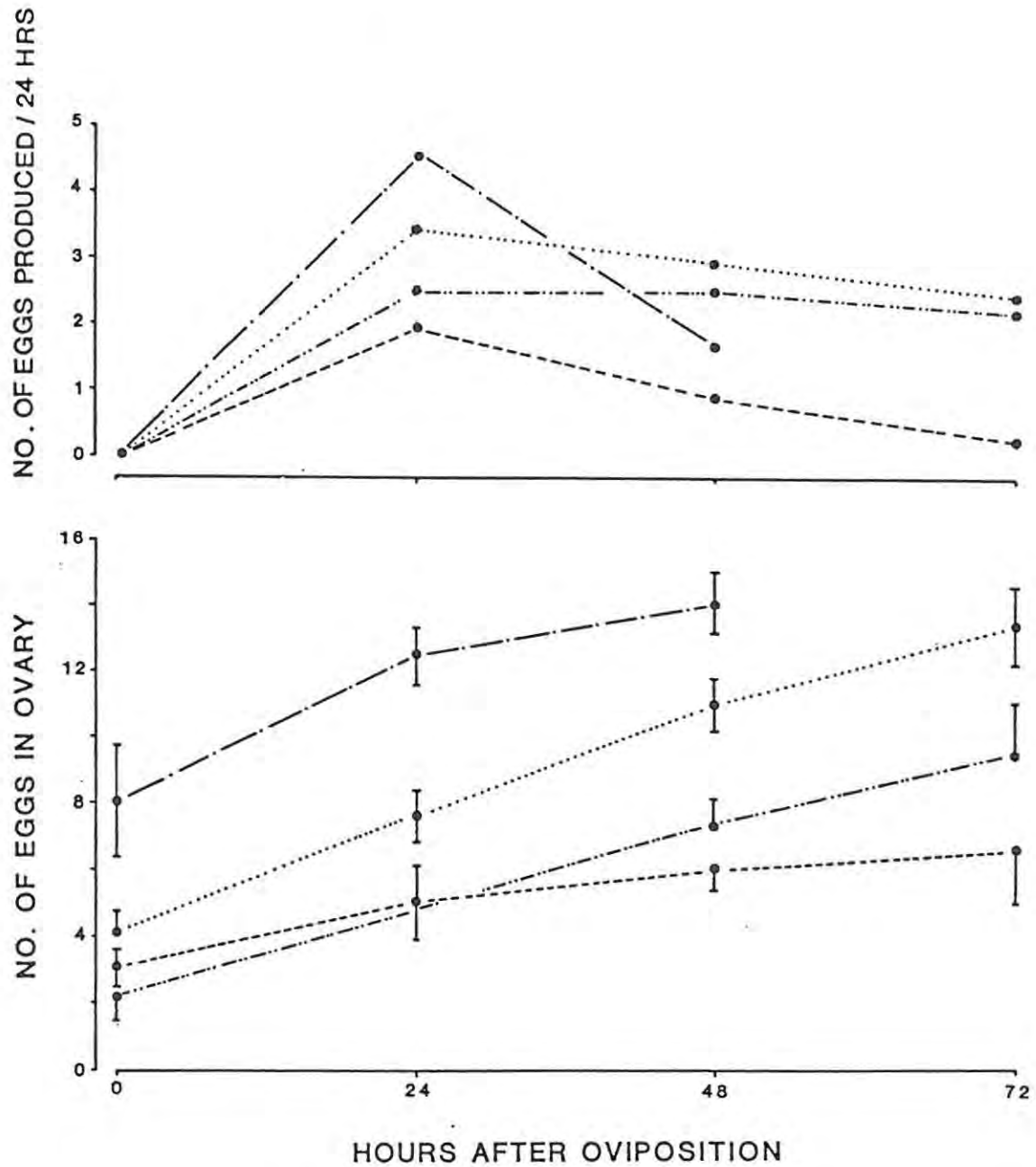


Fig.4.6. Rate of egg replenishment (above) and number of full-sized eggs present in the ovaries (below) of *Coccophagus atratus* females allowed to oviposit one (—), two (·····), three (-·-·-) or four (- - - -) batches of eggs. Data points represent the mean and standard error (lower graph only) for ten individuals where one batch of eggs was deposited, eight individuals where two batches were deposited and six and four individuals respectively where three and four batches of eggs were deposited (total= 96 wasps). Lower numbers of wasps in the latter groups were due to mortality of parasitoids during the experiment.

Discussion

C. atratus females deposited batches of eggs during recognizable bouts of activity. These bouts of oviposition were apparently related to the availability of mature eggs. Peaks in ovipositional activity have been reported in other Hymenoptera, e.g. Coccophagus gurneyi (Compere & Smith, 1932; Cendaña, 1937), Lounsburyia trifasciata (Cendaña, 1937), Coccophagus bartletti Annecke & Insley (Walter, 1984) and Aphidius matricariae Haliday (Giri et. al., 1982). However in these species bouts were neither as pronounced nor as consistent as in C. atratus.

The data presented in this chapter on ovipositional activity in C. atratus, formed the basis for experiments on brood sex ratios of C. atratus, dealt with in the following chapter. Because 83% of ovipositions took place in the first half hour of exposure to hosts, and because bouts of oviposition tended to occur on particular days, experiments were planned so that females were exposed to hosts for only half an hour on days of high ovipositional activity, i.e. the 2nd, 4th, 7th, 10th and 13th days after emergence and at three day intervals thereafter until the female died. It also became possible to determine whether females had ceased ovipositing due to a lack of mature eggs or had stopped for some other reason.

CHAPTER 5

BROOD SEX RATIOS OF INDIVIDUAL C. ATRATUS FEMALES

Sex ratio theory predicts that in solitary parasitic Hymenoptera, where male and female progeny are equally expensive to produce, a population equilibrium sex ratio of 1:1 should exist. Although theories differ in their expectations of the brood sex ratios that should be produced by individual females when the population is at equilibrium, the most acceptable prediction seems to be that of a 1:1 brood sex ratio. Therefore, assuming that male and female offspring are equally expensive to produce, C. atratus females would perhaps be expected to deposit equal numbers of male and female eggs.

Theories predicting 1:1 brood sex ratios do not always specify how such sex ratios are obtained. In most diploid organisms it may be a consequence of random (Mendelian) sex determination (Williams, 1979). However in arrhenotokous organisms, some other mechanism must operate. Hartl (1971) proposed that arrhenotokous females deposit a fixed proportion of unfertilised eggs. They may also perhaps deposit male and female eggs in a fixed sequence. Such sequences have been found in gregarious parasitoids that deposit consistently predictable sex ratios (Mertins, 1980; Waage & Ming, 1984). The possibility of fixed quotas of male and female eggs generates a number of predictions. 1) Virgin females would be expected to limit the number of eggs they deposit, since they can deposit only male eggs. 2) Mated females would be expected not to deposit broods composed either entirely of males or entirely of females. If hosts suitable for only one sex were available, females would be expected to deposit fewer eggs than when hosts suitable for both sexes were available. 3) Sex ratios would be expected not to vary with changes in the relative abundance of hosts suitable for either male or female offspring.

The design of an experiment to test whether these predictions hold for C. atratus is presented in Table 5.1. Within this experiment, three sets of data were collected. 1) The number of eggs deposited by females in each treatment was assessed to determine whether virgin females, and mated females provided with hosts suitable for one sex only, deposited fewer eggs than females provided with hosts suitable for both male and female

offspring. 2) The behaviour of females during exposure to hosts was analysed to determine whether stress on ovipositing females, caused by unnatural conditions of host abundance, affected the number of eggs deposited by them. 3) Sex ratios produced by females provided with hosts suitable for both sexes were analysed to see whether they conformed to the predictions of a 1:1 ratio and to determine whether sex ratios shifted with changes in the relative abundance of hosts suitable for either male or female offspring.

Table 5.1. Design of an experiment to test the response of virgin and mated Coccophagus atratus females to different conditions of host abundance. Each replicate comprised a single adult female which was exposed to hosts for half hour periods on the 2nd, 4th, 7th, 10th and 13th days after emergence and at three day intervals thereafter until the female died.

Condition of females	Hosts suitable for	Number of hosts	Number of replicates
virgin	male	40	10
mated	male	40	10
mated	female	40	10
mated	female:male	(25:25)	10
mated	female:male	(35:15)	15

Number of Eggs Deposited

An analysis of the number of eggs deposited by C. atratus females exposed to the treatments outlined in Table 5.1 is presented in Table 5.2. These results show that there were no significant differences ($p > 0.05$) between treatments in the number of eggs deposited. In all cases the number of eggs deposited on each day of exposure to hosts was similar to the number of eggs deposited by females on the corresponding day in the daily activity studies of the previous chapter. Females, therefore, regardless of whether they were virgin or mated, and irrespective of the type of hosts offered to them, were willing to deposit all their mature eggs in the hosts that were available.

Table 5.2. Number of eggs deposited by Coccophagus atratus females in each of the five treatments outlined in Table 5.1. Figures represent the mean numbers of eggs deposited by ten females in the first four treatments and by fifteen females in the last treatment. One standard error is given in parentheses. Females were exposed to hosts for half hour periods on the 2nd, 4th, 7th, 10th and 13th days after emergence and at three day intervals thereafter until the female died. Treatments varied in the condition of the parasitoid females (virgin/ mated) and the type of hosts offered (suitable for males/ females). Results (F values) of a one way ANOVA to compare egg numbers between treatments as well as the F95 value are also given. There were no significant differences ($P > 0.05$) between treatments.

Condition of female	Hosts suitable for	Days after emergence						Total number of eggs
		2	4	7	10	13	16	
virgin	♂s	9.13 (±1.6)	10.0 (±1.2)	7.7 (±0.2)	5.8 (±1.0)	4.2 (±1.7)	2.0 (±0.4)	32.7 (±2.8)
mated	♂s	5.7 (±0.7)	9.6 (±1.6)	6.7 (±0.9)	5.0 (±0.7)	3.0 (±1.7)	2.5 (±0.5)	23.4 (±2.1)
mated	♀	7.2 (±0.8)	10.2 (±1.0)	9.1 (±1.0)	6.0 (±0.9)	6.8 (±2.3)	4.0 (±1.0)	33.9 (±4.5)
mated	♀:♂ 25:25	8.7 (±1.6)	10.1 (±0.6)	9.5 (±1.3)	6.1 (±0.5)	7.2 (±1.6)	5.8 (±1.5)	38.5 (±6.5)
mated	♀:♂ 35:15	6.3 (±0.5)	7.2 (±0.6)	7.0 (±0.4)	4.8 (±0.5)	5.6 (±0.9)	3.9 (±0.2)	29.4 (±1.9)
F value		1.8	0.1	1.6	0.4	1.1	2.7	2.2
F95		2.53	2.61	2.61	2.69	2.69	2.87	2.53

The readiness of virgin females to deposit complete batches of unfertilised (male) eggs is contrary to the theoretical prediction that virgin females should limit the number of unfertilised eggs they deposit. However, even though females in the laboratory were prepared to oviposit prior to mating, this may not be a frequent occurrence in the field. Many reports state that aphelinids mate soon after emergence (Kuwana, 1934; Cendaña, 1937; Zinna, 1962), in which case virgin females would contribute little to the production of male progeny. Although mating soon after emergence by C. atratus was observed in the laboratory, all attempts to observe mating in the field failed: males were never seen. Therefore, C. atratus females were collected in the field, their spermathecae were dissected out and these were examined for the presence of sperm. Table 5.3 shows that only six out of forty-six females did not have sperm. Of these, three had fewer than five full-sized eggs in their ovaries. It is therefore possible that the latter females had recently emerged and would possibly have been inseminated before they began ovipositing. In any event, the low number of unmated females suggests that virgin C. atratus females do not contribute significantly to the deposition of male eggs in the field.

Table 5.3. Number of Coccophagus atratus females with and without sperm in their spermathecae. Females were collected on Chrysanthemoides monolifera plants from leaves infested with unparasitised Filippia gemina scale insects (hosts for female C. atratus), leaves containing parasitised scale insects (hosts for male C. atratus), and from leaves not infested with scale insects. Presence of sperm in the spermatheca was determined using the methods described in Chapter 2.

Location	With sperm	Without sperm
On hosts for males	18	3
On hosts for females	20	2
On uninfested leaves	2	1

The deposition by mated females of entire broods comprising only males or females is also contrary to the theoretical predictions. This suggests that C. atratus females do not deposit fixed quotas of male and female eggs. However, the lack of ovipositional restraint could have been due to the wasps being constrained in a small arena with hosts suitable for only one sex. In the following section, the behaviour of females during exposure to hosts was analysed to determine whether there were any significant differences in behaviour between treatments.

Behaviour of C. atratus Females

Even though similar numbers of eggs were deposited by C. atratus females under the conditions described in Table 5.1, females may have been reluctant to oviposit and may have done so only because of the unnatural conditions. Unnatural conditions are known to affect other aspects of parasitoid ovipositional behaviour, e.g. parasitoids that do not usually superparasitise hosts, will do so when confined on a limited number of hosts (see van Lenteren, 1981). It has been stressed (van Lenteren et. al., 1978; van Lenteren, 1981) that behavioural observations are necessary to distinguish abnormal behaviour from that normally associated with oviposition. Female C. atratus were therefore observed throughout their exposure to hosts and various aspects of their behaviour were recorded so that any reluctance to oviposit, or signs of stress, could be detected. Behaviour, recorded on a six function event recorder, was divided into six distinct activities, namely; oviposition, probing of hosts, searching for hosts, cleaning, drinking honeydew and sitting on the glass of the arena.

Fig. 5.1 illustrates the relative amount of time spent by C. atratus females in each of the six activities. The results of a Wilcoxon two-sample test (Sokal & Rohlf, 1981) for differences in time allocation between treatments are presented in Table 5.4.

Approximately 50% of the time spent on hosts was taken up with cleaning, drinking honeydew and sitting on the glass of the arena (Fig.5.1). Of these activities, only the time spent drinking honeydew was significantly different between treatments (Table 5.4), specifically between those treatments where hosts for females were present and those where only hosts for males were present. This is because hosts for males did not exude honeydew, and the only honeydew available was a crusty residue

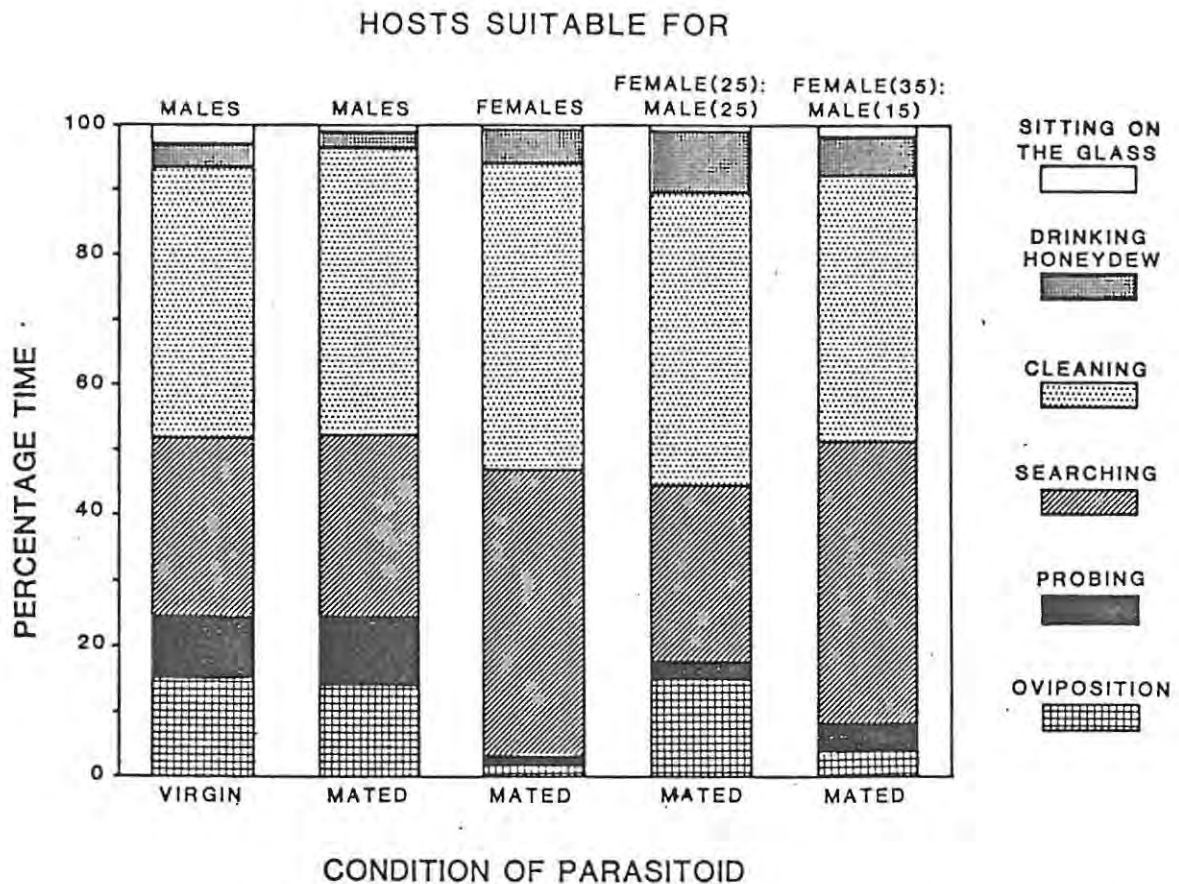


Fig.5.1. Percentage time spent ovipositing, probing, searching, cleaning, drinking honeydew and sitting on the glass of the experimental arena by *Coccophagus atratus* females. The wasps were exposed to hosts for half hour periods on the 2nd, 4th, 7th, 10th and 13th days after emergence and at three day intervals thereafter until the female died. There were five treatments which differed in the condition of the parasitoid female (virgin, mated) and the type of host offered (suitable for females/males). Females were observed throughout their exposure to hosts and their behaviour was recorded on a six function event recorder. The first four columns each represent the mean for ten females exposed to hosts on at least five occasions whereas the last column (right hand) represents the mean for fifteen replicates. Total number of hours observation= 163 hours.

Table 5.4. Results of a Wilcoxon two-sample test for differences between treatments in the amount of time spent ovipositing (O), probing (P), searching (S), cleaning (C), drinking honeydew (H) and sitting on the glass of the experimental arena (G), by Coccophagus atratus females during the experiment outlined in the legend to Fig.5.1. The table is divided into two parts. The U value obtained in the test is presented in the upper right hand section of the table above the diagonal. Differences in behaviour between treatments, designated with asterisks, are given in the lower left hand section of the table below the diagonal. ($P < 0.05 = *$; $P < 0.01 = **$).

treatment	virgin all ♂	mated all ♂			mated all ♀			mated 25 ♀ : 25 ♂			mated 35 ♀ : 15 ♂		
virgin all ♂		58	54	51	100	100	85	72	94	52	100	93	83
		60	67	63	54	68	67	74	70	64	58	51	63
mated all ♂	O P S H C G				100	100	76	72	96	52	100	96	80
					62	56	55	76	64	53	60	53	54
mated all ♀	O** P** S** H C G	O** P** S*						100	104	88	93	100	55
		H C G	H C G					72	56	55	51	62	58
mated 25 ♀ : 25 ♂	O P** S H* C G	O P** S	O** P** S**								100	52	92
		H* C G	H* C G	H C G							69	62	57
mated 35 ♀ : 15 ♂	O** P** S** H C G	O** P** S*	O** P** S	O** P S**				O** P S**					
		H C G	H C G	H C G	H C G			H C G					

surrounding the scale insect mummies.

The remaining 50% of the time was spent ovipositing, probing and searching and it was in these activities that similarities and differences between treatments were most noticeable. There was remarkable similarity between the behaviour of virgin females and mated females provided only with hosts for males (Fig.5.1). However, the behaviour of parasitoids presented only with hosts for males was very significantly different ($p < 0.01$) from the behaviour of parasitoids presented only with hosts for females (Table 5.4). Parasitoids exposed only to hosts suitable for males spent more time on oviposition and probing and less time searching than parasitoids exposed only to hosts suitable for females. Further analysis of data showed that these differences in behaviour were not due to stress, but were the result of normal differences associated with the deposition of male or female eggs. C. atratus females deposited each female egg very rapidly (mean \pm 1S.E. = 5.9 ± 0.16 secs, $n=100$), whereas male eggs took a much longer time to deposit (mean \pm 1S.E. = 63 ± 11.3 , max = 13 mins, $n=100$). Therefore, parasitoids exposed only to hosts suitable for females deposited a batch of eggs in a significantly shorter time than parasitoids exposed only to hosts suitable for males. Similarly, inconsequential probes into hosts suitable for females lasted only a few seconds (mean \pm 1S.E. = 2.7 ± 0.4 secs, $n=100$), whereas inconsequential probes into hosts suitable for males lasted between 3 secs and several minutes (mean \pm 1S.E. = 46.8 ± 7.9 secs, $n=100$). Accordingly, parasitoids exposed only to hosts for females spent less time probing than parasitoids presented only with hosts suitable for males.

C. atratus females exposed to unparasitised scale insects ('female' hosts) spent nearly twice as much time searching as those females exposed to parasitised hosts ('male' hosts). This difference was apparently due to the parasitoid female being very particular in her selection of hosts suitable for females. On average, one out of every three unparasitised scale insects was accepted for oviposition of a female egg, whereas parasitised scale insects were seldom rejected and were often superparasitised. Thus, parasitoids depositing female eggs spent more time searching for acceptable hosts than parasitoids depositing male eggs. Although literature regarding differential host selection in heteronomous hyperparasitoids is scarce, the occurrence of superparasitism of hosts

for males is well documented (Compere & Smith, 1932; Fisher, 1961; Williams, 1972; Jarraya, 1977; Wilk & Kitayama, 1981). This suggests that heteronomous hyperparasitoids may generally be less particular when selecting hosts for males than when selecting hosts for females. The reason for this difference in host selection is not clear, but may occur because females develop as endoparasitoids and therefore have to overcome host immune reactions, whereas males develop as ectoparasitoids and do not have to contend with immune reactions. Parasitoid females may therefore select only those hosts for females which do not appear to have well developed immune reactions. An alternative, more plausible, reason is that parasitoid females may select scale insect hosts that are at a particular stage of their moult cycle.

The results presented in this section show that deposition of entire compliments of eggs did not occur as a result of females being exposed to unnatural conditions of hosts abundance. Differences between treatments can be explained as a consequence of the differences in behaviour when a female deposits a female egg and when she deposits a male egg. This interpretation supports the previous conclusion that female *C. atratus* do not seem to deposit fixed quotas of male and female eggs as predicted from sex ratio theory.

Brood Sex Ratios in the Laboratory

In two of the treatments outlined in Table 5.1, mated *C. atratus* females were exposed to hosts suitable for both female and male larval development. In one of these treatments, hosts were present in a ratio of 25 suitable for females: 25 suitable for males and in the other treatment hosts were present in a ratio of 35 suitable for females: 15 suitable for males. Although the proportion of hosts suitable for females and males differed between the two treatments, there were still sufficient numbers of both host types for parasitoid females to deposit any sex ratio, from an all female ratio to an all male ratio. Therefore, if *C. atratus* females produce precise sex ratios of 1:1, as predicted in theory, equal numbers of male and female offspring should have been obtained from both treatments.

The brood sex ratios obtained in this experiment are presented in Fig. 5.2. These sex ratios were so different between the two treatments that not even the ranges overlapped. Parasitoids exposed to hosts in a 25:25

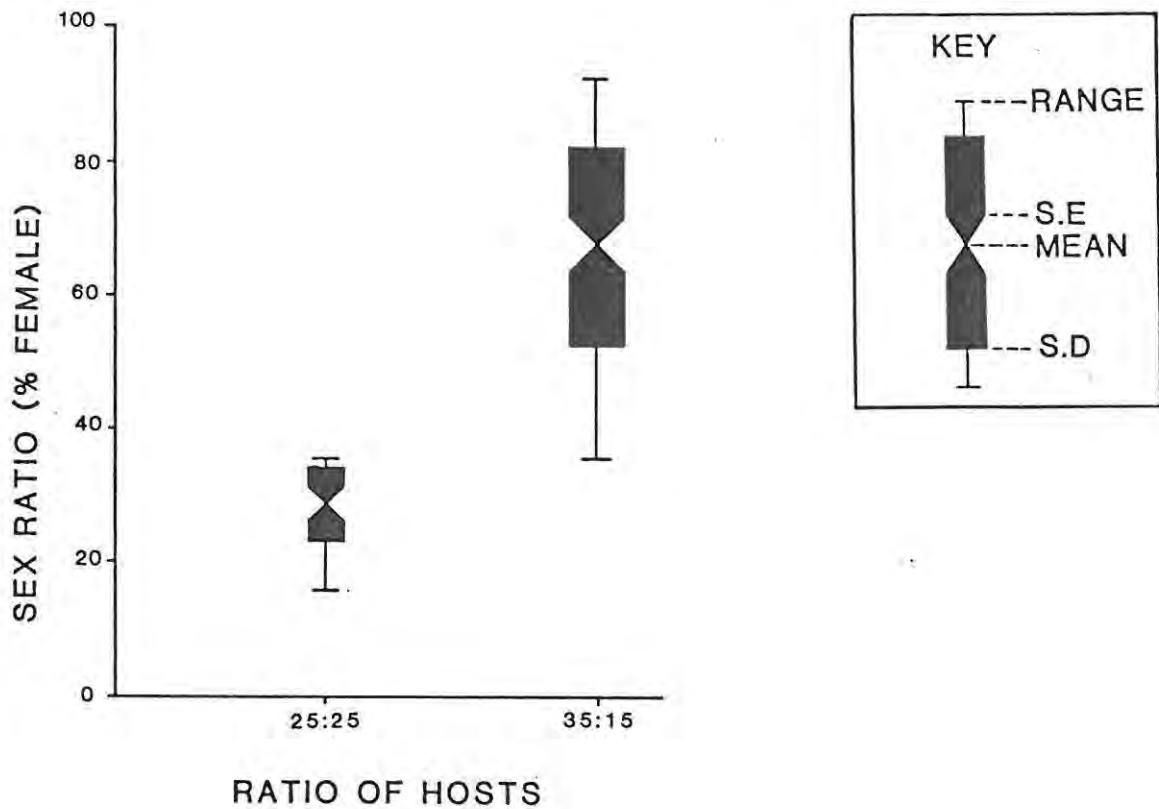


Fig.5.2. Brood sex ratios produced by *Coccophagus atratus* females exposed to hosts suitable for both male and female progeny in ratios of either 25 for female: 25 for male or 35 for female: 15 for male. Data points represent the means, standard errors, standard deviations and ranges for ten replicates where hosts were offered in a 25: 25 ratio and for fifteen replicates where hosts were offered in a 35: 15 ratio. The sex ratios are based on the total number of male and female eggs deposited by females exposed to hosts for half hour periods on the 2nd, 4th, 7th, 10th and 13th days after emergence and at three day intervals thereafter. In all cases oviposition was observed and later confirmed by dissection of hosts thought to be parasitised.

ratio produced male-biased sex ratios (16-38% female) whereas parasitoids exposed to hosts in a 35:15 ratio produced sex ratios that varied from 38-92% female. Both the variability in the brood sex ratios produced by individual females and the shift in sex ratios with changes in host abundance, are contrary to the predictions of a 1:1 brood sex ratio. These brood sex ratios can be more realistically interpreted as the result of random searching by *C. atratus* females which then deposit eggs into any suitable host, regardless of the resultant sex ratio. This conclusion is supported by a runs test for randomness (Sokal & Rohlf,

1981) that was carried out on the oviposition sequence of male and female eggs. In twenty-four out of twenty-five replicates, the sequence of egg deposition was not significantly different from random ($p > 0.05$). The remaining replicate was significant only at the 5% level of significance. Male and female eggs therefore seem to be deposited in a random sequence. Since hosts for males and hosts for females are discrete, this conclusion must mean that females do not search specifically for hosts suitable for either male or female progeny. Also, a χ^2 analysis was carried out to compare sex ratios produced by C. atratus females on each day of exposure to hosts. This analysis showed that sex ratios did not differ significantly ($p > 0.05$) between days. Thus there is no tendency to deposit more of one sex early in the oviposition sequence.

C. atratus therefore seems to differ from those Hymenoptera in which male and female eggs are deposited in a recognizable sequence, e.g. Trichogramma evanescens Westwood (Waage & Ming, 1984), or where more eggs of one sex are deposited early in the oviposition sequence, e.g. Trichogramma chilonus Ishii (Suzuki et. al., 1984), Spalangia endius (Donaldson & Walter, 1984) and some scelionids (Waage, 1982).

However, data on C. atratus sex ratios do coincide with the limited data available on other heteronomous hyperparasitoids. Virgin females have been shown to oviposit without restraint in Coccophagoides utilis (Broodryk & Douth, 1966), Coccophagus caridei (Flanders, 1939) and Coccophagus insidiator (Dalman) (Flanders, 1952b). Also, Coccophagus lycimnia is reported to deposit complete broods of a single sex (Flanders, 1943). Williams (1972) examined the sequence in which male and female eggs were deposited in Phycus seminotus Silvestri. In this species, females always deposited male and female eggs in a sequence that corresponded to the sequence of hosts provided. This was irrespective of the sex of eggs previously deposited. Thus, like C. atratus, P. seminotus does not seem to deposit male and female eggs in either a fixed proportion or a fixed sequence.

The ovipositional behaviour of C. atratus females should have important consequences for field sex ratios. This is examined in the following chapter.

CHAPTER 6

SEX RATIOS OF C. ATRATUS IN THE FIELD

The results of the previous chapter showed that C. atratus females deposit male and female eggs according to the availability of hosts suitable for either male or female progeny, regardless of the resulting sex ratio. This behaviour should lead to population sex ratios that shift with changes in the relative abundance of hosts suitable for males or for females. Such changes in host abundance should be particularly noticeable in a host such as F. gemina, because this scale insect has two discrete generations per year. Therefore, unparasitised second-instar scale insects, suitable for female parasitoids, would be available only for a limited period. Then, once the female parasitoid larvae within the scale insects had developed to the prepupal stage, they would be suitable as hosts for males. Therefore a preponderance of females would be expected initially, followed by a preponderance of males.

In contrast to the above expectations, sex ratio theories predict a 1:1 population sex ratio. Field populations of C. atratus parasitising F. gemina scale insects were therefore examined to determine whether the sex ratio of emerging adults was correlated with the relative abundance of hosts for females and males or whether the sex ratio approached an equilibrium ratio of 1:1.

F. gemina scale insects were sampled from two plant species, Chrysanthemoides monolifera and Cliffortia strobilifera, which, because of differences in sampling technique, are dealt with separately. In each case, F. gemina scale insects were the predominant habitual hosts, so no other species of scale insects were sampled.

Sampling From Chrysanthemoides monolifera

Three large stands of C. monolifera plants were chosen as study sites. Each stand consisted of patchily-distributed plants separated from other such stands by large tracts of open land or built-up areas (Fig.6.1). Within each stand all the plants infested with F. gemina scale insects were located. Scale insects were easily found because they occur in



Fig.6.1. Stand of Chrysanthemoides monolifera plants showing the patchy distribution of plants (see arrows). The stand was surrounded by built up areas.

aggregated infestations on the plants. All scale insects tended to be in a similar stage of development. On C. monolifera, scale insects were found mostly on the leaves rather than on the stems of the plant (Fig.6.2). Consequently, only the leaves were sampled. Of the three stands, one had 254 infested leaves and the remaining two had 139 and 71 infested leaves. Sampling was therefore carried out by collecting 15, 8 and 5 leaves from each stand respectively, once every two weeks. Leaves were selected by giving each infested leaf a number and then using random numbers to choose leaves. Low numbers of leaves were collected from each stand of C. monolifera because this was a destructive sampling programme designed to run for twelve months.

Leaves were processed in the following way. 1) All scale insects present on the leaves were counted and classified according to their instar. 2) Seemingly unparasitised second-instar scale insects were dissected. If not parasitised these scale insects were counted as potential hosts for



Fig.6.2. Chrysanthemoides monolifera leaf infested with Filippia gemina scale insects. Scale insects tended to settle on the leaves rather than the stems of this plant.

females C. atratus. 3) C. atratus and Metaphycus parasitoids that had reached the late larval stage were counted as potential hosts for male C. atratus. 4) All parasitised scale insects, where the parasitoid larva was well developed, were removed from the leaf and placed individually in gelatin capsules so that the sex ratio of adult C. atratus could be ascertained. Young parasitoid larvae were discarded because it was impossible to distinguish C. atratus larvae from other parasitoid species and few of these larvae reached the adult stage when kept in the laboratory.

Sex Ratios

The results from the three collection sites were very similar and a χ^2 analysis (RxC table to test for homogeneity) showed that there was no significant difference between them ($P > 0.05$). They were therefore pooled.

The number of potential hosts (Fig.6.3) was characterised by an initial

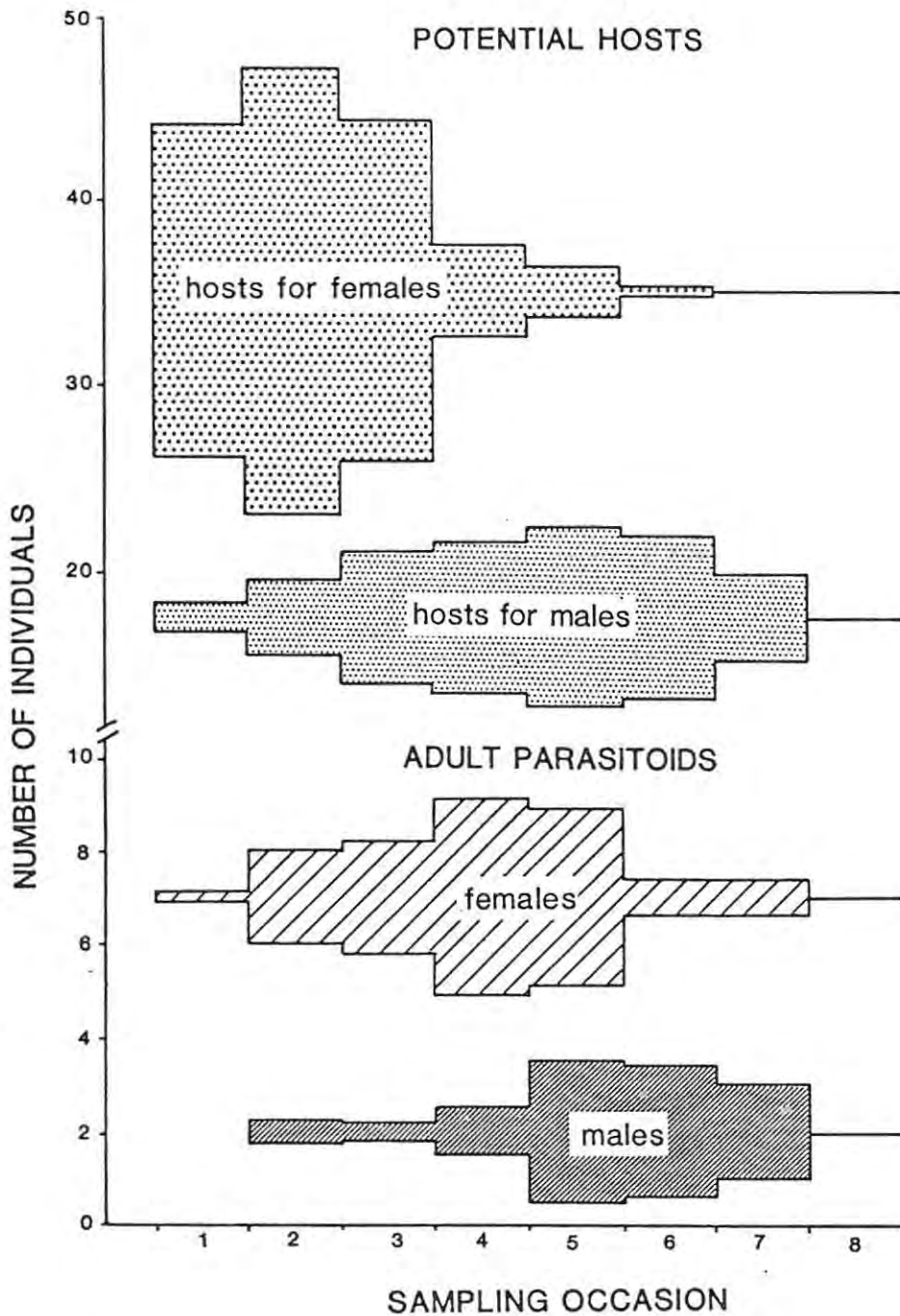


Fig.6.3. Mean numbers of potential hosts for female and male *Coccophagus atratus*, as well as mean numbers of adult *C. atratus* parasitoids obtained from sampling on *Chrysanthemoides monolifera* plants. Samples were collected once every two weeks. Initially twenty-three samples were taken, but this number was gradually reduced until only five samples were collected on the seventh sampling occasion.

peak in hosts for females followed by a decline in these hosts and a simultaneous increase in the number of hosts for males. The emergence of male and female parasitoids followed a similar trend. Females were initially predominant but progressively fewer emerged until males were in excess. This resulted in an extremely variable sex ratio (Fig.6.4) fluctuating from 100% female to 20% female. This change in the proportion of emerging females was due mainly to the discrete generations of the scale insect host. An analysis of the number of first-, second-, and third-instar scale insects present on each sampling occasion (Fig.6.5) showed that there was a clear progression from first-instar to third-instar without any overlap of generations. Few scale insects reached the third-instar because of high levels of parasitism of second-instar scale insects.

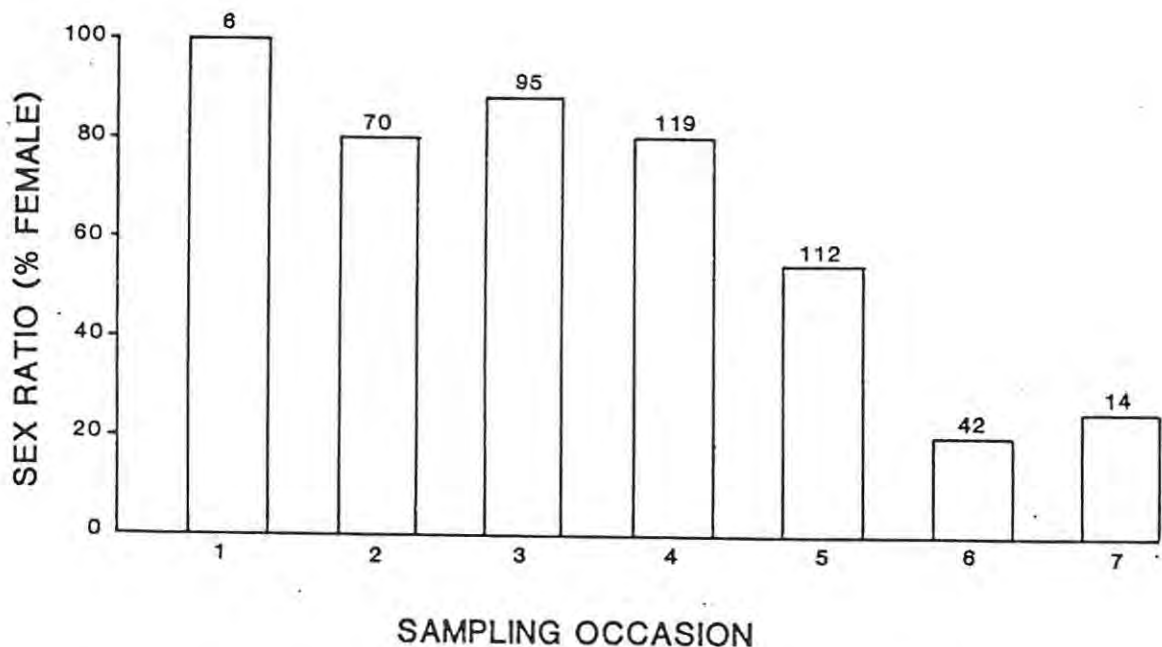


Fig.6.4. Sex ratios of *Coccophagus atratus* adults obtained from parasitised *Filippia gemina* scale insects collected from *Chrysanthemoides monolifera* plants. Samples were taken once every two weeks and details of sampling procedures are given in the text. Numbers above each bar denote the total number of parasitoids collected.

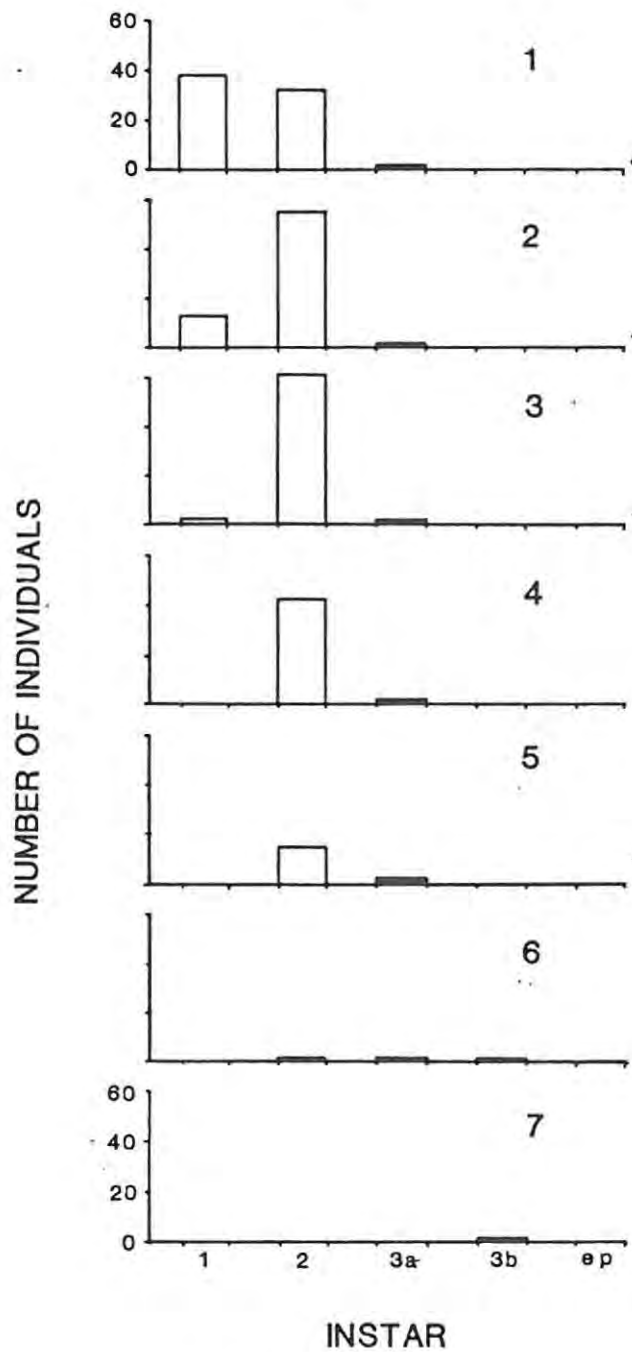


Fig.6.5. Number of 1st-, 2nd- and 3rd-instar and egg packet stage Filippia gemina scale insects present on Chrysanthemoides monolifera leaves collected in the field. The 3rd-instar was divided into (a) pregravid, and (b) gravid, stages. Samples were collected once every two weeks for a period of fourteen weeks. The sampling occasion is denoted by a number (1-7) on the right side of each graph. Few scale insects reached the 3rd-instar because C. atratus parasitoids attacked this stage.

Although this study was intended to run over twelve months, and populations were monitored for this time, the numbers of scale insects dropped markedly during the sampling programme. Four months after the start of sampling, two of the stands were devoid of *F. gemina* scale insects (Fig.6.6) and only a few infestations remained in the surviving population. This decrease in the number of scale insects was not due to destructive sampling since the programme had been designed to run over twelve months. The drop in the number of scale insects was apparently due to the very high rate of parasitism (see Fig.6.7) as well as predation by coccinellid beetles. These mortality factors caused a decrease in the number of scale insects per leaf (Fig.6.7), whereas destructive sampling would only have affected the total number of leaves infested with scale insects.

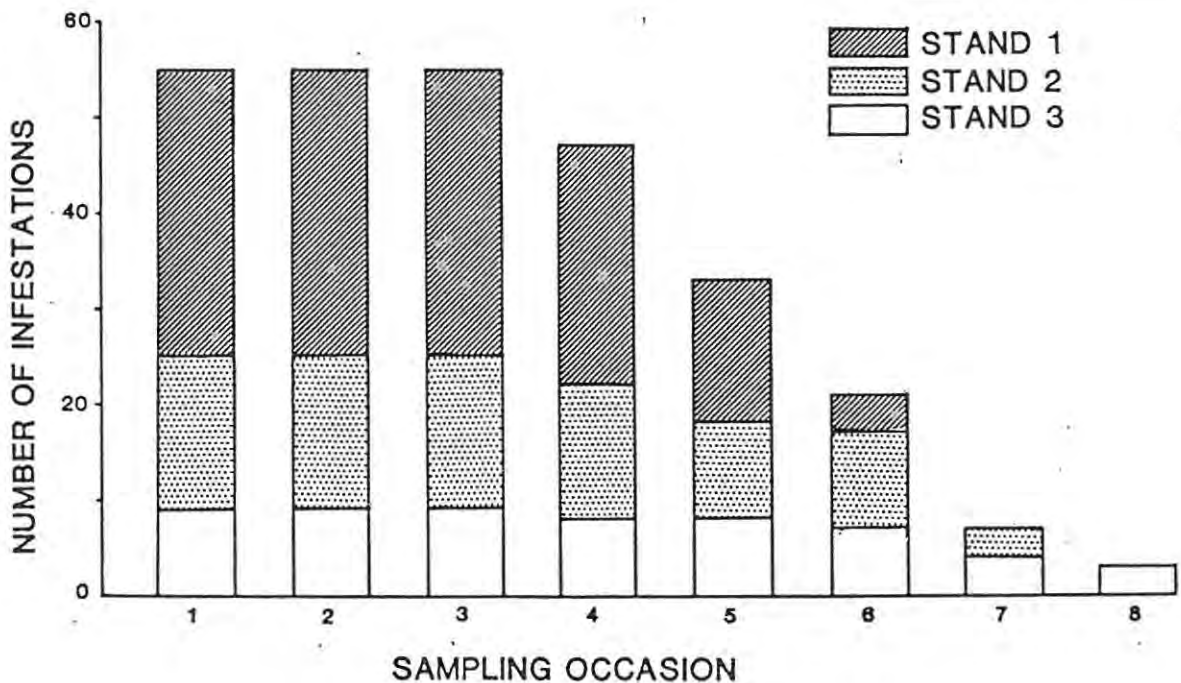


Fig.6.6. Number of infestations of *Filippia gemina* scale insects in three stands of *Chrysanthemoides monolifera* plants. Stands were monitored by counting the number of infestations once every two weeks.

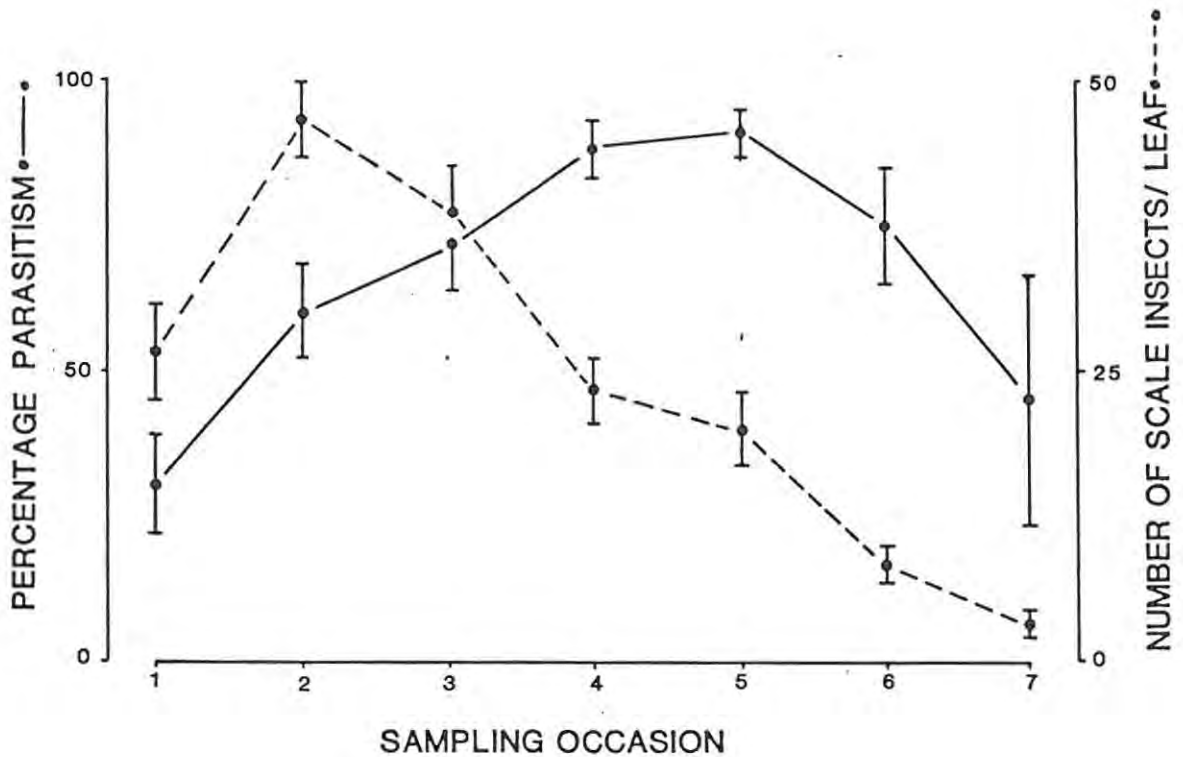


Fig.6.7. Percentage parasitism of Filippia gemina scale insects by Coccophagus atratus parasitoids as well as the number of scale insects present on leaves of Chrysanthemoides monolifera plants. Leaves were collected once every two weeks. Points represent the mean and standard error for: 23 samples for occasions 1, 2 and 3; 20 samples for occasion 4; and 15, 5 and 8 samples for occasions 5, 6 and 7 respectively.

Sampling From Cliffortia strobilifera

A single stand of C. strobilifera, bordering a dam (Fig.6.8), was found to be infested with F. gemina scale insects. Unlike C. monolifera, C. strobilifera has small, narrow leaves (Fig.6.9) and the majority of the scale insects settle on the stems. A survey revealed that 43 stems were infested with scale insects.

To develop a sampling technique, five stems infested with scale insects were collected, and the distribution of scale insects on them was analysed. The stems were divided into 5cm sections and the number of unparasitised first-, second- and third-instar scale insects as well as

the number of parasitised scale insects were counted on each section. A χ^2 analysis showed that there was no significant difference ($P > 0.05$) between any of the 5 cm sections. Therefore, as neither the scale insects nor the parasitoids favoured any particular portion of the stems, 5cm sections, cut from the distal part of scale insect-infested branches, were used as samples. All branches infested with scale insects were given a number, and random numbers were used to select eight stems per sample occasion. Samples were taken once a week and were analysed in the same way as those taken from C. monolifera.



Fig.6.8. Stand of Cliffortia strobilifera plants from which samples of Filippia gemina scale insects were collected. This stand (see arrows), measuring approximately 60m in length and 2m in width, contained 43 infestations of F. gemina scale insects.



Fig.6.9. A branch of Cliffortia strobilifera showing an infestation of Filippia gemina scale insects. The scale insects tended to congregate mainly on the stems of this plant.

The results of this sampling programme (Fig.6.10) show that here, too, numbers of potential hosts for male and female C. atratus changed gradually from a situation where hosts suitable for females were abundant to a situation where hosts suitable for males were more numerous. Again, the pattern of adult emergence corresponded with the pattern of host availability and variable C. atratus sex ratios were obtained (Fig.6.11). In this sampling programme, although few scale insects escaped parasitism, no infestations were entirely extirpated and sampling therefore proceeded for a longer time. There are three possible reasons why the scale insects on C. strobilifera were present for a longer period than those on C. monolifera. 1) Emergence of scale insect crawlers was less synchronised on C. strobilifera (Fig.6.12) than on C. monolifera. Therefore there was input of new hosts for a longer period than on C. monolifera. 2) Coccinellid beetles were not as abundant on C. strobilifera plants, therefore predation was lower. 3) C. strobilifera

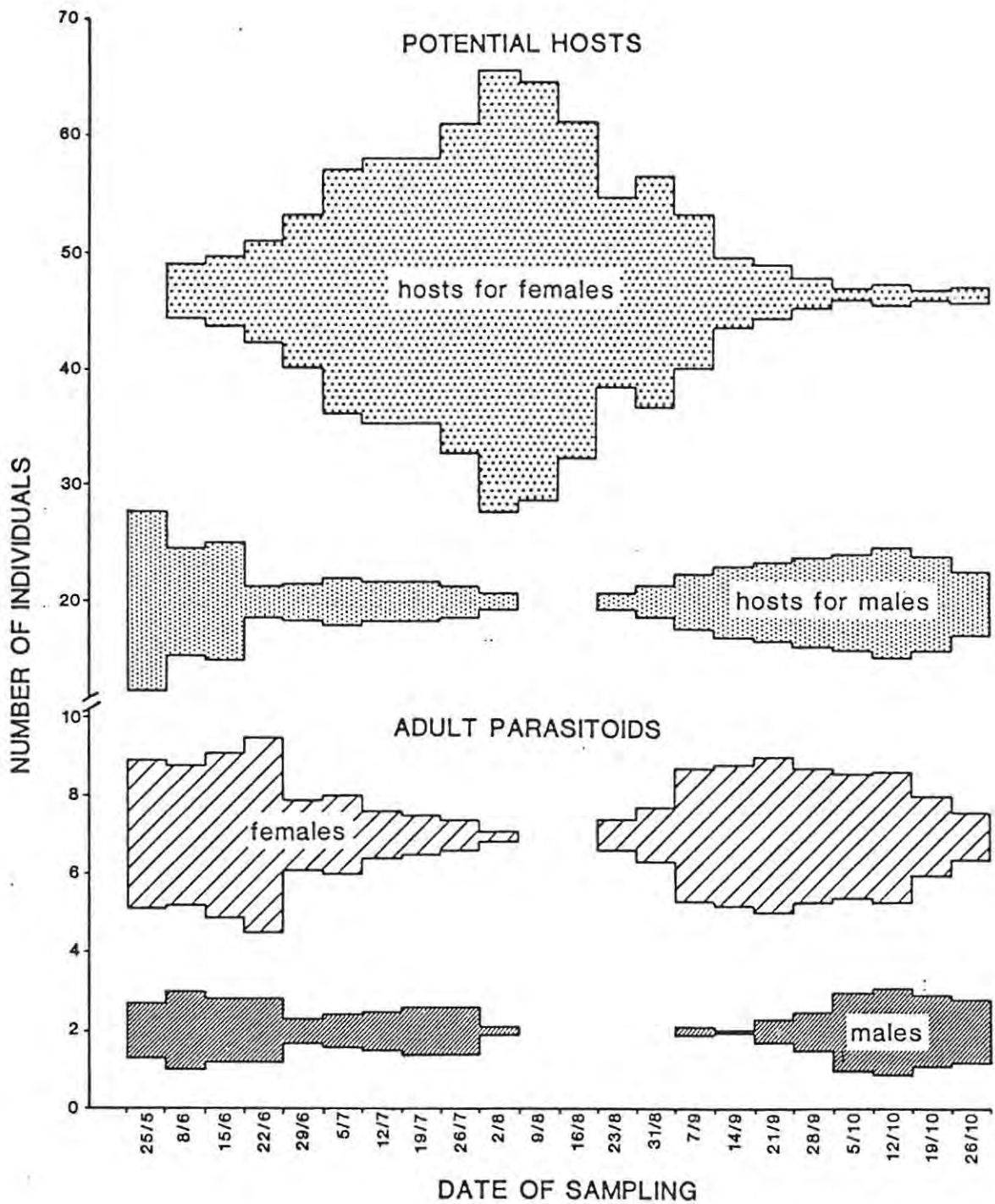


Fig.6.10. Number of potential hosts for female and male *Coccophagus atratus* and number of *C. atratus* adults obtained from sampling on *Cliffortia strobilifera* plants. Unparasitised second-instar *Filippia gemina* scale insects were regarded as potential hosts for females, whereas late larval and prepupal stages of *C. atratus* and *Metaphycus* parasitoids were counted as potential hosts for males. Samples were taken once a week from the 25/5/1984 to the 26/10/1984. Each bar represents the mean for ten samples.

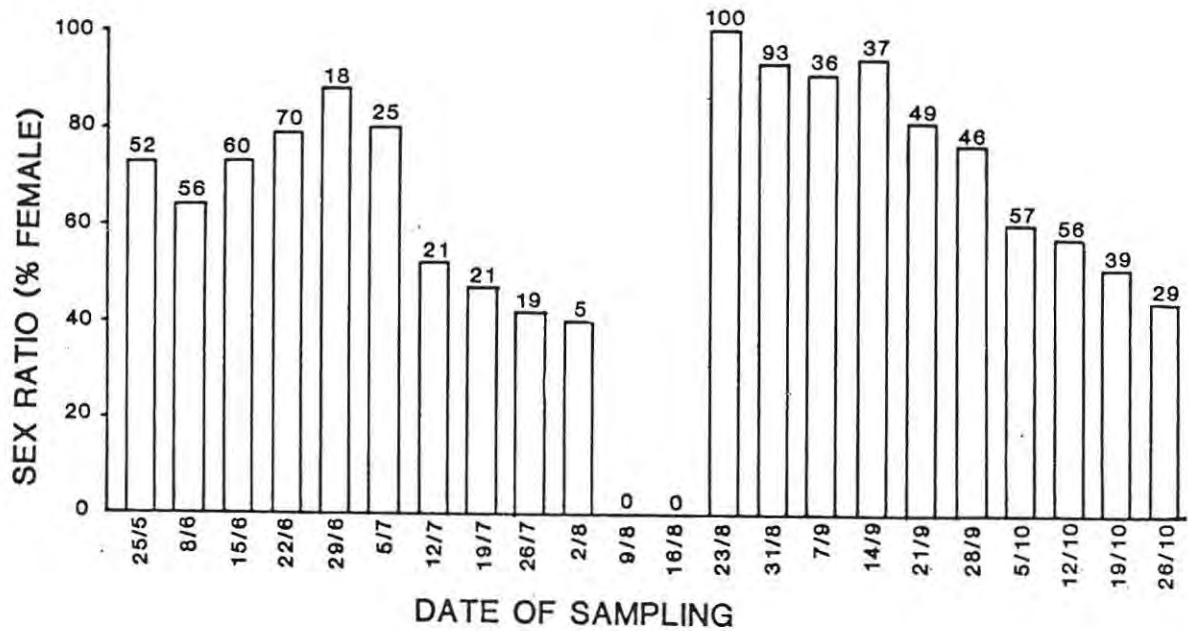


Fig.6.11. Sex ratios of *Coccophagus atratus* adults obtained from parasitised *Filippia gemina* scale insects collected from *Cliffortia strobilifera* plants. Each bar represents the mean for ten samples. Figures above each bar denote the total number of parasitoids obtained. Samples were collected once a week from the 25/5/1984 to the 26/10/1984.

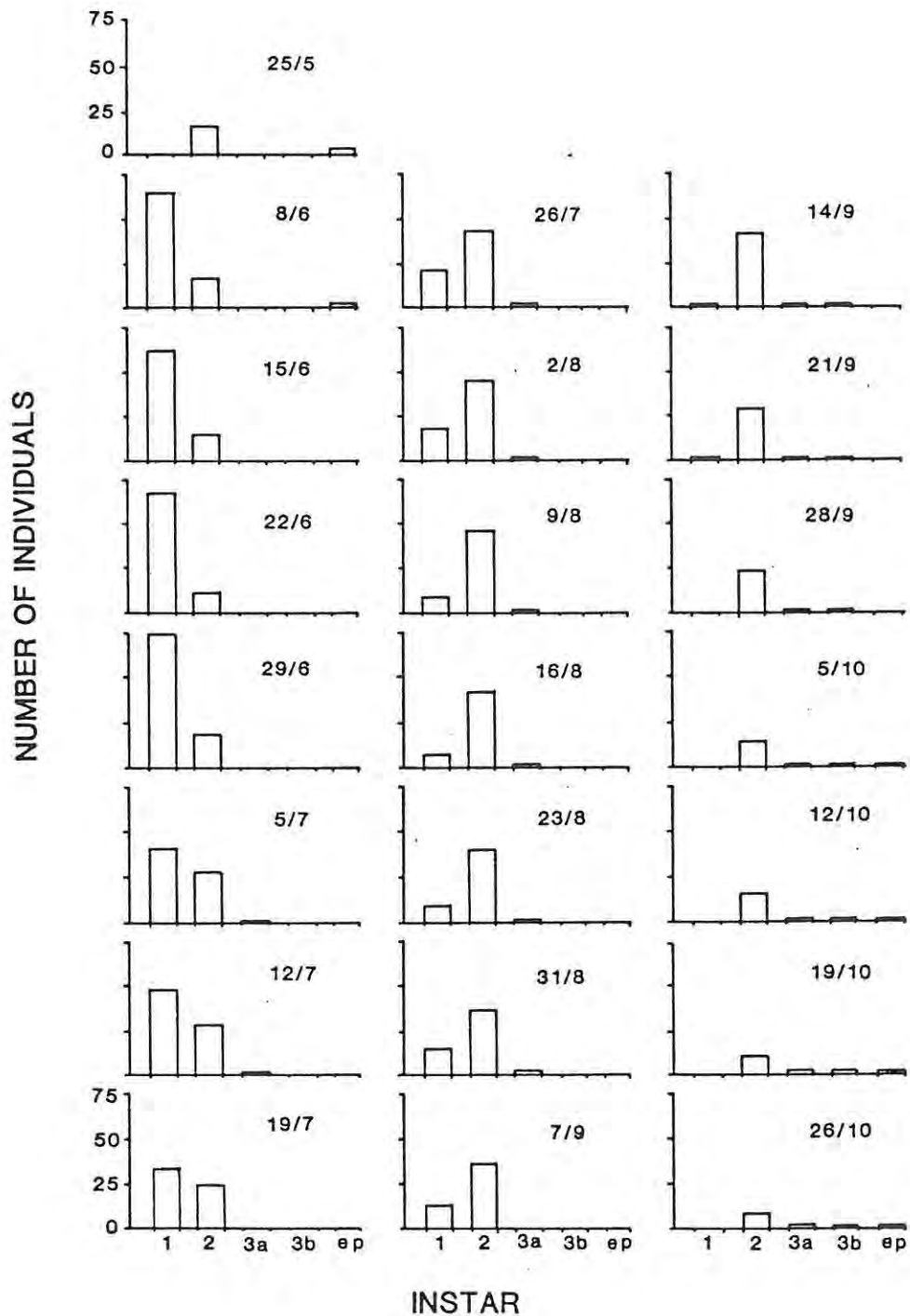


Fig.6.12. Number of 1st-, 2nd- and 3rd-instar and egg packet stage *Filippia gemina* scale insects present on *Cliffortia strobilifera* plants. The 3rd-instar is divided into (a) pregravid and (b) gravid, stages. Samples, comprising 5 cm sections of plant were collected once a week from 25/5/1984 to 26/10/1984. Each bar represents the mean for ten samples.

plants were in a more exposed situation and there may have been less parasitoid activity.

Sex ratios of C. atratus, from collections of scale insects on Chrysanthemoides monolifera and Cliffortia strobilifera, fluctuated coincidentally with changes in the availability of hosts suitable for male and female development. These shifts in sex ratio were expected because individual females exposed to hosts in the laboratory showed no preference for depositing either male or female eggs, and did not produce a precise sex ratio.

Sex ratio changes related to the availability of hosts have been reported in other aphelinids, but are usually quoted without any supporting evidence. Viggiani & Mazzone (1978) reported that sex ratios of Prospaltella lahorensis Howard in the field ranged from 32.5-72.6% female. They attributed shifts in sex ratios to changes in host abundance, but provided no empirical evidence to support this claim. Unquantified data for Prospaltella perniciosi Tower (Chumakova & Goryunova, 1963) and anecdotal evidence for Prospaltella elongata Dozier (Flanders et. al., 1950) suggest that sex ratios in these species also change coincidentally with changes in the abundance of hosts for males and females. Variable sex ratios in the field are therefore probably quite common in heteronomous hyperparasitoids.

CHAPTER 7

DISCUSSION AND CONCLUSIONS

Experiments presented in this thesis were designed to test the hypothesis that Coccophagus atratus, a solitary hymenopteran parasitoid, should deposit male and female eggs in a fixed ratio of 1:1. Predictions based on the hypothesis were as follows. 1) Virgin females were expected to limit the number of eggs they deposited since they can lay only male eggs. Theoretically they should reserve eggs for later production of females, thus ensuring a 1:1 sex ratio. 2) Mated females, exposed only to hosts suitable for either male or female development, should, for similar reasons, have limited the number of eggs they deposited. 3) Mated females exposed to hosts suitable for both male and female development were expected to produce male and female progeny in a ratio that approached 1:1. To test these predictions, C. atratus females were exposed, for the duration of their lives, to the various conditions of host availability described in the predictions.

The results obtained showed that: 1) virgin females deposited complete batches of eggs and did not restrict the number of eggs deposited (Table 5.2); 2) mated females exposed only to hosts suitable for either male or female development deposited their full complement of eggs into the hosts provided, even though this resulted in broods that comprised a single sex (Table 5.2); and 3) where mated females were provided with hosts suitable for deposition of both male and female eggs, sex ratios varied between 16 and 92% female and were not fixed at 1:1 (Fig.5.2). In the latter experiment, when the ratio of hosts suitable for male and female development was altered, sex ratios shifted accordingly. In addition, the sequence in which male and female eggs were deposited was not significantly different from random, which suggests that C. atratus females do not search specifically for hosts suitable for the development of either sex. Clark (1984) found that C. atratus females respond to cues that originate from unparasitised scale insects (mainly honeydew). This suggests that C. atratus females locate patches of hosts by homing in on cues associated with hosts for females, but once on a patch of hosts, females seem to deposit eggs into all available hosts; they do not prefer

to lay female eggs.

In addition to the above experiments, samples of scale insect hosts were collected from the field to assess sex ratios under natural conditions. These samples, taken from four different sites and from two different plant species, revealed that sex ratios of C. atratus adults in the field varied from being extremely female-biased (100% female) to being predominantly male-biased (20% female) (Figs 6.4 & 6.11). Shifts in sex ratio were related to temporal changes in the relative abundance of hosts suitable for male and female development (Figs 6.3 & 6.10).

C. atratus sex ratios therefore do not fulfil the predictions based on the hypothesis of a 1:1 sex ratio. However, deviations from a 1:1 sex ratio are expected under certain circumstances, and consideration of these circumstances forms the bulk of this discussion. Also, data collected during the course of this study were found to have implications for current views on the adaptive significance of heteronomous hyperparasitism. A discussion of the theories proposed to explain the evolution of heteronomous hyperparasitism concludes this chapter.

Sex Ratio Theory

Sex ratio theories can perhaps be divided into three broad categories; 1) those that predict production of males and females in fixed proportions, 2) those that predict facultative manipulation of sex ratios by ovipositing females, and 3) those theories that consider sex ratios to be controlled by the environment. Since theories in these three categories have different predictions for sex ratios that deviate from 1:1, they are dealt with individually. The genetic basis of sex ratios is also considered.

Fixed Sex Ratios

Several sex ratio theories predict that parents should produce male and female offspring in consistent, genetically-fixed ratios (Fisher, 1930; Shaw & Mohler, 1953; Kolman, 1960, Verner, 1965; Hamilton, 1967; Maynard Smith, 1978; Taylor & Sauer, 1980; Charnov, 1982). Equable sex ratios are generally expected, but deviations from unity are predicted to occur under certain circumstances that include the following.

1) Situations where the cost of producing males is not equal to the cost of producing females (Spieth, 1974). In this case, a bias towards the less expensive sex is expected.

2) Differential mortality between males and females.

3) Those mating systems where local mate competition and inbreeding occur frequently. Under such conditions female-biased sex ratios are predicted (Hamilton (1967)

4) The deposition of male eggs onto sibling females by heteronomous hyperparasitoids. Female-biased sex ratios are expected where this occurs (Colgan & Taylor, 1981).

These four cases are dealt with in turn.

Fisher (1930) proposed that parents should invest equally in male and female offspring. This should result in a 1:1 sex ratio where males and females are equally expensive to produce. However, where the cost of one sex is more than that of the other, sex ratios should be biased in favour of the less expensive sex (Spieth, 1974). Unless the cost of producing each sex varies, sex ratio biases caused by unequal costs of male and female production should be unidirectional and constant, i.e. sex ratios should be either consistently female-biased or consistently male-biased. C. atratus sex ratios varied from being extremely female-biased to being predominantly male-biased and are therefore not explained by this theory. In any event, there appears to be no difference in the cost of producing male and female offspring. Male and female eggs are identical prior to fertilisation and the amount of time spent locating and parasitising hosts suitable for males is comparable with that spent on hosts suitable for females (see Fig.5.1).

Differential mortality between male and female offspring could cause a bias in secondary sex ratios (that at emergence) or, if differential mortality is compensated for, it may result in biased primary sex ratios (that at oviposition) (Spieth, 1974). Leigh (1970) and Spieth (1974) have reasoned that adjustments in primary sex ratios in response to differential mortality are unlikely, and this theory also does not account for the variation in brood sex ratios produced by C. atratus females. In addition, the limited data available on mortality of C.

atratus indicates that there is there is no difference in mortality between male and female larvae (Chapter 2).

Where local mate competition and inbreeding are regular features of mating behaviour, female-biased sex ratios are expected (Hamilton, 1967). Female-biased sex ratios may be constant for a species (Waage, 1982) or, in some instances, sex ratios may vary according to the the degree of inbreeding (see Wylie, 1966; Werren 1980, 1983). Since hosts suitable for development of male and female C. atratus are seldom present at the same time in the field, it is unlikely that male and female siblings will develop on the same patch of hosts. Mating between siblings is therefore probably uncommon. Also, sex ratios of C. atratus were male-biased in some situations. Therefore, kin selection theory also does not explain deviations away from 1:1 sex ratios in C. atratus.

Colgan & Taylor (1981) proposed that female-biased sex ratios should occur in those species of heteronomous hyperparasitoids that deposit male eggs onto sibling females. This possibly occurs in parasitoids such as Coccophagus semicircularis and Coccophagus gurneyi, because their females oviposit male eggs into very young parasitoid larvae. However, in C. atratus, male eggs are deposited onto late larval and prepupal stages of conspecific females. Female progeny would, therefore, be suitable for the deposition of male eggs only some weeks after the initial deposition of female eggs. Therefore, it is unlikely that male eggs are deposited onto female siblings, especially since observations of females in the field indicate that adult C. atratus females do not remain on the same host plant for any length of time. In addition, Colgan & Taylor's (1981) theory predicts only that sex ratios should be biased towards females, and does not explain variable sex ratios such as those obtained in C. atratus.

In summary, sex ratios of C. atratus are not predicted by any of the above theories. Sex ratios of this species are therefore examined in the context of facultative sex ratios.

Facultative Sex Ratios

In contrast to the predictions of fixed sex ratios, some sex ratio

theories propose that an ability to vary the sex ratio would be adaptive under certain circumstances. Werren & Charnov (1978) postulated that if the proportion of males and females in a population is not always constant, an ability to detect sex ratio biases and to deposit compensatory sex ratios would be adaptive. A basic assumption of Werren & Charnov's (1978) theory is that females have the ability to assess the present sex ratio before they deposit their offspring. Such an ability was not evident in C. atratus. In the laboratory C. atratus females adjusted their sex ratios in response to changes in the abundance of hosts suitable for development of male and female offspring, and females had no opportunity to assess the sex ratio prior to oviposition. Sex ratios in the field also changed coincidentally with changes in the relative abundance of hosts suitable for male and female development. In contrast to the expectations of Werren & Charnov's theory, the tendency of C. atratus females to oviposit in the most common hosts often resulted in continued production of the sex that was already more numerous (see Figs 6.3 & 6.10).

Many parasitic wasps deposit female eggs in large hosts, male eggs in small ones (Brunson, 1937; Arthur & Wylie, 1959; van den Assem, 1971; Charnov et. al., 1981; Avilla & Albajes, 1984). Chewyreu (1913, in Charnov, 1982) proposed that such behaviour is adaptive because females gain more from the greater quantity of food than males do. These species therefore have essentially different hosts for males and females, a situation analogous to that found in heteronomous hyperparasitoids. Sex ratios in wasps with size-related sex determination also shift with changes in the abundance of large and small hosts (Brunson, 1938), again behaviour comparable with that observed in C. atratus. However, Charnov (1982) believes that sex ratios of species with size-related sex determination should not depend entirely on the availability of hosts, but should, at least to some limited extent, be genetically controlled. This is implicit in his consideration that local mate competition and inbreeding may alter sex ratios produced by wasps with size-related sex determination. Control of sex ratios is apparently lacking in C. atratus in which females deposit entire broods that comprise a single sex and where sex ratios in the field fluctuate from one extreme to the other. Similarly, Hymenoptera with size-related sex determination may not

control their brood sex ratios, e.g. Tiphia popilliavora Rohwer produced sex ratios that ranged between 2 and 67% female in the laboratory and sex ratios that ranged between 27 and 67% female were obtained from the field (Brunson, 1934, 1937, 1938). Females presented with large and small hosts in different proportions and different sequences, adjusted their sex ratios accordingly. The extreme variability of T. popilliavora sex ratios is indicative of the absence of sex ratio control. Experiments similar to those done on C. atratus should perhaps be carried out on a wasp with size-related sex determination to assess the extent of parental control of sex ratios in these species. Results may be more difficult to interpret than those for C. atratus because size thresholds may not be absolute (Chewyreu, 1913; in Charnov, 1982) and this would lead to ambiguous results. For instance, females provided only with small hosts might deposit predominantly, but not exclusively male-biased sex ratios. Such ratios could be interpreted in relation to both a poorly defined response to host size and as production of a genetically-fixed minimum number of female offspring.

Sex ratios of C. atratus are therefore also not explained by theories that predict facultative manipulation of sex ratios. Environmental control of sex ratios is therefore considered in the following section.

Environmental Control of Sex Ratios

Environmental control of sex ratios has been proposed for Hymenoptera in general by Flanders (1939b, 1942, 1952b, 1956, 1964, 1965) and Clausen (1939) and has been intimated for heteronomous hyperparasitoids by Zinna (1961, 1962), Williams (1977), Hassel et. al., (1983) and Viggiani (1984). In heteronomous hyperparasitoids, sex ratios are expected to depend on the frequency with which an ovipositing female encounters hosts suitable for male or female development (Williams, 1977, Hassel, et. al., 1983).

The data obtained on C. atratus sex ratios are best explained by the theory of environmental control since females exert no control over their sex ratios and male and female eggs are not deposited in any fixed proportion or temporal sequence. Data from both the laboratory and the field show that sex ratios depend on the relative abundance of hosts

suitable for male and female development. An analysis of the limited data available on sex ratios in other heteronomous hyperparasitoids (Table 7.1) shows that variable sex ratios occur in at least five other species. Anecdotal comments suggest that these sex ratios are also correlated with changes in host abundance and may therefore be environmentally determined.

As mentioned in the previous section, sex ratios in some Hymenoptera with size-related sex determination may also be determined by host abundance in the environment. This needs further experimental examination.

In recent years sex ratio theories have tended to concentrate on the adaptive significance of sex ratios and have therefore focussed on genetically-based theories. The alternative possibility that sex ratios may be largely determined by the environment, raises questions about the extent of genetic control of sex ratios. This is dealt with in the next section.

Genetic Control of Sex Ratios

All theories that accept an adaptive significance to sex ratios assume that this trait is genetically controlled. This assumption makes it possible to predict selection for specific, adaptive, sex ratios or for adaptive responses of ovipositing females to specific situations. There is, however, very little evidence that sex ratios are genetically controlled in Hymenoptera. The precise sex ratios produced by certain gregarious species suggest that there is some element of genetic control (see Green *et. al.*, 1982), but this has yet to be shown. The only evidence of heritability in hymenopteran sex ratios is that of extrachromosomal inheritance of extreme sex ratios (Wilkes, 1964; Werren *et. al.*, 1981; Skinner, 1982). The two species involved (Dahlbominus fuliginosus (Nees) and Nasonia vitripennis Walker) are probably exceptional in this way. There is no evidence of heritability of sex ratios in solitary parasitoids. It is therefore important to reiterate the conclusions of Donaldson & Walter (1984) that the genetic basis of hymenopteran sex ratios needs further examination. This is re-emphasised here because C. atratus sex ratios do not support genetically based theories.

Table 7.1 Sex ratios of various heteronomous hyperparasitoids. Many anecdotal references to sex ratios of heteronomous hyperparasitoids have been omitted. Parasitoids with variable sex ratios, either in the laboratory or the field are indicated with asterisks.

Species	Sex Ratio	Field collection/ Laboratory experiment	Reference
<u>Coccophagoides utilis</u> * Doutt	variable, 6% female at end of winter	4 field collections	Kennet <u>et. al.</u> , 1966
	60.7 - 73% female	laboratory experiment	Broodryk & Doutt, 1966
<u>Coccophagus caridei</u> (Brèthes)	50% female	single field collection	Valentine, 1964
<u>Coccophagus gossypariae</u> Gahan	variable, but always female-biased	field collections	Ceianu, 1968
<u>Coccophagus semicircularis</u> (Foerster)	69.8 - 80.4% female female-biased	field collections laboratory	Jarraya, 1975 Jarraya, 1977
<u>Physcus debachi</u> Compere & Annecke	90% female	laboratory	Fisher, 1961
<u>Physcus seminotus</u> * Silvestri	variable	laboratory	Williams, 1972
<u>Prospaltella</u> * <u>elongata</u> Dozier	varies at different times of the year	field collections	Flanders <u>et.al.</u> , 1950
<u>Prospaltella</u> * <u>lahorensis</u> Howard	32.5 - 72.6% female	field collections	Viggiani & Mazzone, 1978
<u>Prospaltella</u> * <u>perniciosi</u> Tower	approximately 1:1 80% female 37 - 62% female	1 field collection few field collections field collections	Tower, 1914 Rice, 1937 Chumakova & Goryunova, 1963
<u>Prospaltella smithi</u> Silvestri	77 - 100% female	3 field collections	Kuwana, 1934

Adaptive Significance of Heteronomous Hyperparasitism

The adaptive significance of heteronomous hyperparasitism has been discussed to a limited extent by Flanders (1937, 1959) and Zinna (1961, 1962) and in more detail by Walter (1984). The relevance of data on C. atratus to the proposals of Flanders (1937, 1959) and Zinna (1961, 1962) is discussed below.

Flanders (1937, 1959) believed that heteronomous hyperparasitism resulted in female biased sex ratios because hosts for males were more scarce, and less susceptible to parasitism, than hosts for females. Since females search for hosts, a bias towards this sex would result in an increased searching capacity for a species with heteronomous hyperparasitism and therefore provide a selective advantage over other parasitoid species (Flanders, 1959). In addition to being group selectionist, the proposed advantage gained by heteronomous hyperparasitoids is unrealistic because females of all arrhenotokous species can determine the sex of their offspring and therefore produce female-biased sex ratios (see Walter, 1984). Also, the data on C. atratus showed that sex ratios in the field were, on occasion, male-biased (Figs 6.4 & 6.11). The bias towards males was caused by a shortage of hosts suitable for female development and a preponderance of hosts suitable for male development (Figs 6.3 & 6.10). Flanders' interpretation is therefore untenable.

Zinna (1961, 1962) thought that heteronomous hyperparasitism may have arisen as a means of population self-regulation. He noted that the phytophagous hosts attacked by heteronomous hyperparasitoids often occur in isolated patches ("biotopic islands"; Zinna, 1961) in which the number of hosts is limited. Further, Zinna believed that heteronomous hyperparasitoid females were poor fliers and would therefore be restricted to a single patch of hosts for several generations. Under these circumstances, unrestricted parasitism would rapidly exhaust the supply of hosts. Zinna therefore proposed that heteronomous hyperparasitism, and specifically autoparasitism (deposition of males on conspecific females) could have evolved to control population numbers, thereby preventing over exploitation of the limited resources.

The group selectionist basis of Zinna's (1961, 1962) argument has been criticised by Walter (1984). Zinna's hypothesis is also not supported by field data collected during the course of this study. In C. atratus levels of parasitism were extremely high (up to 100%; Fig.6.7) and the extent of parasitism caused the extinction of several patches of hosts (termed infestations in the text; Fig.6.6). In addition, observations of C. atratus females in the field, carried out by Clark (1984) and myself, indicated that female wasps are competent fliers, and were observed both leaving, and arriving at, host-infested plants. C. atratus females would therefore not be restricted to one patch of hosts for any length of time.

Therefore, data on C. atratus support neither Flanders' (1937, 1959) nor Zinna's (1961, 1962) hypotheses. An alternative hypothesis for the evolution of heteronomous hyperparasitism has been proposed by Walter (1984). Information on C. atratus described in this thesis provides no evidence for or against Walter's hypothesis and it is therefore not discussed further.

To summarise, sex ratios of C. atratus are not predicted by any theories of adaptive sex ratios. They are best explained by the alternative of environmental control of sex ratios. Sex ratios in C. atratus are therefore not adaptive. Herbers (1979) stated that sex ratios in a number of ant species are best explained by theories that do not consider sex ratios to be adaptive, and Williams (1979) has argued that sex ratios in vertebrates may not be adaptive. The adaptive significance of sex ratios and especially the genetic basis of sex ratios needs further examination.

SUMMARY

1) Sex ratio theory predicts that solitary hymenopteran parasitoids should deposit male and female eggs in a ratio of 1:1. Coccophagus atratus was used to test this prediction. C. atratus is suitable for such a study because male and female eggs are deposited in different hosts and an observer can therefore determine the sex of an egg when it is deposited. Sex ratio data collected in this way is not affected by mortality.

2) Studies on daily ovipositional activity were carried out to determine when C. atratus females were most active. Results showed that most ovipositional activity took place on the 2nd, 4th, 7th, 10th and 13th days after emergence. On each occasion of exposure to hosts, most of the eggs (83%) were deposited during the first half hour. The patterns in ovipositional activity were related to the availability of mature eggs in the ovaries of ovipositing females.

3) Predictions derived from sex ratio theory were that: i) virgin females should limit the number of eggs they deposit since they can lay only male eggs; ii) mated females exposed only to hosts suitable for development of one sex were expected to limit their oviposition because host availability restricted them to the deposition of only one sex; iii) mated females, exposed to hosts suitable for both male and female development, were expected to consistently produce male and female offspring in a ratio of 1:1. Observational experiments were carried out in the laboratory to test the response of virgin and mated females to the conditions of host abundance outlined in the predictions. These experiments showed that: i) virgin females deposited all their available eggs into hosts suitable for male development; they did not limit the number of eggs deposited; ii) mated females, exposed only to hosts suitable for development of one sex, similarly did not restrict the number of eggs they deposited; and iii) mated females, exposed to hosts suitable for both male and female development, did not produce sex ratios approaching 1:1. In the latter experiment, sex ratios varied according to the availability of hosts suitable for male and female development and male and female eggs were deposited in a random sequence.

4) Analysis of the behaviour of ovipositing females showed that the number of eggs deposited was not influenced by stress related to the females being confined in small experimental arenas with limited numbers of hosts. Differences in behaviour between treatments were a result of differences in the time associated with deposition of male and female eggs and in searching for hosts suitable for male and female development.

5) Samples of hosts from the field, taken from four different sites, and from two different plant species, showed that sex ratios of C. atratus in the field varied from being extremely female-biased to being predominantly male-biased. Changes in the sex ratio were related to changes in the relative abundance of unparasitised and parasitised scale insects.

6) Sex ratios of C. atratus fit no current theory of adaptive sex ratios. The environmental influence of host availability seems to be the main factor determining sex ratios in this species.

7) Data obtained on C. atratus was found not to support the hypotheses of Flanders (1937, 1959) and Zinna (1961, 1962) regarding the adaptive significance of heteronomous hyperparasitism.

REFERENCES

- ANNECKE, D.P. & INSLEY, H.P. 1974. The species of Coccophagus Westwood, 1833 from the Ethiopian region (Hymenoptera: Aphelinidae). Entomology Memoirs. Department of Agricultural Technical Services. Republic of South Africa, 37: 1-62.
- ARTHUR, A.P. & WYLIE, H.G. 1959. Effects of host size on sex ratio, development time and size of Pimpla turionellae (L.) (Hymenoptera: Ichneumonidae). Entomophaga, 4: 297-301.
- ASKEW, R.R. 1975. The organisation of Chalcid dominated parasitoid communities centered upon endophytic hosts. Evolutionary Strategies of Parasitic Insects and Mites. Ed. P.W. Price. Plenum Press, New York.
- ARZONE, A. 1976. Indagini biologiche su Encarsia tricolor Foerst (Hym. Aphelinidae) parassita endofago di Trialeurodes vaporarium Westw. (Hem. Hom. Aleyrodidae). Bolletino di Zoologia agraria e di Bachicoltura, Ser.ii, 13: 119-129.
- AVILLA, J. & ALBAJES, R. 1984. The influence of female age and host size on the sex ratio of the parasitoid Opius concolor. Entomologia experimentalis et applicata, 35: 43-48.
- BARTLETT, B.R. 1964. Patterns in the host-feeding habit of adult parasitic Hymenoptera. Annals of the Entomological Society of America, 57: 344-350.
- BODMER, W.F. & EDWARDS, A.W.F. 1960. Natural selection and the sex ratio. Annals of Human Genetics, 24: 239-244.
- BRAIN, C.K. 1920. The Coccidae of South Africa v̄. Bulletin of Entomological Research, 11: 1-41.
- BROODRYCK, S.W. & DOUTT, R.L. 1966. II. The biology of Coccophagoides utilis Doutt (Hymenoptera: Aphelinidae). Hilgardia, 37: 233-254.

- BRUNSON, M.H. 1934. The fluctuation of the population of Tiphia popilliavora Rohwer in the field and its possible causes. Journal of Economic Entomology, 27: 514-518.
- BRUNSON, M.H. 1937. The influence of instars of host larvae on the sex ratio of the progeny of Tiphia popilliavora Roh. Science, 86: 197.
- BRUNSON, M.H., 1938. Influence of Japanese beetle instar on the sex and population of the parasite Tiphia popilliavora. Journal of Agricultural Research, 57: 379-386.
- CEIANU, I. 1968. Observations sur le Coccophagus gossypariae Gah. (Hym., Aphelinidae) parasite de la cochenille de l'orme - Gossyparia spuria (Mod.)(Hom., Eriococcidae). Revue Roumaine de Biologie Serie de Zoologie, 13: 307-314.
- CENDAÑA, S.M. 1937. Studies on the biology of Coccophagus (Hymenoptera), a genus parasitic on non diaspidine Coccidae. University of California Publications in Entomology, 6: 337-399.
- CHARNOV, E.L. 1979. The genetical evolution of patterns of sexuality: Darwinian fitness. American Naturalist, 113: 465-480.
- CHARNOV, E.L. 1982. The Theory of Sex Allocation. Princeton University Press.
- CHARNOV, E.L., LOS-DEN-HARTOGH, R.L., JONES, W.T. & VAN DEN ASSEM, J. 1981. Sex ratio evolution in a variable environment. Nature, 289: 27-33.
- CHUMAKOVA, B.M. & GORYUNOVA, Z.S. 1963. Development of males of Prospaltella perniciosi Tow. (Hymenoptera, Aphelinidae), parasite of San Jose' scale (Homoptera, Coccoidea). Entomological Review (English Translation of Entomologicheskoye Obozreniye), 42: 178-181.

- CLARK, M.M. 1984. Host searching behaviour of Coccophagus atratus Compere (Hymenoptera: Aphelinidae). Unpublished MSc thesis, Rhodes University.
- CLAUSEN, C.P. 1939. The effect of host size on the sex ratio of hymenopterous parasites and its relation to methods of rearing and colonisation. Journal of the New York Entomological Society, 47: 1-9.
- CLOUTIER, C., McNEIL, J.N. & REGNIERE, J. 1981. Fecundity, longevity and sex ratio of Aphidius nigripes (Hymenoptera: Aphidiidae) parasitising different stages of its host, Macrosiphum euphorbiae (Homoptera: Aphididae). Canadian Entomologist, 113: 193-198.
- COLGAN, P. & TAYLOR, P. 1981. Sex-ratio in autoparasitic Hymenoptera. American Naturalist, 117: 564-566.
- COLWELL, R.K. 1981. Group selection is implicated in the evolution of female-biased sex ratios. Nature, 290: 401-404.
- COMPERE, H. & SMITH, H.S. 1932. The control of citrophilus mealybug, Pseudococcus gahani, by Australian parasites. Hilgardia, 17: 585-618.
- COPLAND, M.J.W. 1976. Female reproductive system of the Aphelinidae (Hymenoptera: Chalcidoidea). International Journal of Insect Morphology and Embryology, 5: 151-166.
- CROZIER, R.H. 1975. Animal Cytogenetics. 3: Insecta 7: Hymenoptera. Gebruder Borntraeger, Berlin.
- DE LOTTO, G. 1974. New species of Filippia Targioni Tozzetti 1868 (Homoptera: Coccidae) from South Africa. Journal of the Entomological Society of South Africa, 37: 207-214.

- DONALDSON, J.S. & WALTER, G.H. 1984. Sex ratios of Spalangia endius Walker (Hymenoptera: Pteromalidae) in relation to current theory. Ecological Entomology, 9: 395-403
- FINNEY, G.L. & FISHER, T.W. 1964. Culture of entomophagous insects and their hosts. In: Biological Control of Insect Pests and Weeds. Ed. P. de Bach. Chapman & Hall, London.
- FISHER, R.A. 1930. The Genetical Theory of Natural Selection. Oxford University.
- FISHER, T.W. 1961. Biology of Physcus sp. (Eulophidae, Aphelininae) from Burma. Journal of Economic Entomology, 54: 444-446.
- FLANDERS, S.E. 1936. Remarkable phenomena of reproduction in the parasitic hymenoptera. Journal of Economic Entomology, 29: 468.
- FLANDERS, S.E. 1937. Ovipositional instincts and developmental sex differences in the genus Coccophagus. University of California Publications in Entomology, 6: 403-422.
- FLANDERS, S.E. 1939a. The propagation and introduction of Coccophagus heteropneusticus Comp. a parasite of Lecaniine scale insects. Journal of Economic Entomology, 32: 888-890.
- FLANDERS, S.E. 1939b. Environmental control of sex in hymenopterous insects. Annals of the Entomological Society of America, 32: 11-26.
- FLANDERS, S.E. 1942. Oosorption and ovulation in relation to oviposition in the parasitic hymenoptera. Annals of the Entomological Society of America, 35: 251-266.
- FLANDERS, S.E. 1952a. A parasite of the European Elm scale established in California. Journal of Economic Entomology, 45: 1078-1079.
- FLANDERS, S.E. 1952b. Biological observations on parasites of the black scale. Annals of the Entomological Society of America, 45: 543-549.

- FLANDERS, S.E. 1956. The mechanism of sex-ratio regulation in the (parasitic) hymenoptera. Insectes Sociaux, 3: 325-334.
- FLANDERS, S.E. 1959. Differential host relations of the sexes in parasitic Hymenoptera. Entomologia Experimentia et Applicata, 2: 125-142.
- FLANDERS, S.E. 1964. "Male producing factor" in the wasp Dahlbominus. Science, 145: 726-727.
- FLANDERS, S.E. 1965. On the sexuality and sex ratios of hymenopterous populations. American Naturalist, 99: 489-494.
- FLANDERS, S.E., BARTLETT, B.R. & FISHER, T.W. 1961. Coccophagus basalis (Hymenoptera: Aphelinidae): its introduction into California with studies of its biology. Annals of the Entomological Society of America, 54: 227-237.
- FLANDERS, S.E., GRESSIT, J.L. & DE BACH, P. 1950. The Glover's scale parasite, Prospaltella elongata established in California. Citrus Leaves, 30: 32-33.
- FORD, R.L.E. 1943. On collecting and rearing parasitic Hymenoptera with special reference to the genus Apantales (Hym. Braconidae). Proceedings of the Royal Entomological Society of London, A, 18: 89-94.
- GERLING, D. & BAR, D. 1971. Reciprocal host-parasite relations as exemplified by Chrysomphjalus aonidum (Homoptera: Diaspididae) and Pteroptrix smithi (Hymenoptera: Aphelinidae). Entomophaga, 16: 37-44.
- GIRI, M.K., PASS, B.C., YEARGAN, K.V. & PARR, J.C. 1982. Behaviour, net reproduction, longevity and mummy stage survival of Aphidius matricariae (Hym. Aphidiidae). Entomophaga, 27: 147-153.

- GORDH, G. & DE BACH, P. 1976. Male inseminative ability in Aphytis lingnanensis (Hymenoptera: Aphelinidae). Canadian Entomologist, 108: 583-589.
- GREEN, R.F. 1980. Optimization of the sex ratio when family size and sex ratio can vary. University of California Technical Report no. 66: 1-33.
- GREEN, R.F., GORDH, G & HAWKINS, B.A. 1982. Precise sex ratios in highly inbred parasitic wasps. American Naturalist, 120: 653-665.
- HAMILTON, W.D. 1967. Extraordinary sex ratios. Science, 156: 477-484.
- HARTL, D.L. 1971. Some aspects of natural selection in arrhenotokous populations. American Zoologist, 11: 309-325.
- HARTL, D.L. & BROWN, S.W. 1970. The origin of male haploid genetic systems and their expected sex ratio. Theoretical Population Biology, 1: 165-190.
- HASSEL, M.P., WAAGE, J.K. & MAY, R.M. 1983. Variable parasitoid sex ratios and their effect on host-parasitoid dynamics. Journal of Animal Ecology, 52: 889-901.
- HERBERS, J.M. 1979. The evolution of sex ratio strategies in Hymenopteran societies. American Naturalist, 114: 818-834.
- HOELSCHER, C.E. & VINSON, S.B. 1971. The sex ratio of a hymenopterous parasitoid, Camponotus perdistinctus, as affected by photoperiod, mating, and temperature. Annals of the Entomological Society of America, 64: 1373-1376.
- HOFVANG, T. & HÅGVAR, E.B. 1975a. Fecundity and oviposition period of Aphidius platensis Brèthes (Hym., Aphidiidae) parasitising Myzus persicae Sulz. (Hom., Apididae) on paprika. Norwegian Journal of Entomology, 22: 113-116.

- HOFVANG, T. & HÅGVAR, E.B. 1975b. Developmental rate, longevity, fecundity and oviposition period of Ephedrus cerasicola Stary' (Hym., Aphidiidae) parasitising Myzus persicae Sulz. (Hom., Aphididae) on paprika. Norwegian Journal of Entomology, 22: 15-22.
- JARRAYA, A. 1975. Contribution à l'étude des interactions hôte parasite chez Coccus hesperidum L. (hom. Coccidae) et son parasite Coccophagus scutellaris Dalman (hym. Aphelinidae). 1. Etude expérimentale du comportement de ponte du parasite. Archives Institut Pasteur de Tunis, 4: 415-456.
- JARRAYA, A. 1977. Note sur le sex-ratio de Coccophagus scutellaris Dalman (Hym. Aphelinidae). Fruits, 32: 607-610.
- KENNET, C.E., HUFFAKER, C.B. & FINNEY, G.L. 1966. III. The role of an autoparasitic Aphelinid, Coccophagoides utilis Doult, in the control of Parlatoria oleae (Colvee). Hilgardia, 37: 255-282.
- KFIR, R. & LUCK, R.F. 1979. Effects of constant and variable temperature extremes on sex ratio and progeny production by Aphytis melinus and A. lingnanensis (Hymenoptera: Aphelinidae). Ecological Entomology, 4: 335-344.
- KOCHETOVA, N.I. 1978. Factors determining the sex ratio in some entomophagous Hymenoptera. Entomological Review (English Translation of Entomologicheskoye Obozreniye), 56: 1-5.
- KOLMAN, W.A. 1960. The mechanism of natural selection for the sex ratio. American Naturalist, 94: 373-377.
- KUWANA, I. 1934. Notes on a newly imported parasite of the spring whitefly attacking citrus in Japan. Proceedings of the 5th Pacific Scientific Congress, 5: 3521-3523.
- LEGNER, E.F. 1977. Temperature, humidity and depth of habitat influencing host destruction and fecundity of Muscoid fly parasitoids. Entomophaga, 22: 199-206.

- LEIGH, E.G. 1970. Sex ratio and differential mortality between the sexes. American Naturalist, 104: 205-210.
- MAYNARD SMITH, J. 1978. The Evolution of Sex. Cambridge University Press, Cambridge.
- MERTINS, J.W. 1980. Life history and behaviour of Laelius pedatus, a gregarious bethylid ectoparasitoid of Anthrenus verbasci. Annals of the Entomological Society of America, 73: 686-693.
- RICE, P.L. 1937. A study of the insect enemies of the San Jose scale (Aspidiotus perniciosus Comstock) with special reference to Prospaltella perniciosi Tower. Abstracts of Doctoral Dissertations, Ohio State University, 24: 267-278.
- SANDLAN, K. 1979. Sex ratio regulation in Coccygomimus turionella Linnaeus (Hymenoptera : Ichneumonidae) and its ecological implications. Ecological Entomology, 4: 365-378.
- SHAW, R.F & MOHLER, J.D. 1953. The selective significance of the sex ratio. American Naturalist, 87: 337-342.
- SKINNER, S.W. 1982. Maternally inherited sex ratios in the parasitoid wasp Nasonia vitripennis. Science, 215: 1133-1134.
- SOKAL, R.R. & ROHLF, F.J. 1981. Biometry. The Principles and Practice of Statistics in Biological Research. 2nd ed. Freeman, San Francisco.
- SPIETH, P.T. 1974. Theoretical considerations of unequal sex ratios. American Naturalist, 108: 837-849.
- SUZUKI, Y., TSUJI, H. & SASAKAWA, M. 1984. Sex allocation and effects of superparasitism on secondary sex ratios in the gregarious parasitoid Trichogramma chilonis (Hymenoptera: Trichogrammatidae). Animal Behaviour, 32: 478-484.

- TAYLOR, P.D. 1981. Intra-sex and inter-sex sibling interactions as sex ratio determinants. Nature, 291: 64-66.
- TAYLOR, P.D. & SAUER, A. 1980. The selective advantage of sex ratio homeostasis. American Naturalist, 116: 305-310.
- TOWER, D.G. 1914. Notes on the life history of Prospaltella perniciosi Tower. Journal of Economic Entomology, 7: 422-432.
- VALENTINE, E.W. 1964. Differential host relations of the sexes in some New Zealand parasitic Hymenoptera. The New Zealand Entomologist, 3: 6-8.
- VAN DEN ASSEM, J. 1971. Some experiments on sex ratio and sex regulation in the pteromalid Lariophagus distinguendus. Netherlands Journal of Zoology, 21: 373-402.
- VAN LENTEREN, J.C. 1981. Host discrimination by parasitoids. In: Semiochemicals: their role in pest control. Eds D.A. Nordlund et.al., Wiley & Sons, New York.
- VAN LENTEREN, J.C., BAKKER, K. & VAN ALPHEN, J.J.M. 1978. How to analyse host discrimination. Ecological Entomology, 3: 71-75.
- VERNER, J. 1965. Selection for the sex ratio. American Naturalist, 99: 419-421
- VIGGIANI, G. 1984. Bionomics of the Aphelinidae. Annual Review of Entomology, 29: 257-276.
- VIGGIANI, G. & MAZZONE, P. 1978. Morfologia, biologia e utilizzazione di Prospaltella lahorensis How. (Hym. Aphelinidae), parassita esotico itrodotto in Italia per la lotta biologica al Dialeurodes citri (Ashm.). Bolletino del Laboratorio di Entomologia Agraria "Filippo Silvestri", 35: 99-161.
- WAAGE, J.K. 1982. Sib-mating and sex ratio strategies in scelionid wasps. Ecological Entomology, 7: 103-112.

- WAAGE, J.K. & MING, N.S. 1984. The reproductive strategy of a parasitic wasp. I. Optimal progeny and sex allocation in Trichogramma evanescens. Journal of Animal Ecology, 53: 401-415.
- WALKER, I. 1967. Effect of population density on the viability and fecundity of Nasonia vitripennis Walker (Hymenoptera: Pteromalidae). Ecology, 48: 294-301.
- WALTER, G.H. 1983a. 'Divergent male ontogenies' in Aphelinidae (Hymenoptera: Chalcidoidea): a simplified classification and a suggested evolutionary sequence. Biological Journal of the Linnean Society, 19: 63-82.
- WALTER, G.H. 1983b. Differences in host relationships between male and female heteronomous parasitoids (Aphelinidae: Chalcidoidea): a review of host location, oviposition and pre-imaginal physiology and morphology. Journal of the Entomological Society of Southern Africa, 46: 261-282.
- WALTER, G.H. 1984. The evolution of heteronomous host relationships in Aphelinidae (Hymenoptera: Chalcidoidea), with special reference to the biology of Coccophagus bartletti Annecke & Insley. Unpublished Phd thesis, Rhodes University.
- WERREN, J.H. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. Science, 208: 1157-1159.
- WERREN, J.H. 1983. Sex ratio evolution under local mate competition in a parasitic wasp. Evolution, 37: 116-124.
- WERREN, J.H. & CHARNOV, E.L. 1978. Facultative sex ratios and population dynamics. Nature, 272: 349-350.
- WERREN, SKINNER, S.W. & CHARNOV, E.L. 1981. Paternal inheritance of a daughterless sex ratio. Nature, 293: 467-468.

- WILKES, A. 1964. Inherited male producing factor in an insect that produces its males from unfertilised eggs. Science, 144: 305-307.
- WILK, B.M. & KITAYAMA, C.Y. 1981. Host stage preference for deposition of male eggs by Coccophagus cowperi (Hym. Aphelinidae). Entomophaga, 26: 313-318.
- WILLIAMS, G.C. 1979. The question of adaptive sex ratio in outcrossed vertebrates. Proceedings of the Royal Society of London, B, 205: 567-580.
- WILLIAMS, J.R. 1972. The biology of Phycus seminotus Silv. and P. subflavus Annecke & Insley (Aphelinidae), parasites of the sugar cane scale insect Aulacaspis tegalensis (Zhnt.) (Diaspididae). Bulletin of Entomological Research, 61: 463-484.
- WILLIAMS, J.R. 1977. Some features of sex-linked hyperparasitism in Aphelinidae (Hymenoptera). Entomophaga, 22: 345-350.
- WILSON, D.S. & COLWELL, R.K. 1981. The evolution of sex ratios in structured demes. Evolution, 35: 882-987.
- WYLIE, H.G. 1966. Some mechanisms that affect the sex ratio of Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae) reared from superparasitised housefly pupae. Canadian Entomologist, 98: 645-653.
- WYSOKI, M. 1977. Overwintering, oversummering, and the effect of extreme temperature and humidity levels on the parasitic wasp Hungariella peregrina (Hymenoptera, Encyrtidae). Phytoparasitica, 5: 15-25.
- ZINNA, G. 1961. Ricerche sugli insetti entomofagi. II. Specializzazione entomoparassitica negli Aphelinidae: Studio morfologico, etologico e fisiologico del Coccophagus bivittatus Compere, nuoro parasitta del Coccus hesperidum L. per l'Italia. Bolletino del Laboratoria di entomologia agraria 'Filippo Silvestri', 19: 301-358.

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. Bolletino del Laboratorio di entomologia agraria 'Filippo Silvestri', 20: 73-182.

APPENDIX: SEX RATIOS OF SPALANGIA ENDIUS

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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

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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,

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 $23 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ r.h. Experiments were continued daily until each female died ($\bar{x} = 5.5$ days; $\text{SE} = 0.13$; $n = 180$). 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

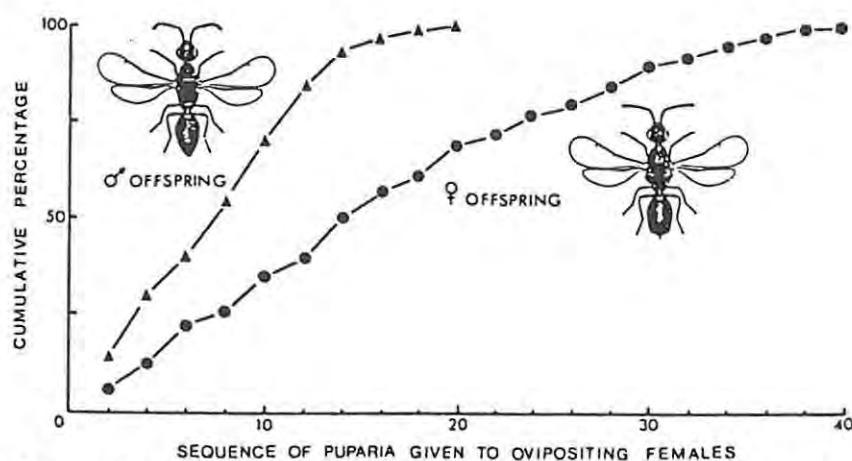


FIG. 1. Cumulative percentages of males and females that emerged from housefly puparia parasitized by *Spalangia endius*. The combined results of 120 females each ovipositing in a sequence of forty housefly puparia are included.

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.

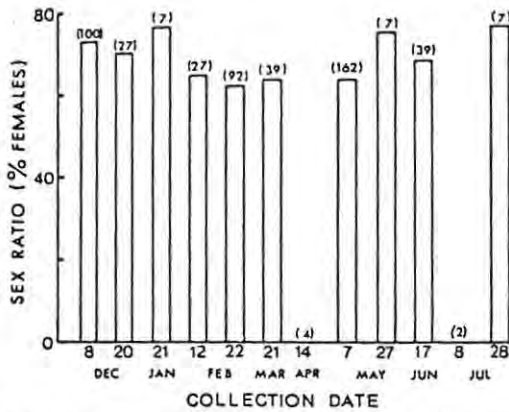


FIG. 2. The sex ratios (% females) of *Spalangia endius* collected from December 1980 to July 1981. (Sampling was continued for a further 6 months but wasp numbers during this period were too low to be included.) Figures in parentheses represent total numbers of *S. endius* collected per sampling occasion.

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).

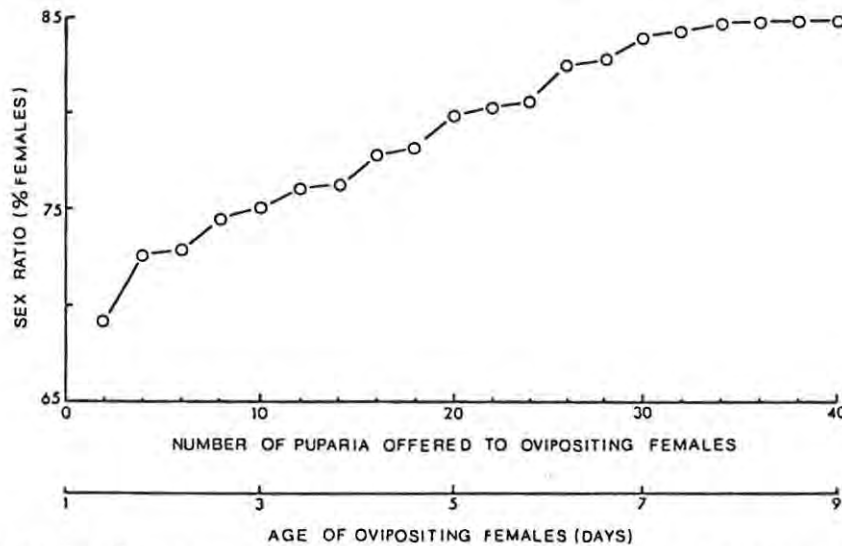


FIG. 3. The effect of female longevity, and therefore number of hosts attacked, on the sex ratio (% females) of *Spalangia endius*. All offspring of 120 females are included, and puparia were presented singly or in twos.

(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 perdinctus* (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 consist-

ently have female-biased sex ratios (e.g. *Hungariella peregrina* Compere; Wysoki, 1977) should be investigated more thoroughly.

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References

- Brunson, M.H. (1937) The influence of the instars of host larvae on the sex ratio of progeny of *Tiphia popilliavora* Rohr. *Science*, **86**, 197.
- Charnov, E.L. (1979) The genetical evolution of patterns of sexuality: Darwinian fitness. *American Naturalist*, **113**, 465–480.
- Charnov, E.L. (1982) *The Theory of Sex Allocation*. Princeton University Press.
- Charnov, E.L., Los-den Hartogh, R.L., Jones, W.T. & van den Assem, J. (1981) Sex ratio evolution in a variable environment. *Nature*, **289**, 27–33.
- Colwell, R.K. (1981) Group selection is implicated in the evolution of female-biased sex ratios. *Nature*, **290**, 401–404.
- Crozier, R.H. (1975) *Animal Cytogenetics. 3: Insecta 7: Hymenoptera*. Gebrüder Borntraeger, Berlin.
- Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*. Oxford University Press.
- Flanders, S.E. (1946) Control of sex and sex-limited polymorphism in the Hymenoptera. *Quarterly Review of Biology*, **21**, 135–143.
- Flanders, S.E. (1965) On the sexuality and sex ratios of hymenopterous populations. *American Naturalist*, **99**, 489–494.
- Hamilton, W.D. (1967) Extraordinary sex ratios. *Science*, **156**, 477–484.
- Hoelscher, C.E. & Vinson, S.B. (1971) The sex ratio of a hymenopterous parasitoid, *Campoletis perdinctus*, as affected by photoperiod, mating, and temperature. *Annals of the Entomological Society of America*, **64**, 1373–1376.
- Hulley, P.E. (1983) A survey of the flies breeding in poultry manure, and their potential natural enemies. *Journal of the Entomological Society of Southern Africa*, **46**, 37–47.
- Legner, E.F. (1976) Low storage temperature effects on the reproductive potential of three parasites of *Musca domestica*. *Annals of the Entomological Society of America*, **69**, 435–441.

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- Legner, E.F. (1977) Temperature, humidity and depth of habitat influencing host destruction and fecundity of Muscoid fly parasites. *Entomophaga*, **22**, 199–206.
- Legner, E.F. & Gerling, D. (1967) Host feeding and oviposition on *Musca domestica* by *Spalangia cameroni*, *Nasonia vitripennis* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) influence their longevity and fecundity. *Annals of the Entomological Society of America*, **60**, 678–691.
- Mackauer, M. (1976) An upper boundary for the sex ratio in a haplodiploid insect. *Canadian Entomologist*, **108**, 1399–1402.
- Morgan, P.B. (1965) Breeding and rearing *Spalangia endius* Walker. *Environmental Entomology*, **4**, 609–611.
- Morgan, P.B., Patterson, R.S. & Le Breque, G.L. (1976) Host parasitoid relationship of the house fly *Musca domestica* L. and the protelean parasitoid, *Spalangia endius* Walker (Hymenoptera: Pteromalidae and Diptera: Muscidae). *Journal of the Kansas Entomological Society*, **49**, 483–488.
- Sandlan, K. (1979) Sex ratio regulation in *Coccygomimus turionella* Linnaeus (Hymenoptera: Ichneumonidae) and its ecological implications. *Ecological Entomology*, **4**, 365–378.
- van den Assem, J. (1971) Some experiments on sex ratio and sex regulation in the pteromalid *Lariophagus distinguendus*. *Netherlands Journal of Zoology*, **21**, 373–402.
- Waage, J.K. (1982a) Sex ratio and population dynamics of natural enemies—some possible interactions. *Annals of Applied Biology*, **101**, 159–164.
- Waage, J.K. (1982b) Sib-mating and sex ratio strategies in scellionid wasps. *Ecological Entomology*, **7**, 103–112.
- Werren, J.H. (1980) Sex ratio adaptations to local mate competition in a parasitic wasp. *Science*, **208**, 1157–1159.
- Werren, J.H. (1983) Sex ratio evolution under local mate competition in a parasitic wasp. *Evolution*, **37**, 116–124.
- Wylie, H.G. (1966) Some mechanisms that affect the sex ratio of *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) reared from superparasitised housefly pupae. *Canadian Entomologist*, **98**, 645–653.
- Wysoki, M. (1977) Overwintering, oversummering, and the effect of extreme temperature and humidity levels on the parasitic wasp *Hungariella peregrina* (Hymenoptera, Encyrtidae). *Phytoparasitica*, **5**, 15–25.

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