Optimal selection and exploitation of hosts in the parasitic wasp *Colpoclypeus florus* (Hym., Eulophidae)

Promotoren: dr. J. C. van Lenteren, hoogleraar in de entomologie, in het bijzonder de oecologie der insekten
dr. K. Bakker, hoogleraar in de dierlijke oecologie aan de Rijksuniversiteit Leiden

L. J. Dijkstra

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PROEFSCHRIFT
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STELLINGEN

1. Een uitspraak over het al of niet optreden van superparasitisme bij gregaire parasitoiden kan pas worden gedaan, wanneer het verband tussen de verhouding van legsel- en gastheergrootte en de totale fitness van het nakomelingschap op een gastheer bekend is. Het sub-maximaal zijn van de fitness van individuele nakomelingen, noch het optreden van mortaliteit hoeft te wijzen op superparasitisme.

   Smith, H.S., 1916. Journal of Economic Entomology, 9: 477-486.
   Salt, G., 1934. Proceedings of the Royal Society (B), 114: 455-476.
   Klomp, H.& J.T. Wiebes (eds), 1979. Sluipwespen in relatie tot hun gastheren. Pudoc, Wageningen, p. 189.
   Lenteren, J.C. van & P. DeBach, 1981. Netherlands Journal of Zoology, 31: 504-532.

2. De opvatting van Vinson dat de ontwikkeling van een geparasiteerde gastheer alleen van belang is voor de parasitoid is onjuist.

   Vinson, S.B., 1975. In: P.W. Price (ed). Evolutionary strategies of parasitic insects and mites. Plenum Press, New York, p. 14.

3. De term "frass" moet worden gereserveerd voor materiaal van de waardplant dat ten gevolge van fourageeraktiviteit van bijvoorbeeld insekten is aangetast, maar niet het darmkanaal is gepasseerd.

   Southwood, T.R.E., 1975. Ecological Methods. Chapman & Hall, Londen. p. 229-230.

4. De sluipwesp Colpoclypeus florus kan plagen van de vruchtbladroller Adoxophyes orana helpen voorkomen, maar kan ze niet bestrijden.

   dit proefschrift.

5. Vleugelpatroon- en kleur van volwassen vruchtbladrollers (Adoxophyes orana) vormen waarschijnlijk een nabootsing van vraatsporen van de rupsen.

6. De vergoedingsregeling van natuurtechnische maatregelen als opgenomen in de Beschikking Bosbijdragen 1983 dient te worden aangevuld met een vergoedingsregeling voor de opbrengstderving, die van het treffen van deze maatregelen het gevolg is.

   Beschikking Bosbijdragen 1983. Ministerie van Landbouw en Visserij. Staatscourant 1983, 244.

7. Door de beslissing om op wildakkers mais te telen wordt de kans gemist om deze tevens als akkeronkruidreservaten in te richten en te beheren.

8. Personeelsadvertenties voor het voortgezet onderwijs vermelden ten onrechte niet de maximaal gewenste diensttijd van de kandidaat.

9. Op grond van de door Bakker & Boeve aangevoerde argumenten verdient het gebruik van het woord stinsplant de voorkeur boven stinzenplant.

   Bakker, P.& E. Boeve, 1985. Stinzenplanten. Terra, Zutphen.

10. Gezien de onjuiste suggesties die aangaande het vóórkomen van de poelsnip en de zonnebloem in ons land worden gedaan door de Nederlandsche Bank, heeft deze instelling behoefte aan biologische deskundigheid.

L.J. Dijkstra
"Optimal selection and exploitation of hosts in the parasitic wasp Colpoclypeus florus (Hym., Eulophidae)"

Wageningen, 13 juni 1986.

„Be warned that if you wish, as I do, to build a society in which individuals cooperate generously and unselfishly towards a common good, you can expect little help from biological nature. Let us try to ***teach*** *generosity and altruism, because we are born selfish. Let us understand what our own selfish genes are up to, because we may then at least have the chance to upset their designs, something which no other species has ever aspired to."*

Richard Dawkins –
The Selfish Gene

Aan mijn ouders

Summary

This study deals with the question how an insect parasitoid can maximize its fitness through adaptation of its reproductive behaviour. It concentrates on the behaviour of a parasitoid after it has encountered a host. Optimal exploitation of individual hosts is emphasized rather than a maximization of the number of parasitized hosts.

In Chapter I the topic of optimization of behaviour is introduced in relation to the study of insect parasitoids. The choice of the experimental animals is explained and behavioural alternatives of the parasitoid are discussed. In this study the number of granddaughters is taken as a measure of fitness.

The chalcidoid wasp *Colpoclypeus florus* (Hym., Eulophidae) is a gregarious ectoparasitoid of larvae of at least 32 species of leafrollers (Lep., Tortricidae; Table 2.1). Host plants are predominantly trees and shrubs. The parasitoid has a west palearctic distribution (Fig. 2.1) and is rare in natural or semi-natural habitats. However, *C. florus* can be found in abundance in intensively cultivated habitats. In the Netherlands they are found especially in apple orchards, during outbreaks of the summer fruit tortrix moth, *Adoxophyes orana*. Efforts to control *A. orana* with mass releases of the parasitoid had not been successful. However, the parasitoid is considered as promising by those working on integrated control and more biological information was required.

In Chapter 2 the parasitization behaviour, development and phenology of the parasitoid is described. The experimental host (*A. orana*), general techniques and conditions are also described. Field experiments were carried out in an experimental apple orchard. Unlike many internal and external parasitoids, *C. florus* has the unusual habit of ovipositing beside instead of on or in the host. This offers the opportunity to manipulate the eggs and hosts separately. In addition, the number of hosts parasitized by an individual

in the field is low, about 2-3 hosts per female, and the time taken to parasitize one host is long (average 13-28 h in the laboratory at 21 °C and about twice as long in the field, in summer). Thus, *C. florus* is particularly suitable for studies on how it optimizes exploitation of individual hosts.

Three stages in the parasitization process were analysed in detail.

(a) The first problem concerned the host size selection for oviposition (Chapter 3 and 4). It was hypothesized that only the most profitable hosts are selected for oviposition. Only the first of five larval instars of *A. orana* is rejected for oviposition by the parasitoid. In the laboratory, proportion of hosts accepted, clutch size, survival of pre-adults, proportion females and parasitization time increase with host weight (Tables 3.3 and 3.5). As a result the profitability of hosts (defined as the fitness gained per unit of time or per egg) is correlated with host acceptance, but the profitability threshold of host acceptance is low (Fig. 3.2). It was shown that this threshold is not influenced by changes in the length of the pre-encounter period with hosts. *C. florus* is assumed to be unable to measure host density, therefore size preference may be genetically fixed and be an adaptation to low host densities. Host acceptance is the same in the laboratory and the field. In the field, however, nearly all parasitized hosts are fourth and fifth larval instars (Table 5.5). It was shown that the parasitoids during the host-habitat location phase have a preference for the young leaves in the outer layer of the canopy where the larger hosts predominate (Fig. 4.4). These larger hosts are also more conspicuous to the parasitoids during host location. The combined effect of host-habitat location and host location could explain why in the field few small hosts are parasitized resulting in a host size selection favouring the most profitable host sizes (Table 4.7). Assuming a genetically fixed optimal host choice, a first theoretical estimate of the host encounter rate in the field was made.

(b) The second question dealth with how many eggs were to be laid near each selected host (Chapter 5). It was hypothesized that the clutch size would maximize the profitability from a selected host. The parasitoid was found to adapt her clutch size to the weight of the host, apparently using a factor related to the host width. This results in a curvi-linear relationship between host weight and clutch size (Fig. 5.2). Experimentally the number of eggs per host was manipulated, and the number and individual weights of offspring were determined. Although pre-adult mortality in the laboratory, as well as in the field is high (50-60 %), density dependent mortality of juveniles does not occur in the normal clutch sizes (Fig. 5.16). However, competition for food among the juveniles always occurs, which results in body weights attained not being maximal (Fig. 5.17). Longevity and fecundity of a female are positively related to her weight (Fig. 5.12 and 5.14). Thus the clutch size per host size ratio affects total longevity and fecundity of the offspring. Two strategies are discussed. The first requires a low number of eggs per host and results in maximum offspring fecundity per egg laid. The second requires a high number of eggs per host and results in maximum offspring longevity per parasitized host. It was shown that *C. florus* produces a clutch size per host size ratio which cannot be explained by either of these strategies. It is assumed that female parasitoids will obtain just that body weight which will allow her to invest all her eggs during her lifetime. With this assumption, a second theoretical estimate of the host encounter rate in the field was made. This second estimate falls within the range of that assuming optimal host choice. Therefore, host size selection and clutch size per host size ratio are both optimal in the same range of host encounter rate.

(c) The third problem concerned that of sex allocation (Chapter 6). The sex ratio of adult *C. florus* is female biased and the proportion of males decreases with increasing clutch size (Table 6.5). The number of males per clutch is

constant, or may slightly increase with increasing clutch size. During development males and females do not suffer differential mortality (Table 6.3). The insemination capacity of males increases with increasing body weight (Fig. 6.2). A male has sufficient time available and, assuming that he has obtained a mean body weight, he has just enough insemination capacity to inseminate all sisters of his clutch even in the largest clutches. It is suggested that fitness of males cannot be increased by a higher body weight, since females outside the clutch are seldom encountered. Using a newly developed cytological technique, it was demonstrated that male eggs are laid at the end of an ovipositional sequence and not at random throughout egg laying (Fig. 6.4). This points to a precise sex ratio mechanism. A model assuming such a mechanism, and using the information that one adult male from a clutch of eggs is sufficient to inseminate all females, accurately predicted the actual number of unfertilized eggs in a clutch (Fig. 6.6). It is suggested that in species where host size is clearly limited and is estimated by the parasitoid before oviposition males are allocated at the end of a clutch.

In Chapter 7 the main conclusions are given and discussed. This study gives insight into how a parasitic wasp tackles the different difficulties which arise in optimizing its reproductive behaviour. Although constraints operate at different levels of the parasitization process, clear examples of optimal behaviour (as defined in this study) are found in *C. florus*. For an understanding of some aspects of the behaviour, the information collected about the field situation appeared to be essential.

Contents

1. INTRODUCTION

1.1 Optimization of behaviour in parasitic wasps

Evolution by means of natural selection favours those organisms best adapted to their environment, whether in morphology, physiology or behaviour. Ultimately, natural selection operates only through differential reproductive success, thus eventually adaptation can only be expressed in terms of reproducing offspring. The ability to survive and to reproduce, generally referred to as fitness, is maximized by natural selection.

Since the environment is subject to change, an organism has also to change to remain well adapted to its environment. If it is assumed that the rate of change in individuals required to reach maximum fitness in a certain environment is greater than the rate of change of the environment itself, then the average adaptation of a population to its environment should be similar to that which results in maximum fitness.

The present study deals with the question how an insect parasitoid can maximize its fitness through adaptation of its reproductive behaviour.

Specifying the behaviour which leads to the highest reproductive success is a problem of optimization. In reviewing optimal foraging theories, Schoener (1971) defined three steps in the procedure for finding the optimal solution for a certain behavioural trait:

1) choosing a currency: what is to be maximized (or minimized) to maximize fitness?
2) choosing the appropriate cost-benefit functions: what is the mathematical form of the set of expressions with the currency as the dependent variable?
3) solving for the optimum: what are the extrema of the cost-benefit function?

Insect parasitoids are suitable subjects for testing optimization of behaviour, because foraging and reproduction

in these insects are closely linked. In most studies on optimal foraging of predators, the net rate of energy intake is assumed to be a measure of reproductive success (Pyke, Pulliam & Charnov, 1977) and there are good arguments for the selection of this as a currency (Schoener, 1971). But this assumption is unnecessary if the result of behavioural alternatives can be measured in terms of differential reproductive success itself.

Much attention has been paid to the study of host-searching behaviour, in which it is assumed that the number of parasitized hosts is maximized (Hubbard & Cook, 1978; Waage, 1979; Galis & Van Alphen, 1981; Stamp, 1982). This leaves the question unanswered to what extent exploitation of the host, once it is parasitized, can be considered to be optimal. The present study focuses on the behaviour of a parasitoid after it has encountered a host, and the benefit of possible behavioural alternatives is expressed in a currency which is the best possible measure of fitness (see Sections 1.2 and 1.3).

The gregarious ectoparasitoid *Colpoclypeus florus* (Walker) has been used as the experimental animal. Unlike many internal and external parasitoids, *C. florus* has the unusual habit of ovipositing beside the host instead of on or in it. This provides the opportunity in experiments to manipulate both parasitoid eggs and hosts without disturbing either. For instance, parasitoid clutch size and host size can be varied independently. Also, the effect of stinging the host and the effect of the presence of eggs on host mortality and host discrimination can be studied separately. In addition, the number of hosts parasitized by an individual female is low, and the time taken to parasitize one host is long. Thus, this parasitoid is particularly suitable for studies on optimization of the exploitation of individual hosts rather than a maximization of the number of parasitized hosts. This choice of both parasitoid and host also resulted from the need expressed by those working on integrated control of pests for more biological information about the

parasitoids of an important pest insect, the summer fruit tortrix moth, *Adoxophyes orana* (Fischer von Röslerstamm). This is a persistent pest in apple trees (De Jong, 1980; Gruys, 1982). Its parasitoid complex has been studied extensively in several European countries, and *C. florus* has been found to be one of its commonest and most widespread parasitoids. However, efforts to control *A. orana* with mass releases of this parasitoid have not been successful (Gruys, 1982).

Although several accounts of the life history of *C. florus* have been published (Janssen, 1958; Karczewski, 1962; Dalla Montà & Ivancich Gambaro, 1973), in the course of the present study additional biological information was collected. Since most of this information is of relevance to interpretation of the results of the present study, it is presented in Chapter 2.

1.2 Behavioural alternatives for the exploitation of hosts

The cost-benefit functions which are to be determined and finally solved for the optimum, depend largely on the range of possible behaviour and the constraints which operate on the animal.

When an inseminated gravid female *C. florus* encounters a larva of *A. orana*, firstly, she either accepts or rejects the host for oviposition. This may depend on the following factors:

- preference of the parasitoid for hosts of a certain size and stage of development;
- preference of the parasitoid for the period of time elapsed since the last moult of the host;
- defensive behaviour of the host;
- acceptance of hosts which have already been parasitized (host discrimination).

In this study, hosts were standardized so that they were offered to the parasitoids unparasitized and 1-2 days after

moulting. Thus only the effects of the preference for host size and stage of development and the defensive behaviour of the host were investigated.

In theories of optimal diet choice, the density of the most profitable host types influences the decision to accept less profitable host types (Mac Arthur & Pianka, 1966; Krebs, 1978, for a review). In the present study, it was assumed that the parasitoid had no opportunity to measure the required densities because of the generally low number of host encounters under field conditions. This assumption is discussed in Chapter 3.

After acceptance of a host the female needs to determine how many eggs to lay at one parasitization. Gregarious parasitoids are known to produce clutches of varying size. Usually, the size of a clutch is related to the host size, but as yet the ratio of clutch size to host size in gregarious parasitoids has not been shown to be optimal.

This problem is very similar to that discussed by Pianka (1976): whether the limited amount of material for egg production should be divided among a lot of small eggs or a few large eggs. In the present study, the size of the egg has been taken to be constant, but the size of the resulting adult to vary with the amount of host material allocated. The size of adult insect parasitoids is known to vary widely within one species (see for instance, Wilbert, 1965). Thus the problem largely concerns the relationship between the size of an adult and its reproductive success. The proportion of reproductive and non-reproductive tissue of adult weight (whether optimal or not) is not considered to be affected by parasitoid behavioural alternatives.

Finally, the parasitoid needs to decide the sex ratio of the eggs produced. Most hymenopterous insects have an arrhenotokous sex determination mechanism and the number of fertilized eggs is influenced by many factors (Kochetova, 1974). Arrhenotoky has no genetic advantage over thelytoky in obligatory sibmating (that is, mating between brothers and sisters) while in thelytoky the allocation of host material

to males is not necessary. However, in this study the sex determination system is excluded from the problem of optimization and considered to be a constraint of the studied animal.

As these three decisions i.e. host acceptance, number of eggs and sex ratio influence the fitness of the parasitoid and its offspring, fitness needs to be measured as a function of these behavioural alternatives.

1.3 How to measure fitness

Maximizing fitness not only means that the number of offspring should be maximized, but also the reproduction capacity of the progeny. This makes certain demands on characteristics such as sex, fecundity, longevity, the skill to find and to parasitize hosts. Since only females search for hosts and oviposit near them, and since they can only do this by investing time and eggs, the offspring should be mainly daughters, which together represent the maximum total longevity and fecundity. These daughters have to be inseminated to be able to produce females in their turn. Consequently, a minimum proportion of the offspring have to be sons. By maximizing the total longevity, fecundity and chance of insemination of her daughters, the parent wasp indirectly maximizes the number of her granddaughters. Assuming that the behaviour of mother and daughter will be same in this respect, the number of granddaughters represents the ultimate reproductive success of a decision-making wasp, and thus is a measure of its fitness.

2. DESCRIPTION OF PARASITOID, HOST AND ENVIRONMENTAL CONDITIONS

2.1 Colpoclypeus florus

2.1.1 Nomenclature and descriptions

This species was first described by Walker (1839: 127) under the name *Eulophus florus* for the British specimen. It was described in more detail by Lucchese (1941), who thought it to be both a new genus and a new species, *Colpoclypeus silvestrii*. This name was widely used during the 1950s and early 1960s even though the material from Italy used by Lucchese appeared to be morphologically identical to that described by Walker (Graham, 1959). The combination, *Colpoclypeus florus* (Walker) made by Graham is now commonly used. It is the only species of the genus.

All stages have been described and drawings of the egg, larval stages, pupa and adult are presented by Lucchese (1941: 34-39).

2.1.2 Geographical distribution and habitat

Colpoclypeus florus has a west palearctic distribution (Fig.2.1). In addition to records of the species on the Isle of Wight, Great Britain (Walker, 1839) and Province of Campania, Italy (Lucchese, 1941), it has also been found in Hongary (Erdös, 1951), Poland (Erdös, 1951; Karczewski, 1962), Öland, Sweden (Jansson, 1954), West Germany (Janssen, 1958), Czechoslovakia (Boucek, 1959), East Germany (Auersch, 1960),Montpellier, France (C.I.L.B., 1960), Moldavian S.S.R. (Talitzki, 1961), Morocco (Delucchi, 1962), Veronese, North Italy (Ivancich Gambaro, 1962), Bulgaria (Nikolova & Natskova, 1966), Ukrainian S.S.R. (Pykhova, 1968), Azerbaidzhan and Armenian S.S.R. (Boucek & Askew, 1968), East Spain (Limon de la Oliva & Blasco Pascual, 1973; Albajes, Bordas & Vives, 1979), the Netherlands (Evenhuis, 1974), Switzerland and

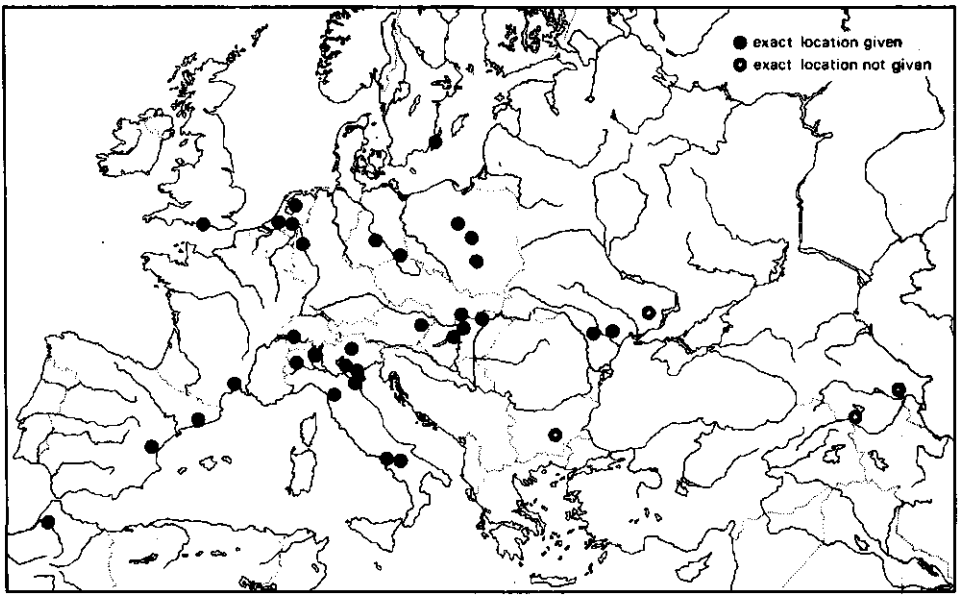

Fig. 2.1 Geographical distribution of *Colpoclypeus florus* based on published records.

Austria (Carl, 1976).

Most published records concern intensively cultivated habitats such as orchards, vineyards and strawberry fields where under certain conditions *C. florus* can be found in abundance. In natural or semi-natural habitats, the species is rarely observed and is likely to occur at a very low density. The exception to this is the reference of Karczewski (1962) to the species being numerous in the deciduous undergrowth of a pine forest in Poland. In the Netherlands, *C. florus* has been reported only once outside orchards and surrounding windsheds, in spite of an intensive search during the last decade (H.H. Evenhuis and M.J. Gijswijt, pers. comm.).

2.1.3 Hosts and host plants

Although polyphagous, *C. florus* is confined to hosts of a small number of related genera almost exclusively of the

family of leafrollers (Lep., Tortricidae). Host plants are all flowering plants which do not, however, belong to a special systematic group but are predominantly trees and shrubs. The hosts and host plants when known are listed in Table 2.1.

2.1.4. Host searching

C. florus is a gregarious ectoparasitoid on the larvae of the host species given in Table 2.1. The caterpillars of the Tortricidae are characterized by their tunnel-shaped webs which they make by rolling a single leaf or by joining together several leaves of the host plant. The tunnel has one or more openings on both sides to allow the caterpillar to feed on the surrounding leaf or leaves (Fig. 2.2d).

Little is known about the way *C. florus* finds its host. Field observations are difficult because the wasps are very small, and not easy to follow during their searching behaviour. Laboratory studies have indicated that searching behaviour is a long lasting procedure at least in the laboratory, even though host and parasitoid are fairly near to each other as compared with the field situation. Damaged apple leaves most probably are an attractant (J.C. van Veen, pers. comm.). The effect of the size of the host and its location on the host plant on host-searching behaviour are discussed in Chapter 4.

2.1.5 Parasitization

The following description of parasitization is based mainly on observations in the laboratory under experimental conditions (see Section 2.3) with fourth larval instars of *A. orana* as hosts.

Following an encounter with a complex of host and host plant, the female wasp shows an arrestment response (Vinson, 1975), which is most likely in response to olfactory stimuli.

Table 2.1 Hosts* and host plants of *Colpoclypeus florus*

Host**	Host plant***	Reference****
Tortricidae, Tortricinae		
Acleris schalleriana (Linnaeus)		Bouček, 1959; Čapek, 1963
A. lipsiana (Denis & Schiffermüller)		Karczewski, 1962
A. maccana (Treitschke)		Karczewski, 1962
Adoxophyes orana (Fischer von Röslerstamm)	*Malus sylvestris* Miller (apple)	Janssen, 1958
	Pyrus communis L. (pear)	Dalla Montà & Ivancich Gambaro, 1973
Aphelia paleana (Hübner)		Bouček & Askew, 1968
Archips xylosteana (Linnaeus)		Bouček, 1959; Talitzki, 1961
A. crataegana (Hübner)	*Corylus avellana* L. (hazel)	Russo, 1966
A. rosana (Linnaeus)		Talitzki, 1961
A. podana (Scopoli)	apple	Janssen, 1959
Argyrotaenia pulchellana (Haworth)	apple	C.I.L.B., 1960; Ivancich Gambaro, 1962
	pear	Dalla Montà & Ivancich Gambaro, 1973
	Prunus domestica L. (plum)	L. dalla Montà (pers. comm.)
Cacoecimorpha pronubana (Hübner)	*Citrus* spp.	Limon de la Oliva & Blasco Pascual, 1973
Choristoneura lafauryana (Ragonot)	*Fragaria x ananassa* Duch. (strawberry)	Ivancich Gambaro, 1968
Cnephasia stephensiana (Doubleday)		Karczewski, 1962
Clepsis spectrana (Treitschke)	apple	Gruys & Vaal, 1984
Epichoristodes acerbella (Walker)	*Dianthus caryophyllus* L. (carnations)	Fenili, 1977
Eulia ministrana (Linnaeus)		Karczewski, 1962
Lozotaenia forsterana (Fabricius)		Karczewski, 1962
Pandemis corylana (Fabricius)	apple	Domenichini, 1963
P. dumetana (Treitschke)	strawberry	Ivancich Gambaro, 1968
P. cerasana (Hübner)		Bouček, 1959; Talitzki, 1961
P. heparana (Denis & Schiffermüller)	apple	Janssen, 1959
	Prunus persica Batsch (peach)	Scaramozzino & Ugolino, 1979
Philedonides lunana (Thunberg)		Karczewski, 1962
Ptycholoma lecheana (Linnaeus)	apple	Gruys & Vaal, 1984
Sparganothis pilleriana (Denis & Schiffermüller)	*Vitis vinifera* L. (vine)	Pykhova, 1968
Syndemis musculana (Hübner)		Bouček, 1959; Karczewski, 1962
Tortricidae, Olethreutinae		
Ancylis comptana (Frölich)	strawberry	Ivancich Gambaro, 1968
A. myrtillana (Treitschke)	*Vaccinium myrtillus* L. (bilberry)	Karczewski, 1962

Table 2.1 (cont.)

Host**	Host plant***	Reference****
Griselda myrtillana (Humphreys & Westwood)	bilberry	Karczewski, 1962
Gypsonoma minutana (Hübner)	*Populus* sp. (poplar)	Delucchi, 1962
Hedya nubiferana (Haworth)	apple	Domenichini, 1963
Olethreutes lacunana (Denis & Schiffermüller)	bilberry	Karczewski, 1962
	strawberry	Ivancich Gambaro, 1968
Pardia cynosbatella (Linnaeus)	*Rosa damascena* Miller (oil-bearing rose)	Nikolova & Natskova, 1966
Rhopobota unipunctana (Haworth)	apple	Lucchese, 1941
Spilonota ocellana (Denis & Schiffermüller)		Talitzki, 1966
Cochylidae		
Eupoecilia ambiguella (Hübner)		Talitzki, 1966
Zygaenidae		
Adscita budensis Speyer & Speyer		Bouček & Askew, 1968

* Names of hosts are according to Lempke (1976).

** A host-record by Erdös (1951) of larvae of *Rhabdophaga rosaria* (Loew) (Dipt., Cecidomyiidae) is withdrawn by himself (see: Jansson, 1954), while a record of larvae of *Athalia cordata* Lepeletier (Hym., Tenthredinidae) as host of '*Colpoclypeus* sp.' (Nikolova, 1972) can be regarded as at least doubtful.

*** The following references were found without the precise name of the host:
Salix sp. (willow) - Walker, 1839; Erdös, 1951.
Microlepidopteron sp. - *Rosa* sp. (rose) - Jansson, 1954.
Fagus sylvatica L. (beech) - Erdös, 1956.
Acleris sp. - *Quercus* sp. (oak) - Bouček, 1959; Čapek, 1963.

**** The two host-records by Gruys & Vaal (1984) are from wasps released in spring.

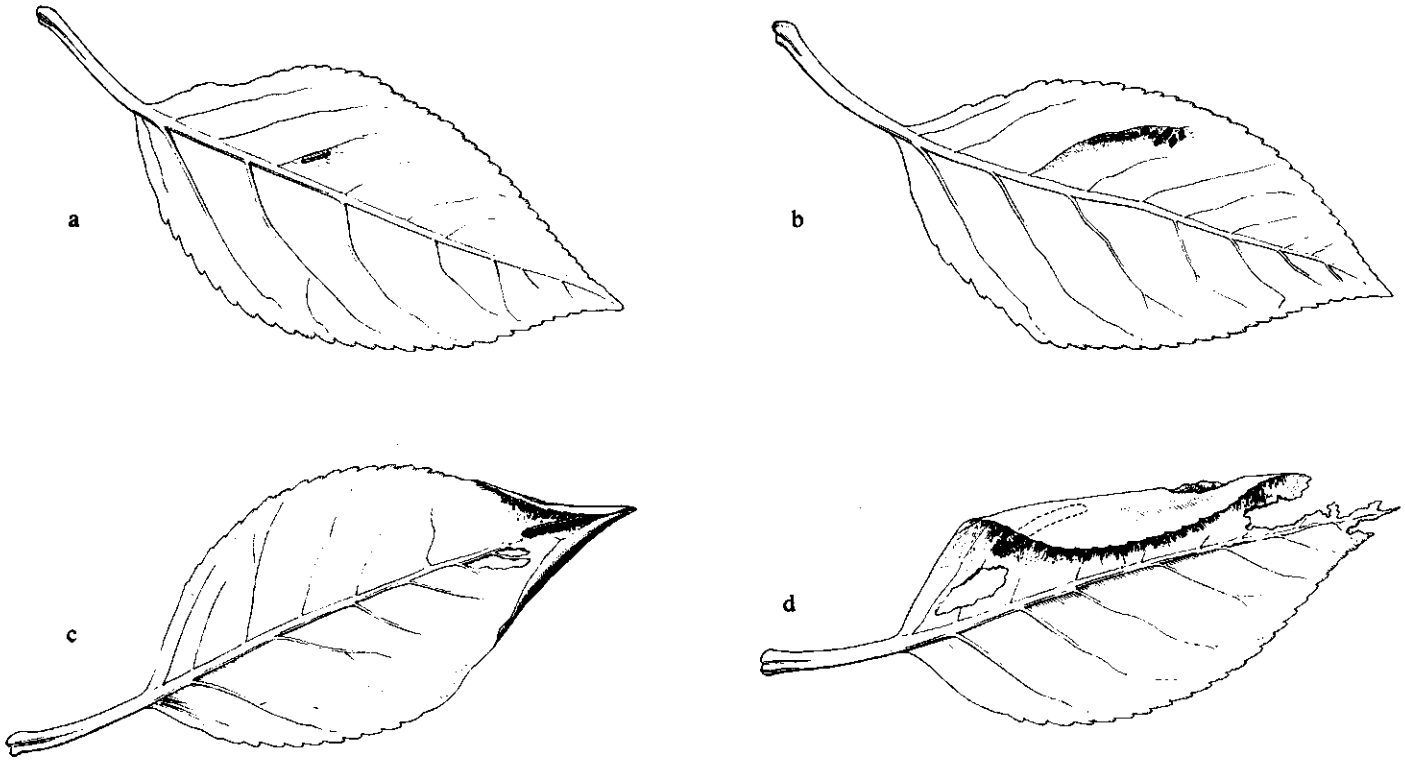

Fig. 2.2 Apple leaves with typical web forms of the larvae of *Adoxophyes orana*: a. first larval instar, b. second larval instar, c. early fourth larval instar, d. early fifth larval instar.

This response consists of short periods of immobility during which the antennae may be alternately slowly moved up and down, alternated with periods of walking during which the antennae are moved quickly.

When the wasp finds an entrance to a tunnel, she may remain there for some time, but may continue walking if the host is foraging through another opening at that time. When the host approaches the parasitoid while feeding or spinning, the wasp shrinks back, often bringing the ovipositor into its sting position (Fig. 2.3). Direct contact between host and parasitoid is not necessary to evoke this sting posture. A slowly moving pointed object, such as a needle or a pencil, can also act as a stimulus for this response in this stage of parasitization. Sometimes even motionless objects, such as droppings of the host, can serve as an olfactory stimulus. Nevertheless, the visual stimuli of the moving host are regarded as the cue for the sting posture, once the wasp is in constant range of the strong olfactory stimuli of the complex of host and host plant.

Fig. 2.3 Female of *Colpoclypeus florus* displaying sting posture to a host, fourth instar larva of *Adoxophyes orana*.

Usually, a wasp can strike the host with its ovipositor from a position on the edge of the web near to one of the entrances but sometimes it bites a new opening in the web. Stinging the host itself is done in short volleys of numerous stings, each volley lasting approximately one second. Usually, it is difficult to see whether the host has been penetrated, but sometimes the piercing of the head capsule is very obvious, namely when the wasp has to retract its ovipositor by pulling or when the host responds to the sting with a trembling movement of the frontal part of the body. All host stinging is directed to the head capsule of the caterpillar, but no preferential area could be distinguished within the head capsule.

Host stinging may be completed within an hour after encountering a complex of host and host plant, but usually it continues at short intervals until after the first egg is deposited. It is not clear whether more than one strike is necessary for parasitization to result in adult offspring. However, a host which is not stung is unsuitable for further parasitoid development. When eggs are transferred to such a host, the hatched larvae do not settle on the host.

A stung host from which the eggs have been removed dies after about a month in a characteristic prepupal-like shape and position without having passed through further moults. Apart from the trembling mentioned above, the short-term effect on the host of stinging is expressed in quantitative behavioural changes only after three days. Thus, if a substance is injected during host stinging, it is not directly paralyzing or deadly poisonous to the host, as is reported in the case of *C. florus* parasitizing *Choristoneura lafauryana* (Dalla Montà & Ivancich Gambaro, 1973). However, qualitative behavioural changes are visible within a few hours after host stinging. The host becomes increasingly insensitive to mechanical disturbance, which allows the parasitoid to enter the tunnel-shaped part of the web. Moreover, the host remodels this tunnel into a closed cocoon by the time the parasitoid eggs have hatched.

In the fairly long period subsequent to the first stinging of the host, the wasp exhibits behaviour which in outline is not very different from that immediately following the first encounter and which is directed towards the host and its web. Host measuring (see Chapter 5) and host discrimination are assumed to take place during this period.

Usually the first egg is laid between 8 and 24 h after encounter. The eggs are always laid in the web. When the host is remodelling his web, eggs may become stuck to his skin or setae by chance, explaining why wasps sometimes were thought to lay eggs on the hosts (Lucchese, 1941, on *Rhopobota unipunctana*).

Before oviposition, the wasp probes the web in several places. She extends her ovipositor between the threads of the web with short backward and downward movements of her body, and the ovipositor can sometimes be seen probing the web laterally. When she has found an appropriate place, the probing procedure is followed by egg laying.

Without changing posture, she lays one or more often two eggs between the threads of the web. An egg does not pass through the entire length of the ovipositor but leaves it in its proximal part (Fig. 2.4). The probing procedure then starts again and is followed by another oviposition. This is

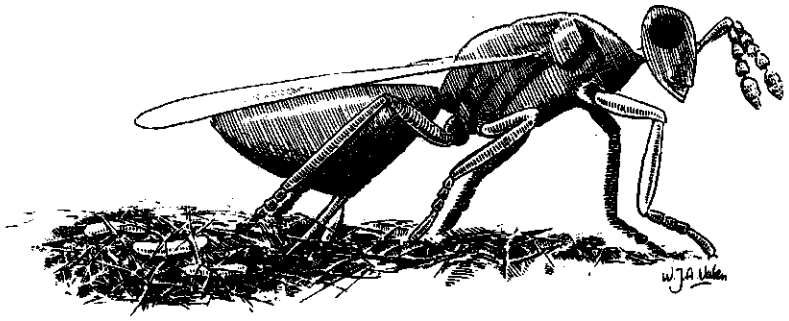

Fig. 2.4 Ovipositing female of *Colpoclypeus florus* with ovipositor in probing position and egg leaving the proximal part of the ovipositor.

continued until all the eggs have been deposited. Laying on average five eggs per hour, the wasp lays in total one to 48 eggs depending on the host size, in one or more loose clusters. Shortly after completion of oviposition, the wasp leaves the host and its web. The average length of the period between encounter and departure (parasitization time) is 13 to 28 h, depending on host size.

2.1.6 Development

A scheme of development of *C. florus* under laboratory conditions (see Section 2.3) is shown in Fig. 2.5. Eggs hatch 2-3 days after oviposition, and the freshly hatched larvae can be found on the host within a few hours. They move actively to the inner surface of the cocoon and are picked up accidentally by the setae of the moving host or they crawl along the legs or setae of the host. The wasp larvae settle on the outside of the integument, preferentially in the intersegmental folds and the spaces between the thoracic and

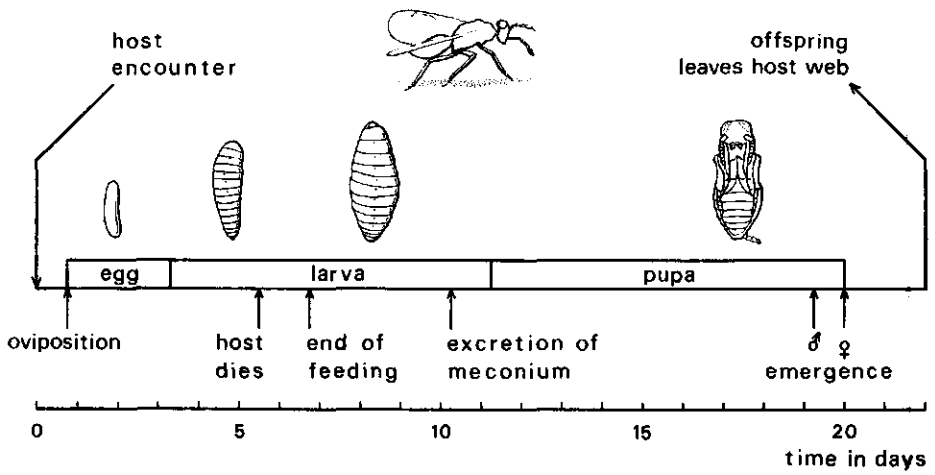

Fig. 2.5 Scheme of development of *Colpoclypeus florus* under laboratory conditions at a temperature of 21°C.

the prolegs. They move regularly to new sucking spots, leaving small black scars on their host. Within a very short period the larvae become the colour of their host, mostly bright or yellowish green, and continue to feed for about 3.5 days. At the beginning of this feeding period, the host is already very lethargic as a result of the delayed effect of host stinging. However, the direct cause of death is the continuous sucking of the parasitoid larvae. The host dies in the second half of the feeding period.

Cannibalism of feeding larvae in cases of food shortage has been mentioned by Janssen (1958), but this was not observed during the present study. The end of the feeding period is marked by the fact that the larvae are no longer attached to the host by their mouth parts. Their gradual change in colour from green to grey indicates that digestion and the formation of tissues are proceeding. About 3.5 days after the end of the feeding period, the meconium is excreted and white prepupae are formed.

A prepupal stage of about one day is followed by a pupal stage of approximately eight days, during which the pupae gradually become dark brown in colour. As a result of crawling in the grey larval stage, the prepupae and pupae are scattered throughout the cocoon, attached to it by the hardened meconium, and in the case of the pupae, especially by the sticky remnants of the larval-pupal moult.

2.1.7 Emergence and mating

Emergence of adult wasps is protandric (males first), and time difference is less than one day. Sex ratio is spanandrous (female biased), and males are smaller than females. Although observations have been published suggesting facultative thelytokous parthenogenesis (Dalla Montà & Ivancich Gambaro, 1973), during the present study evidence was collected pointing to an arrhenotokous parthenogenetic sex determination (see Chapter 6).

Courtship, copulation and insemination of the females

takes place in the cocoon of the host (see Chapter 6), and after several days the adults escape through holes that they make in the web with their mandibulae.

2.1.8 Temperature sensitivity

Temperature is a decisive factor in the length of the parasitization period, the duration of pre-adult development, and the duration of the period in the cocoon as newly emerged adults (Table 2.2). The mean development period between oviposition and emergence is given as 13-14 days at 26°C by Dalla Montà & Ivancich Gambaro (1973).

Table 2.2 Parasitization time, duration of pre-adult development and duration of the stay in the cocoon as newly emerged adults of *Colpoclypeus florus* at various constant temperatures

Temperature (°C)	Parasitization time (hours)	(n)	Pre-adult development (days)	(n)	Duration of stay in cocoon (days)	(n)
	mean ± SE		mean ± SE		mean ± SE	
12	123 ± 3.3	41	40 ± 0.85	19	7.8 ± 0.51	16
15	113 ± 4.0	41	29 ± 0.45	35	4.3 ± 0.41	34
18	47 ± 2.5	42	20 ± 0.30	34	3.1 ± 0.27	34
21	18 ± 1.3	47	19 ± 0.18	44	2.7 ± 0.14	38
24	15 ± 1.2	43	13 ± 0.13	39	2.2 ± 0.27	39
27	12 ± 0.9	44	11 ± 0.13	21	2.6 ± 0.26	21

2.1.9 Life span and food of the adult parasitoid

The reported average life span of female wasps in the laboratory varies widely, depending on the temperature, availability of honey, and opportunity for oviposition (Table 2.3). A constant supply of honey can lengthen the life span considerably, but the supply of water seems to have little effect on it. Higher temperatures and opportunity for oviposition may have a shortening effect on the life span. The effect of body size of females on their life span is discussed in Chapter 5. For males, life span is shorter under comparable laboratory conditions. Host feeding is never observed.

Table 2.3 Average life span of females of *Colpoclypeus florus* under various experimental conditions given as the approximate time-intervals (days) at 50% survival

Experimental conditions				Average lifespan (days)	Number of females tested	Reference
honey	water	hosts	temp. (°C)			
first 2 days only	-	-	20	5	175	Soenarjo, 1979
first 2 days only	ad lib.	-	20	6	135	Soenarjo, 1979
ad lib.	-	-	20	24	110	Soenarjo, 1979
ad lib.	-	-	21	21	100	present study
-	ad lib.	-	21	11	169	present study
ad lib.	ad lib.	-	21	21	100	present study
ad lib.	ad lib.	ad lib.	21	17	94	present study
ad lib.	ad lib.	ad lib.	26	6	no data	Dalla Montà & Ivancich Gambaro, 1973

2.1.10 Host discrimination

Both Dalla Montà & Ivancich Gambaro (1973) and Evenhuis (1974) have suggested that very large egg clutches (Evenhuis counted up to 70 eggs on one host) could be the result of the activity of more than one female. In the present study, far more (up to 198) eggs and up to four female parasitoids were found per host in the field in contrast to the laboratory studies in which this type of superparasitism was precluded (48 eggs at most per host). It is probable, if it exists, that host discrimination does not prevent superparasitism under conditions where the chance of encountering hosts already parasitized is high.

2.1.11 Multiparasitism and hyperparasitism

Multiparasitism by *C. florus* and *Meteorus ictericus* Nees (Hym., Braconidae) is mentioned by Janssen (1958). In the present study hosts multiparasitized by *C. florus* and one of the following parasitoids were collected:
Meteorus ictericus (Nees)
Scambus brevicornis (Gravenhorst) (Hym., Ichneumonidae)
Teleutaea striata (Gravenhorst) (Hym., Ichneumonidae)
Mesochorus sp. (Hym., Ichneumonidae)
Bracon sp. (Hym., Braconidae)

Descriptions of the sampling method, the frequency and the rearing of these multiparasitizations are given in Subsection 5.4.2.

Hyperparasitism has only been reported by Lucchese (1941) who found larvae of *C. florus* parasitized by *Pleurotropis* sp. (Hym., Eulophidae).

2.1.12 Phenology

In the Netherlands, *C. florus* is abundant on *A. orana* in July and August, but in spite of polyphagous behaviour recorded and the variety of leaf rollers present during

spring and autumn, it is found only sporadically throughout the remainder of the year (Gruys, 1982). From rearing experiments (Gruys, 1982; Gruys & Vaal, 1984) it has been concluded that its yearly cycle consists of four or five generations from April to October. A preference for large leaf roller larvae which are not available in autumn has been put forward as the reason for the shortage of wasps at that time (Gruys, 1982). This is supported by the results of the present study (see Chapters 3 and 4). In a fairly natural habitat in Poland where *C. florus* has three or four generations on various leaf roller species of bilberry between May and September, the hibernating last instars of *Syndemis musculana*, *Ancylis myrtillana* and *Eulia ministrana* are used for hibernation and as a result the population density is high in spring (Karczewski, 1962). In Italy, eight generations occur per year between March and November on leaf rollers of strawberry, *Argyrotaenia pulchellana* being the main host used for hibernation (Dalla Montà & Ivancich Gambaro, 1973). However, Janssen (1958), observed *C. florus* using *A. orana* third instars for hibernation in West Germany. Evenhuis (1974) did not collect hibernating stages of *C. florus* in orchards in the Netherlands, probably because his last collection date for the year (13 September) was too early. He supposed that *C. florus* hibernated outside the orchard. Although he collected many potential hosts in different habitats no parasitoids were found (Evenhuis, 1980).

In the present study, hibernating larvae were obtained by introducing large hosts (*A. orana*) to the orchard late in the season. Thus, the results of the present study agree with those of Gruys (1982) that under normal conditions there are few suitable hosts for hibernation of the parasitoid in orchards in the Netherlands, and as a result the population density in spring is very low. In this connection, it may be important that the only two host species of *C. florus* which hibernate on apple as fully grown larvae or as pupae, *Argyrotaenia pulchellana* and *Syndemis musculana* (Chapman, 1973), and would provide opportunity for the parasitoid to

hibernate are respectively absent or unimportant in orchards in western Europe. Immigration from outside the orchard is unlikely to be important to build up the spring population (Evenhuis, 1974; M.J. Gijswijt, pers. comm.). Synchronizing mechanisms other than temperature are not known between *C. florus* and its hosts. The wasp does not show oviposition periods which are governed by host availability. Two generations can occur on one host generation (Karczewski, 1962; Carl & Zwölfer, 1965) and probably this is the effect of a much shorter rate of development of the parasitoid than most of its hosts.

2.2 Adoxophyes orana

The summer fruit tortrix, *A. orana*, has been used as the host for *C. florus* throughout this study, for the reasons given in Chapter 1. Since *A. orana* is a serious pest in apple in several West European countries, much attention has been paid to its biology. *A. orana* has a palearctic distribution (for a distribution map, see Barel, 1973: 4) and the larvae are extremely polyphagous (Janssen, 1958; Barel, 1973). Nevertheless, damage only occurs in well-kept commercial orchards (Janssen, 1958; De Jong & Minks, 1981), and if routine chemical control is discontinued, other tortricids become more important leaving *A. orana* as a fairly rare species (Gruys, 1982).

In the Netherlands, *A. orana* produces two generations per year and hibernates as a second or third instar larva in spun hibernacula in rough places on the branches. Diapause induction occurs when daylength is less than 16 h (Ankersmit, 1968). In April, the larvae become active again, and feed on buds and young leaves. Their first flight lasts about four weeks and begins between the first and the third week of June, depending on the weather. The summer generation larvae feed on the young leaves of shoots and the older instars also on the fruit. The duration of the second flight is about six weeks and starts in the month of August. The larvae of

the winter generation also feed on the fruit but make less deep scars. Photographs of characteristic damage are presented in Janssen (1958) and Barel (1973).

For the present study, the life history of the larval stage is of importance. On the basis of Janssen (1958) and the observations of others including the present study it may be summarized as follows:

From measurements of the width of the head capsules of a group of larvae collected in the field, Janssen concluded that there are five larval instars in the summer generation and six or seven in the winter generation. The duration of each instar in the field is very short compared with the long oviposition period during a flight so most instars could be found at the time. This only applies to the summer generation.

The effect of temperature on the developmental rate of the various stages has been investigated by De Jong & Minks (1981: Fig. 3). The egg masses which consist of 20 to 150 eggs are deposited on the upper smooth side of an apple leaf. The ovipositing females probably prefer densely foliated trees or parts of trees (Janssen, 1958; G. Vanwetswinkel, pers. comm.). All the larvae of an egg mass hatch at about the same time and disperse immediately, thus usually resulting in one larva per leaf (Barel, 1973). The web of a first instar larva is a narrow tunnel along one of the leaf ribs (Fig. 2.2a). The second and third instar lengthen and widen this tunnel on one side into a covering web (Fig. 2.2b). From the fourth instar onwards young leaves are folded at the tips (Fig. 2.2c) or at the margins, ultimately producing leaf rolls (Fig. 2.2d) or in clusters of leaves spun together.

During larval development, *A. orana* migrates to young leaves (Janssen, 1958), this resulting in an individual making several webs. This was also observed in the present study (see also Chapter 4). Barel (1973) sometimes observed the making of a new leaf roll for pupation.

The larvae are very sensitive to disturbance. They react

with a lively backward winding movement, sometimes letting themselves down along a selfspun thread. The web is kept clear from droppings, which are shot away through one of the openings.

2.3 Environmental conditions

2.3.1 Field conditions

All field experiments were carried out in an experimental orchard, De Schuilenburg near Lienden, the Netherlands. In 1965, this orchard was planted with five varieties of apple raised as spindlebushes on dwarfing root stocks. Only fungicides were sprayed during the experiments, and out of these an integrated pest management programme was followed. In the summer of 1980, temperature and relative humidity were measured inside a web of a fourth larval instar of *A. orana* during a period of six days of varying weather conditions and compared with standard meteorological data. Mean temperature was always about 2°C higher in the web than temperature recorded at a weather station 8 km away. Since the developmental rate of *A. orana* in relation to temperature is known (De Jong & Minks, 1981), host development in the field could be predicted using weather station data (see Chapter 4). Inside the web, the relative humidity was never below 45% for more than 3 h and the mean was 75%. Experimental conditions in the laboratory were adapted as far as possible to those in the host webs in the field.

2.3.2 Rearing technique

In the rearing room used for both hosts and parasitoids, the temperature was 21°C ± 1°C, relative humidity 55% ± 5%, and there was continuous light. Hosts used were from a strain kept in the laboratory for more than 25 generations. Parental stock of *A. orana* was reared continuously on a wheat germ diet modified as described by Ankersmit (1968). The

composition of the diet was casein 79 g; wheat germ 68 g; sugar 79 g; brewer's yeast 68 g; linseed oil 18 ml; choline chloride 8 g; sorbic acid 3.5 g; methyl parahydroxybenzoate 2.3 g; agar 58 g; water 1.85 l; and leaf pulp 450 g.

Leaf pulp was prepared by mixing 0.5 kg of fresh apple leaves and 1 l of water in a commercial blender (Waring) for 3 min. It was then stored until use at -30°C. Hosts fed on a diet containing leaf pulp were of a more natural colour than those fed on a diet without.

From this parent stock, newly hatched larvae were transferred to glass vials (14 mm diameter and 55 mm high) containing 2.5 ml diet and plugged with cotton wool. The diet was prepared according to the recipe of Ankersmit (1968) with the omission of Wesson's salt, choline chloride and carboxy-methylcellulose, and the addition of 150 g of leaf pulp. The hosts reared individually in this way produced five larval instars (L_1 - L_5) with developmental periods of 5, 4, 3, 4, and 8 days respectively. Unless otherwise stated, hosts reared in these vials were used in experiments.

Identical glass vials, each containing one L_5 from the parental stock and one leaf of the garden privet (*Ligustrum ovalifolium* Hassk.) were used to rear the parasitoids. In order to allow the caterpillars to make a web, the vials were prepared one day in advance of the parasitoids being introduced.

After introducing one female parasitoid to each vial, they were arranged horizontally in polyethylene boxes, on wooden shelves above a layer of water, and covered with window glass except for 2 cm on both sides. Stored this way, the relative humidity in the parasitization vials was 95-100% during the first five days, decreasing to 70-80% at the time of emergence of the adult parasitoids. Adults from several parasitizations were put together in one vial to increase the chance of all females being mated, and were provided with honey.

Once a year (approximately every 12^{th} generation), the laboratory strain was gradually and totally replaced by

wildtype *C. florus* from several orchards throughout the country in order to minimize the effects of artificial selection. To minimize genetic drift, at least 100 wildtype females were used each year.

3. ACCEPTANCE OF HOSTS OF DIFFERENT PROFITABILITY

3.1 Introduction

In the field, most larval instars of *A. orana* are present at the same time. Settlement of larvae occurs after dispersal from the oviposition site, thus larvae from egg clutches of different ages may mingle in the canopy of an apple tree. As only one larva settles per leaf, simultaneous encounters of the parasitoid with more than one host are rare. Hence, hosts of different size and age are encountered separately. In this chapter, the results of experiments on the process of host acceptance are presented and discussed. How factors such as size of the host, influence the rate of encounter with various host larval instars, and may affect the results of the host acceptance process are discussed in Chapter 4.

An encounter, as defined here, begins when an arrestment response is evoked in the parasitoid by the complex of host and host plant. Usually, this happens after contact with web, frass, or droppings of the host. A host is considered to be accepted when the parasitoid has deposited one or more eggs.

As available larval instars of the host differ in weight, they are likely to offer unequal amounts of food to parasitoid offspring. Hence it is expected that the available hosts differ in profitability, and that the most profitable hosts are preferred by the parasitoid. Three decisions made by the parasitoid which will affect the ultimate benefit of a parasitization, are: which hosts to accept; how many eggs to lay; and how many eggs to fertilize. In this chapter, the first of these is dealt with. Thus the ultimate currency, this being the number of granddaughters, cannot yet be calculated. The clutch size and the sex ratio, if not optimized in some way, are therefore considered in this chapter also to be constraints.

Thus the number of daughters arising from a host is used as an indication of the benefit of a parasitization. This can be calculated for a host type as follows

$$d_i = c_i\,(1-m_i)(1-r_i)$$

where d = number of daughters
i = type of host
c = clutch size
m = proportion pre-adult mortality
r = sex ratio $\left(\frac{♂}{♂ + ♀}\right)$ of adult offspring

As the cost of parasitizing a certain host may vary with host type, the profitability may be expressed as a cost-benefit ratio. The parasitoid expends both time and eggs on parasitizing a host, but only one of these will be the limiting factor in a particular situation and therefore should be used as measure of cost. In general, time is the limiting factor when the host encounter rate (HER), as a measure for the number of hosts encountered per unit travel time, is low, and number of eggs is the limiting factor when HER is high. Estimation of HER under field conditions is beyond the scope of this study. When there is no reason to use one particular cost factor as a measure of profitability, the results of host-acceptance experiments are discussed in terms of both expressions of profitability.

Where HER is high, each egg laid should give the maximum yield of daughters. Hence, the profitability of parasitizing a certain host of type i in this situation will be

$$d_i/c_i = \frac{c_i\,(1 - m_i)\,(1 - r_i)}{c_i} = (1 - m_i)\,(1 - r_i)$$

In this case, the profitability of a host type depends

only on pre-adult mortality and the adult sex ratio on that host type.

Where HER is low, time spent parasitizing hosts should yield the maximum number of daughters. Let tp_i be the time necessary to sting a host of type i and to complete oviposition (parasitization time). Then tp_i is the total duration of a visit to a host which is parasitized (t_i) minus the time spent with potential hosts, that is those which are rejected (tr). The profitability of parasitizing a host of type i in this situation is

$$d_i/tp_i = \frac{c_i\ (1 - m_i)\ (1 - r_i)}{t_i - tr}$$

Here the profitability of a host type does not only depend on m_i and r_i but also on the ratio c_i/tp_i or the mean time necessary to deposit one egg near that particular host type. If these measures of profitability operate in specific ranges of HER, then for optimum host-acceptance strategy, the parasitoid should be able to estimate HER. Also when one measure of profitability is always correct, because of either a permanent low or a permanent high HER in the field, it is important for the parasitoid to be able to estimate the changing relative HERs of the different host instars to optimize host acceptance. The optimal diet of predators has been shown to depend not only on the relative profitabilities of the host types but also on the encounter frequencies of the most profitable host types (MacArthur & Pianka, 1966). In the present study, it appears that *C. florus* is not able to estimate total HER, or even estimate the HERs of the different host types. Firstly the number of possible parasitizations during the lifetime of a female was estimated from the ratio of mean potential fecundity to clutch size. Secondly, the effect of prolonging the pre-encounter period was investigated thus simulating different values of HER on host acceptance (experiment I).

3.2 Ability to estimate host encounter rates

3.2.1 Materials and methods

Number of parasitizations. Mean fecundity was measured by dissecting seven-day old adult female wasps in an isotonic fluid. The females were provided with honey and had never oviposited. The ovarioles were placed on a microscope slide and the eggs counted using a stereomicroscope (magnification 50x). Only fully grown eggs were counted. Other experiments showed that fecundity measured in this way did not differ greatly from fertility (realized fecundity) when sufficient hosts were available (Subsection 5.5.3.).

Mean clutch size was calculated from a field sample consisting of parasitized larvae of the five instars of *A. orana*. The experimental design minimized the chance of superparasitism (see Subsection 5.4.1., experiment XI).

Effect of a prolonged pre-encounter period (experiment I). A group of 500 female parasitoids from the laboratory strain was given the following treatment. On the first day after emergence they were put together with about 75 males for 24 h. From experience, it was known that this would ensure insemination of almost all the females. On the second day after emergence, the females were separated from the males and divided in two equal groups. One larva was offered to each of the females of the first group, as described in Subsection 2.3.2. The second group was provided with honey and stored in separate vials in the rearing room for a week. At the end of this period, they were offered larvae of *A. orana*.

About 50 individuals of each of the five larval instars of *A. orana* were used for each parasitoid group. They were reared individually and each day the width of the head capsules was measured to determine the time of ecdysis. Larvae were used for the experiment during the first day after ecdysis. The female parasitoids of both groups were

given 24 h to parasitize the larva offered and then removed from the experimental vials.

After rearing the larvae for at least three weeks the number of caterpillars which produced adult parasitoids was counted. In later experiments, a technique for counting the eggs was developed.

3.2.2 Results and discussion

Number of parasitizations. Each of the two ovaries consisted of seven ovarioles, each containing about three fully grown eggs and a number of smaller oocytes in different stages of development. The mean number of fully grown eggs was 38.8 per female (SD = 8.7; n= 79). The mean clutch size of the field sample was 16.2 (SD = 9.2; n = 118). Thus the number of parasitized hosts during the lifetime of the female was estimated to be 2.4. Even under favourable conditions (Subsection 5.5.3, experiment XIV) mean fecundity did not exceed 40.6 eggs, thus it may be concluded that the average number of parasitizations performed by a female parasitoid is very low. Hence it is unlikely that a female can measure HER from the information she obtains from a large number of encounters, unless overall acceptance of encountered hosts is very low.

Effect of a prolonged pre-encounter period (experiment I). The feasibility that the parasitoid uses the travel time before a host encounter as a measure of HER has been considered. On the basis of the number of caterpillars giving rise to adult parasitoids in two pre-encounter periods, it is concluded that the wasp showed no difference in host acceptance towards one of the five larval instars of the host (Table 3.1). Prolonging the time before an encounter had no effect on the chance of a female parasitoid accepting a host.

The period preceding the first encounter with a host as used in this experiment may normally be an inappropriate

Table 3.1 Effect of the length of pre-encounter period on number of hosts resulting in adults of *Colpoclypeus florus*

Larval instar	Pre-encounter period of 1-2 days			Pre-encounter period of 8-9 days			χ^2 2 x 2, $\alpha = 0.05$
	hosts tested	hosts resulting in adult parasitoids		hosts tested	hosts resulting in adult parasitoids		
	(n)	(n)	(%)	(n)	(n)	(%)	
L_1	48	0	0	38	0	0	-
L_2	52	0	0	48	1	2	p = 0.5
L_3	54	8	15	49	5	10	p = 0.844
L_4	52	31	60	58	39	67	p = 0.261
L_5	53	23	43	54	32	59	p = 0.076

measure of HER. Since in the field this period includes travel from the place of emergence to the host patch, it may be longer than the mean travel time between hosts of the same patch. Nevertheless, first encounters are important in the small total number of encounters, and if HER is used for optimization of host acceptance, then it should be estimated before and be available at the first host acceptance decision.

It can be questioned whether the period of confinement of the wasp in a vial in the laboratory has the same effect on host acceptance as a period in the field without finding hosts. In the field more time is spent flying, and thus more energy is used. This could be compensated for by increasing the period in the vial in the laboratory. Furthermore, performance of aspects of the host-searching behaviour in the field in the absence of host cues may have a different effect.

The difference between the proportion of success (the proportion of accepted hosts resulting in adult parasitoids) of the two groups of females may have masked a possible difference in acceptance based on egg deposition. This is unlikely because the number of hosts offered which pass a successive moult, and consequently have not been stung, did not differ very much in the two groups of wasps.

Confining parasitoid and host for a period may have undesirable effects. It may result in a number of forced parasitizations, or it may interrupt parasitizations unnecessarily. The results of a subsequent experiment (experiment *III*) have shown that interruption of parasitization was important on hosts of the fourth and fifth instar, but not on the smaller hosts. Since at a lower HER more host larval instars should be included in the diet of the parasitoid, the acceptance of hosts of the second and third instar especially is expected to differ. Maturation of older wasps, which causes them to carry out parasitization in less time than the young wasps, can only influence acceptance of hosts of the fourth and fifth instar and therefore does

not detract from the conclusions of this experiment. However, for comparison of the fractions of different host stages accepted, a better method should be designed.

3.3 Acceptance and profitability of the larval instars of the host

3.3.1 Materials and methods

To prevent interruption of parasitization in laboratory experiments, a funnel was designed to fit over the entrance to a parasitization vial (Fig. 3.1). A female wasp escaped within 5 min via the funnel when only a leaf was present in the vial (n = 16). The funnel also prevented superparasitism in vials stored close together, because the wasps had very little chance of entering the vials from outside.

The period during which the vials were closed (that is before the funnels were fitted) prevented enforced parasitization and meanwhile ensured an encounter between parasitoid and host. This period was set at 8 h. In a subsequent experiment (experiment II) is was shown that a period of 8 h is not too long.

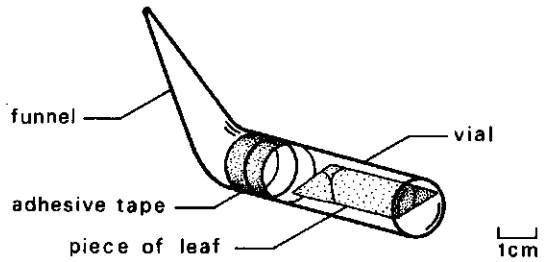

Fig. 3.1 Parasitization vial with funnel to prevent both enforced and double parasitization.

Estimated rejection time (experiment II). Six groups of about 20 female parasitoids each were mated on the first day after emergence by supplying them with three males, and each female was placed together with a host (L_4) in a vial on the second day. Each group was kept in the vials for a different length of time, i.e. 15 and 30 min, 1, 2, 4 and 8 h, after which funnels were fitted to the vials to enable the female parasitoids to escape. Every 30 min, the number which had escaped was recorded, and after two days, the number of egg clutches was counted.

Acceptance of the five larval instars of A. orana (experiment III). Five groups each of about 100 female parasitoids were mated on the first day after emergence by supplying them with about 15 males. On the second day after emergence, one larva of *A. orana* was offered to each female parasitoid as described in Subsection 2.3.2. For each group of females, 100 individuals of one of the five larval instars of *A. orana* were used. They were reared individually and were used less than one day after ecdysis by recording the width of head capsules. Before being placed in a parasitizing vial, each larva except L_1 was weighed. The vials were opened and the funnels fitted 8 h after introduction of the parasitoid. The number of parasitoids which had escaped was recorded each hour. After one had escaped, the number of eggs laid in the vial was counted under a stereomicroscope (magnification 25x) provided with a cold light source, and the vial was closed again with a cotton plug. These vials were stored in the rearing room and in the fourth week after the start of the experiment, the number of adult offspring in each vial was counted and sexed.

3.3.2 Results and discussion

Estimated rejection time (experiment II). The varying periods during which the vials were closed were used to determine the effect of the duration of enforced

confinement with the host on the proportion of accepted hosts. No relationship could be discerned between period in the vial and host acceptance (Table 3.2). Apparently, there is no chance of enforced parasitization in a period of 8 h, whereas a period as short as 15 min may suffice to ensure an encounter between parasitoid and host.

It was expected that the range of time periods during which the vials were closed would divide into two regarding the time between the end of the enforced confinement and the departure of those females which had rejected their hosts. In longer confinement periods the enforced stay was expected to be longer than the rejection time and the parasitoids would escape shortly after the funnels were fitted. In the shorter confinement periods, the enforced stay would be shorter than the rejection time and after the funnels were fitted a parasitoid rejecting a larva would remain in the vial for the period required to reject it.

The time between the end of the enforced stay and the departure of those parasitoids which rejected their hosts (Table 3.2) did not differ significantly in the six periods (Kruskal & Wallis, $p > 0.25$). The weighted mean was 1 h 20 min ($n = 49$). It is not clear why this period was not as short as the escape from a vial without host (less than 5 min), at least in the upper part of the range. Most parasitoids escaped within half an hour of opening the vial, but some remained for a considerable period before the host was rejected. It can be concluded that mean rejection time, which in this experiment is the period of enforced confinement and of the mean period between the end of the enforced confinement and the actual departure of the wasp was at least 0 + 1 h 20 min (extrapolated to a period of enforced stay = 0) and not more than 15 min + 1 h 20 min = 1 h 35 min, as suggested from the the group left for 15 min in the vial. It seems unnecessary to make a more exact estimation, because the average parasitization time was much longer. Hence, the rejection time (tr) was estimated to be 1.5 h.

Table 3.2 Effect of period of confinement of *Colpoclypeus florus* and host on host acceptance and on the period between end of confinement and departure of parasitoids rejecting hosts

Period of confinement of parasitoid and host (h)	Number tested	Proportion of accepted hosts	Period between end of confinement and departure of parasitoids rejecting hosts (h)	Number of hosts rejected
0.25	16	0.69	0.9	5
0.5	23	0.78	3.8	5
1	24	0.42	1.4	14
2	21	0.76	0.3	5
4	22	0.64	0.4	8
8	26	0.54	1.5	12
			weighted mean = 1.3 h	n = 49

Acceptance of the five larval instars of A. orana (experiment III). Host acceptance of *C. florus* is affected greatly by the larval instar of the host as suggested by the percentage of hosts resulting in adult parasitoids given in Tabel 3.1. This is confirmed by the number actually parasitized (Table 3.3). These measures of host acceptance differ, not only because of pre-adult mortality of the parasitoid offspring but also because of significant differences between the number of hosts resulting in adult parasitoid offspring (Table 3.4). This can be ascribed to the methods used. A number of parasitoids (mainly on L_4 and L_5) required more than 24 h to complete parasitization. Interruption as happened in experiment I apparently affects the proportion of successful parasitizations. Thus the method used in experiment III (that is 8 h confinement in a closed vial followed by free escape in the funnel) was used in subsequent experiments where the experimental design permitted.

From the host acceptance as given in Table 3.3, only the smallest of the five instars tested was always rejected by the parasitoid. Profitability of this instar, which cannot be calculated on basis of parasitization results, was assumed to be zero because its weight is about the same as that of the smallest viable parasitoid.

Table 3.3 Host instar dependent parasitization results of *Colpoclypeus florus* in the laboratory

Larval instar	L_1	L_2	L_3	L_4	L_5
Number tested	98	98	98	96	105
Mean weight (mg)	< 0.1	0.3	1.3	5.3	17.4
Proportion accepted by parasitoid	0	0.27	0.57	0.85	0.86
Mean clutch size of parasitoid	-	3.2	5.1	11.1	19.3
Proportion of parasitoid eggs becoming adult	-	0.11	0.14	0.41	0.40
Sex ratio of adults $(\frac{\sigma}{\varphi + \sigma})$	-	0.56	0.34	0.23	0.12
Parasitization time (h)	-	13.1	20.1	18.0	27.5

Table 3.4 Effect of the experimental method on number of hosts giving rise to adults of *Colpoclypeus florus*

Larval instar	8 h confinement followed by free escape of parasitoid			24 h confinement followed by interruption of parasitization			χ^2 2 x 2, $\alpha = 0.05$
	hosts tested	hosts giving rise to adult parasitoids		hosts tested	hosts giving rise to adult parasitoids		
	(n)	(n)	(%)	(n)	(n)	(%)	
L_2	98	9	9	52	0	0	p = 0.060
L_3	98	18	18	54	8	15	p = 0.759
L_4	96	75	78	52	31	60	p = 0.029
L_5	86	75	87	53	23	43	p<< 0.001

The second and third larval instar of the host show intermediate acceptance, whereas the fourth and fifth instar have an equal high value. Thus it may be concluded that the number of encounters with a host during the lifetime of a female parasitoid is not likely to be much higher than the estimated number of parasitizations during its lifetime.

Before discussing the relationship between host acceptance and host profitability, the justification of classifying hosts according to instar, as in Table 3.3, should be considered in terms of whether this reflects the parasitoid's perception of the host. During this study, it became clear that this was not the case and that a classification according to host weight was preferable. Host acceptance and the measures of profitability as calculated for each weight class are given in Table 3.5.

The relationship between profitability and host acceptance is shown in Fig. 3.2. Both profitability measures

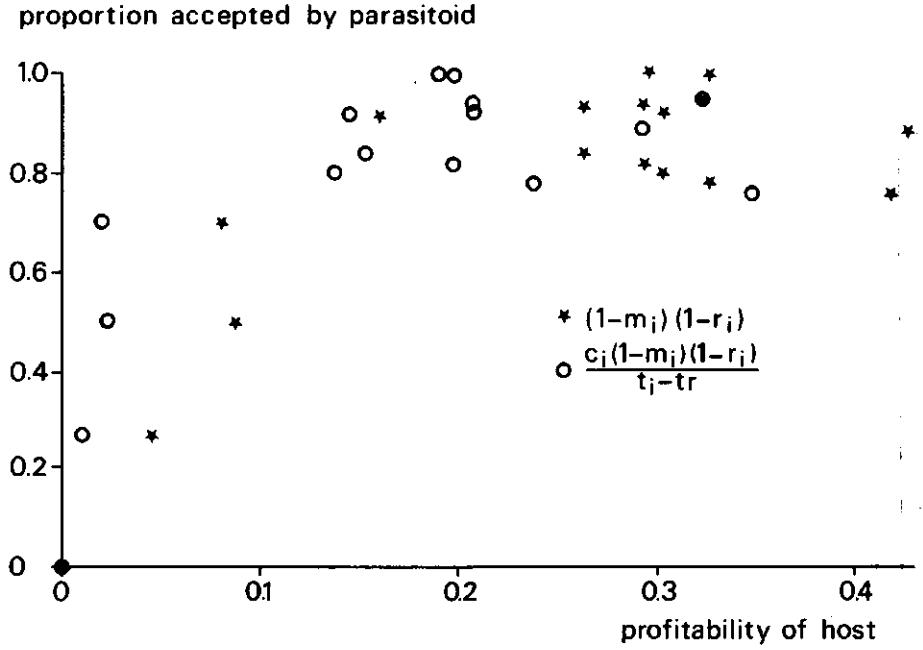

Fig. 3.2 Relationship between profitability of hosts and acceptance by *Colpoclypeus florus*.

Table 3.5 Two measures of profitability (d_i/c_i and d_i/tp_i) for hosts of different weight, based on mean clutch size (c_i), proportion of pre-adult mortality (m_i), sex ratio of adults (r_i) and parasitization time ($tp_i = t_i - tr$) of *Colpoclypeus florus*

Weight class (mg)	Number tested	Proportion accepted by parasitoid	c_i	m_i	r_i	$tp_i = t_i - tr$ (h)	$\frac{d_i}{c_i}$	$\frac{d_i}{tp_i}$
0.1 - 0.5	95	0.27	3.2	0.89	0.56	11.6	0.048	0.013
0.6 - 1.5	72	0.50	4.9	0.85	0.40	17.4	0.090	0.025
1.6 - 2.5	30	0.70	5.6	0.90	0.17	19.9	0.083	0.023
2.6 - 3.5	12	0.92	8.8	0.57	0.29	12.5	0.305	0.215
3.6 - 4.5	18	0.94	9.9	0.60	0.26	13.6	0.296	0.215
4.6 - 5.5	25	0.80	10.5	0.55	0.32	22.6	0.306	0.142
5.6 - 6.5	17	0.82	12.7	0.65	0.16	18.5	0.294	0.202
6.6 - 7.5	13	1.00	12.8	0.61	0.16	21.3	0.328	0.197
7.6 - 8.5	9	0.78	13.3	0.62	0.14	17.8	0.327	0.244
8.6 - 11.5	13	0.92	18.1	0.81	0.15	19.5	0.162	0.150
11.6 - 14.5	17	0.76	17.1	0.54	0.09	20.2	0.419	0.354
14.6 - 17.5	27	0.89	17.8	0.53	0.09	25.4	0.428	0.300
17.6 - 20.5	20	0.95	21.7	0.63	0.12	21.5	0.326	0.329
20.6 - 23.5	19	0.84	20.9	0.67	0.20	35.3	0.264	0.156
23.6 - 26.5	6	1.00	20.8	0.68	0.07	30.5	0.298	0.203

seem to show a similar relationship to host acceptance. There is a rapid increase in level of host acceptance in the lower part of the profitability range to as high as 80 - 100 % in the higher part of the range. Thus hosts of relatively low profitability were as equally well parasitized as hosts with a threefold higher profitability, and only very small larvae were not used as host.

There may be two reasons for the profitability threshold of host acceptance being rather low. Firstly, HER of the most profitable hosts may be so low that many less profitable hosts have to be included in the host range. Since it is very likely that an individual parasitoid cannot measure HER, and thus cannot adapt her strategy to a change in HER, it could be proposed that in the course of its evolution a species becomes adapted to low host densities, or at least to low HER. However, this hypothesis needs to be tested by measuring HERs in the field. If the relative HERs of the different instars in the field were known, the mean travel time between hosts could be estimated on the basis of the assumption that the accepted host instars, which all lie above a certain profitability threshold, form an optimal set. In Section 4.4, relative HERs are given and the mean travel time between hosts in the known set of accepted host instars (under the assumption of optimal host choice) is calculated. Secondly, the difference between the chance of encountering a small host and a large host may be so large, that it is unnecessary to differentiate between them. In the field, a small number of unprofitable hosts could be encountered and parasitized, without greatly affecting the overall profitability.

In addition to these two arguments for acceptance of hosts of relatively low profitability, attention must be paid to the effect of the defensive behaviour of the caterpillar on acceptance. If an otherwise profitable host prevents oviposition by defending itself, the measured host acceptance would be lower than that predicted by these measures of profitability. Hence a serious effect is only to be expected from the smallest host larvae. However, such behaviour could

only be observed in fourth and fifth larval instars, a high proportion of which were nevertheless accepted. These larvae sometimes reacted by spitting gut contents in the direction of the wasp, or by snapping at it. This resulted in a breakdown of parasitization only when the parasitoid was killed or injured. This rarely occurs with wasps in a normal condition. The lively backward winding movement, often displayed by a caterpillar after disturbance, was not observed during parasitization.

4. HOST ENCOUNTER RATE AND HOST ACCEPTANCE IN THE FIELD

4.1 Introduction

Experiments on host acceptance carried out in the laboratory showed (Chapter 3) that apart from the smallest hosts, most hosts were accepted by the parasitoid, even when they gave rise to only very few parasitoid offspring. Thus the profitability threshold of accepting a host is low. Two explanations for this may be offered. Firstly the species is adapted to low host densities in the field, or at least to a low host encounter rate (HER). Secondly, the HER with hosts of low profitability is very low compared with those of high profitability, thus the few parasitizations on hosts of low profitability are not likely to be a disadvantage to the parasitoid.

As the first explanation would require measurement of absolute HER in the field which is extremely difficult, the more fruitful approach seemed to be to concentrate on the second explanation for which determination of relative HERs in the field was sufficient. Differences in HERs with hosts of varying profitability are likely to have a considerable effect on the numbers of these hosts actually parasitized. Since it is very likely (Chapter 3) that the acceptance of hosts of different profitability is not affected by HER, the second mentioned possibility may also operate at low host densities. Thus the two explanations are not mutually exclusive.

The result of preliminary field sampling on *Adoxophyes orana* indicated that the proportion of fourth and fifth instars parasitized was too high in comparison with that of second and third instars to be explained by the difference in host acceptance alone. It is assumed here that in the laboratory experiment the chance of encountering a host offered in a vial is equal to one. Detailed sampling to estimate mortality of juvenile parasitoids in the field (see Section 5.4) yielded a large number of hosts of different

instars which had been available simultaneously for parasitization. None of the second instar larvae were parasitized (n = 49). The ratio of the proportion of third, fourth and fifth instars parasitized was 1:4:6 (with n = 79, 195 and 231 respectively), whereas differences in host acceptance in the laboratory could only explain a ratio of L_2: L_3: L_4: L_5 = 1:2:3:3 (see Table 3.2).

Three factors which may cause differences in the relative HERs with the various host larval instars are:

- the relative densities of the various instars in the area (availability);
- the location of the various instars on the food plant with respect to the microhabitat preference of the parasitoid (accessibility);
- the chance of the various instars in the microhabitat being discovered by the parasitoid (conspicuousness).

Considering availability, the relative densities of instars of *A. orana* vary considerably throughout the summer. During the period of the summer generation of *A. orana* potential hosts (second to fifth larval instar) are available from mid-June to mid-September. In the beginning of this period only the smaller instars are found. From mid-July most of the instars occur simultaneously and by about mid-August only a few larger hosts are left. Thus availability depends largely on the period considered, and consequently depends on the degree of synchronization of parasitoid and host. Gruys (1982) found *C. florus* to be most numerous in July and August, when the larger hosts prevail. However, this is probably attributable to the availability of suitable hosts and not to synchronization, since the incidence of *C. florus* was measured by determining the proportion of parasitized caterpillars. In the present study synchronization could not be determined from catches of adult parasitoids during the summer generation of the host because parasitoid density in the field was too low. Therefore, the study focused on the two other factors affecting the relative HERs of host larval instars. Although the concepts of accessibility and

conspicuousness partly overlap, the former is considered to express spatial compatibility, and the latter to be mainly affected negatively by camouflage or positively by the production of kairomones.

Field experiments to determine the effects of accessibility and conspicuousness of hosts on their chance of being encountered by the parasitoid are discussed. These experiments were carried out in the experimental orchard De Schuilenburg, between 9 July and 19 September 1982 (for description of field conditions, see Subsection 2.3.1).

4.2 Host acceptance in the field and laboratory

4.2.1 Introduction

Since it is very difficult to count encounters between hosts and parasitoids in the field, the number of accepted hosts (that is, hosts with eggs of the parasitoid) was counted. As this measure does not take into account the host-acceptance process in the field, which may differ from acceptance in the laboratory, host acceptance in the field was tested (experiment IV).

4.2.2 Materials and methods

Hosts were reared individually in the laboratory, during which time the head capsules were measured daily to determine ecdysis. About 50 larvae of each of four instars (L_2-L_5) were used. Within the first day after ecdysis, the larvae were placed on apple trees of the variety Jonathan. Each larva was placed on a separate leaf on the outside of the canopy. Equal numbers of each instar were placed on the same day, on the same tree, and with the same exposure to the sun. The leaves were folded slightly by means of a staple. Leaves prepared in this way were very attractive to the larvae, which soon started their spinning activities. A small enclosure was fitted over the leaf containing a larva (Fig. 4.1).

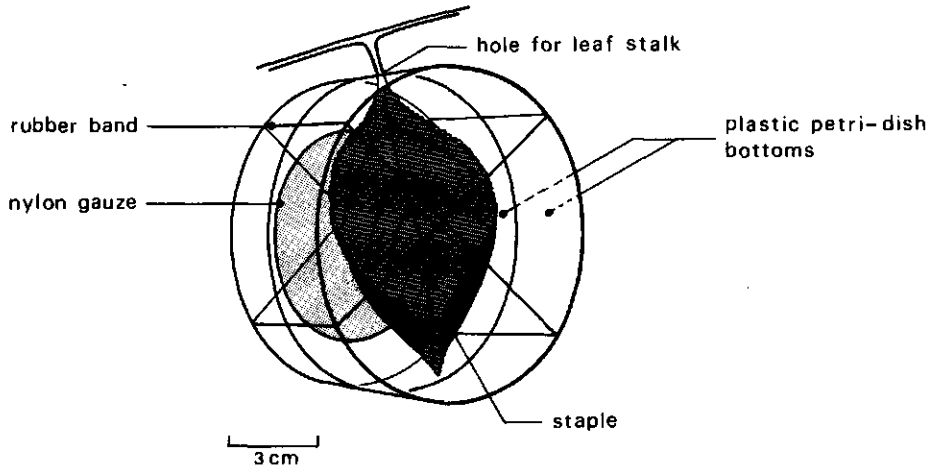

Fig. 4.1 Enclosure to confine parasitoid and host on leaf of apple tree during field experiments.

Within a day, a larva had completed a web and had begun to feed. At that time a female parasitoid was placed on each web with the aid of a wetted fine paint brush. The parasitoids were reared and mated in the laboratory (see Subsection 2.3.2) and were less than a week old when placed on the web. All parasitoids introduced had displayed an arrestment response, usually within 1 min, before the enclosure was fitted again. Two days after introduction of the parasitoids, their presence in the enclosures was noted and the enclosures were removed. Although the enclosures were intended to confine both host and parasitoid (as shown in Chapter 3 this does not lead to enforced parasitizations), most of the parasitoids escaped through the hole in the enclosure made for the leaf stalk. It was investigated whether this influenced the proportion of hosts accepted.

The leaves with hosts were collected in vials (23 mm diameter and 97 mm high), plugged with cotton, and taken to the laboratory. The presence of eggs laid by the parasitoid, and its presence in the web was noted for each host.

4.2.3 Results and discussion

The proportion of the various instars accepted in the field is given in Table 4.1. These data were used to calculate the proportion of hosts encountered, expressed as the proportion of hosts parasitized in the field:

$$\frac{\text{number of parasitized hosts}}{\text{total number of hosts}} =$$

$$= \frac{\text{number of accepted hosts}}{\text{number of encountered hosts}} \times \frac{\text{number of encountered hosts}}{\text{total number of hosts}}$$

$$\rightarrow \text{proportion encountered} = \frac{\text{proportion parasitized}}{\text{proportion accepted}}$$

No significant differences were found in the proportion of hosts accepted in the field and in the laboratory except for the third instar (Table 4.1). This may be explained by the fact that the caterpillars grow faster in the field than in the laboratory, which may increase their profitability at the time of host encounter. The greatest effect of this phenomenon on host acceptance is to be expected in the third instar, because a small increase in weight and profitability had been found to have a considerable effect on acceptance of second and third instars only (see Fig. 3.2) and absolute growth rate was faster in the third than in the second instars.

For each instar no significant differences were found with respect to host acceptance between those wasps which had escaped from the enclosures and those which had not (Table 4.2). Apparently the parasitoid, before she tries to escape, first examines the host. The sooner a host is rejected, the more time the parasitoid may spend trying to escape. Thus, more wasps escaped when they were offered a second instar.

Table 4.1 Acceptance of hosts by *Colpoclypeus florus* in the field and in the laboratory (experiments IV and III)

Larval instar	In the field (experiment IV)			In the laboratory (experiment III)			χ^2 2 x 2, $\alpha = 0.05$
	number tested	number accepted	proportion accepted	number tested	number accepted	proportion accepted	
L_2	52	10	0.19	98	26	0.27	p = 0.424
L_3	50	43	0.86	98	56	0.57	p = 0.001
L_4	48	45	0.94	96	82	0.85	p = 0.234
L_5	50	43	0.86	105	90	0.86	p = 0.841

Table 4.2 Effect of escape of *Colpoclypeus florus** on acceptance of hosts in the field (experiment IV)

Larval instar	Parasitoids remaining after two days		Parasitoids escaping within two days		χ^2 2 x 2, $\alpha = 0.05$
	number tested	number of hosts accepted	number tested	number of hosts accepted	
L_2	11	3	41	7	p = 0.370
L_3	24	20	26	23	p = 0.824
L_4	18	16	30	29	p = 0.964
L_5	26	24	24	19	p = 0.176

* The parasitoids and host larval instars were confined for a period of two days in an enclosure fitted over single tree leaves containing them.

4.3 Distribution of host instars and host encounters in the canopy

4.3.1 Introduction

Accessibility depends on the position of the hosts on the food plant and on the searching behaviour of the parasitoid. The distribution of instars in the canopy of an apple tree was investigated in experiment V. Janssen (1958) referred to a migration to young leaves during larval development. In the summer, new leaves grow mainly in the upper and outer parts of the canopy. While most of the leaf buds open in spring to form short rosettes, some continue to grow to form long internodia and new leaves during the summer. These long shoots are mainly found on the outside of the canopy. Short shoots, including flowering shoots, are confined to the older branches throughout the canopy. Most of the larger caterpillars can be found on long shoots in the second half of July (F. Vaal, pers. comm.). In experiment V wasps were released to investigate the combined effect of accessibility and conspicuousness on the relative HERs with the various instars.

4.3.2 Materials and methods

The required numbers of each larval instar in the field were obtained by artificial infection of trees with egg masses of *A. orana*. The shape of the tree, the place of attachment of the egg masses or the pressure of parasitism at a particular time and place may all have affected the distribution of the various host instars in the tree. To minimize these effects, each tree sampled contained second, third, fourth and fifth instars. Although repeated sampling of the same tree disturbs the remaining larvae, egg masses can be introduced without disturbance. Egg masses of *A. orana* reared in the laboratory were used. Black egg masses indicating that they would hatch within one day were cut from

their substrate (plastic sheet) and either introduced in the field immediately, or stored for several days at 10°C.

Ten apple trees of the variety James Grieve, all with well-developed canopies (about 2.5 m diameter, and about 0.5-3.0 m above the ground) were used. Each week for five weeks, commencing 9 July, two trees were each infected for the first time with ten egg masses (a week-series). The egg masses were attached to the leaves by a staple through the leaf and the plastic sheet to which they were attached. They were positioned in the trees in natural oviposition sites, namely densely foliated parts, easily accessible from outside the canopy and usually about 1.5-2.0 m above the ground (G. Vanwetswinkel, pers. comm.).

The time intervals between successive infections of the same week-series were calculated using the mean day (9.00-21.00 Middle European Time) and night (21.00-9.00 Middle European Time) temperature from a weather station 8 km away and a temperature development table for the larval period (De Jong & Minks, 1981). The times of ecdysis of the larval instars were interpolated from our own laboratory data. Thus the same tree was reinfected after about 17%, 29% and 46% of the total duration of larval development of the first infection (Fig. 4.2). The egg masses of the successive infections were attached to the leaves in the same positions in the canopy to minimize their effect on the distribution of the larvae. The larval densities thus created were typical of those naturally observed (G. Vanwetswinkel, pers. comm.). At 71% larval development, the four instars were present simultaneously in the two trees of a week-series. Parasitoids were released onto one of these two trees, which were at least 25 m apart, and the other was left untreated.

Parasitoids were reared and mated in the laboratory (see Subsection 2.3.2) and were less than a week old when released. They were released at three distances from the stem: in the first week-series at four points 1 m from the outside of the canopy; in the second and fourth week-series

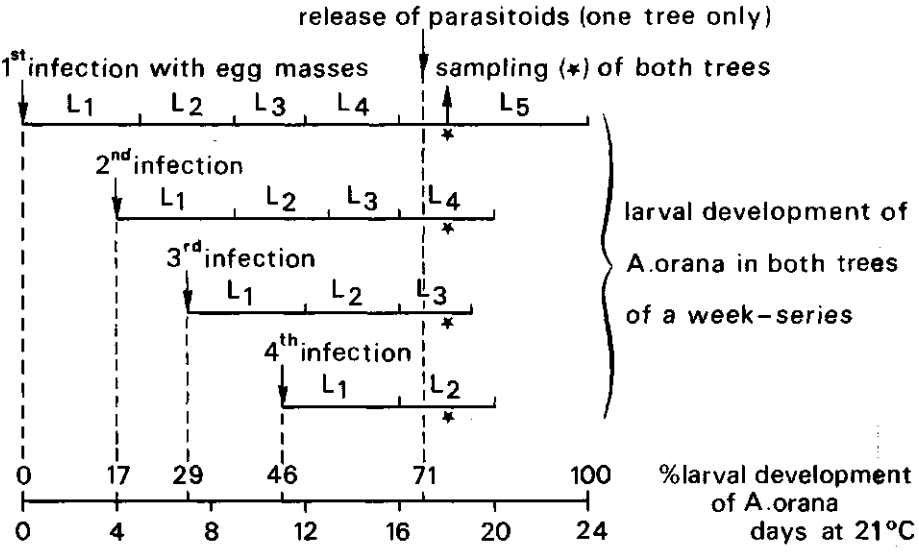

Fig. 4.2 Schematic representation of experiment V. Only one out of five week-series is given, consisting of two trees infected in the same way.

at opposite ends in the outer layer of the canopy; and in the third and fifth week-series at one point in the centre of the canopy less than 0.1 m from the stem. Each point was 1,5 m above the ground and about 100 female parasitoids were released from each point in a period of 20 min.

Two or three days after release of the parasitoids both trees of a week-series were sampled. As shown in Fig. 4.3, the canopy was divided by eye into six parts. An outer and inner layer along the radius of the canopy were subdivided into a lower (below 1 m), middle and upper layer (above 2 m from the ground). A sample comprised a section through all these parts, consisting of between a fifth and a quarter of the canopy. The leaves were picked individually and searched for caterpillars. Leaves with caterpillars were placed in glass vials (23 mm diameter and 97 mm high) and the part of origin and the type of branch to which the leaf was attached noted. A distinction was made between short and long shoots.

Leaves from long shoots were divided into fresh young and older leaves; the outermost six leaves of a long shoot were arbitrarily defined as young leaves.

In the laboratory, the larval instars of the caterpillars were determined from measurement of the head capsule, and parasitization by *C. florus* indicated by the presence of eggs in the web noted.

Caterpillar density in various parts of the canopy was expressed by the number of caterpillars per thousand leaves. The number of leaves in each part of the canopy was estimated from the sample of quarter section of the canopy of another similar five trees.

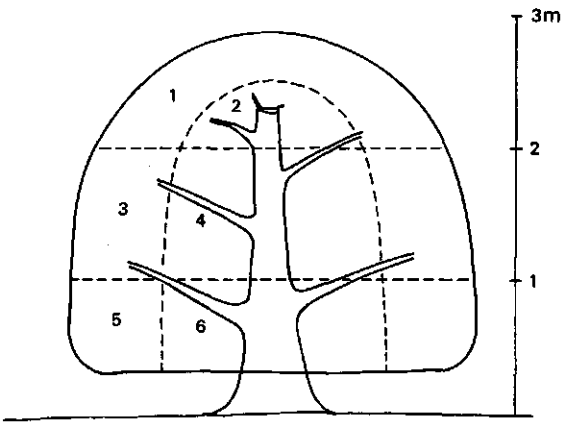

Fig. 4.3 Schematic longitudinal section of the canopy of an apple tree showing the six parts distinguished: 1 upper part, outer layer; 2 upper part, inner layer; 3 middle part, outer layer; 4 middle part, inner layer; 5 lower part, outer layer; 6 lower part, inner layer.

4.3.3 Results and discussion

The ten tree-samples contained in total the leaf material of 2.6 trees and contained a total of 1533 caterpillars. Five other tree-samples of a total size of 1.3 tree contained a total of 12 388 leaves, thus the density of caterpillars (second to fifth instar only) was on average 1533 x 1.3/2.6 x 12 388 = 62 caterpillars per thousand leaves.

The various types of instars were not evenly distributed throughout the parts and branch types of an apple tree distinguished (Table 4.3). The short shoots and the older leaves of the long shoots contained mainly caterpillars of the second and third instar, and the young leaves of the long shoots contained almost as many caterpillars of the fourth and fifth instar as smaller caterpillars. This difference is still more marked when considering the outer layer only. The outer layer of the tree as a whole also had a slightly higher proportion of caterpillars of fourth and fifth instar than the inner layer. Even though this may be explained by the fact that the outer layer contained more young leaves, the difference was still significant (χ^2 2 x 4; $p < 0.025$) even when the young leaves were excluded.

Most of the caterpillars (85%) were found in the middle layer of the canopy. This cannot be explained by the difference in size and number of leaves between the three layers because caterpillar density (caterpillars per leaf) was also more than twice as high in the middle layer as in the lower and upper layer of the canopy. This can most likely be explained by the position of the egg masses in the tree. Most of the positions, which corresponded with the description of natural oviposition sites, were about at 1.5-2.0 m above the ground. However, it is not clear whether *A. orana* has a preference for oviposition at this height. No reference to the distribution of egg masses of *A. orana* was found in the literature. Therefore, the factor height above the ground was omitted from further analysis, and attention

Table 4.3 Distribution of four larval instars of *Adoxophyes orana* in the canopies of apple trees* in the field (experiment V)

	Short shoots				Long shoots								Total			
					older leaves				fresh leaves							
	L_2	L_3	L_4	L_5	L_2	L_3	L_4	L_5	L_2	L_3	L_4	L_5	L_2	L_3	L_4	L_5
Outer layer upper (1)**	1	4	7	2	11	16	4	4	12	18	21	12				
middle (3)	90	53	23	6	176	106	32	9	31	39	52	14				
lower (5)	9	17	6	0	11	5	0	0	3	4	6	2				
total***					298	201	72	21	46	61	79	28	344	262	151	49
Inner layer upper (2)	2	3	4	0	3	1	1	1	1	0	0	0				
middle (4)	236	134	30	10	128	78	12	3	15	13	8	0				
lower (6)	4	7	3	0	8	12	1	0	3	3	3	0				
total***					381	235	51	14	19	16	11	0	400	251	62	14
Total	342	218	73	18	337	218	50	17	65	77	90	28	744	513	213	63

* Data are a summation of 10 tree samples, and represent in total 2.6 trees.

** The figures in brackets correspond to the sections of the tree canopy given in Fig. 4.3.

*** The older leaves of short and long shoots were added together.

focused on the middle layer of the canopy.

The uneven distribution of larval instars throughout the middle layer of the canopy is shown in the schematic longitudinal section in Fig. 4.4. The two parts containing young leaves especially in the outer layer had a higher proportion of larger instars. Since not only the proportion but also the density of larger instars increased towards the outside of the canopy it would seem that older caterpillars had a preference for the outer layer, especially for the tips of long shoots.

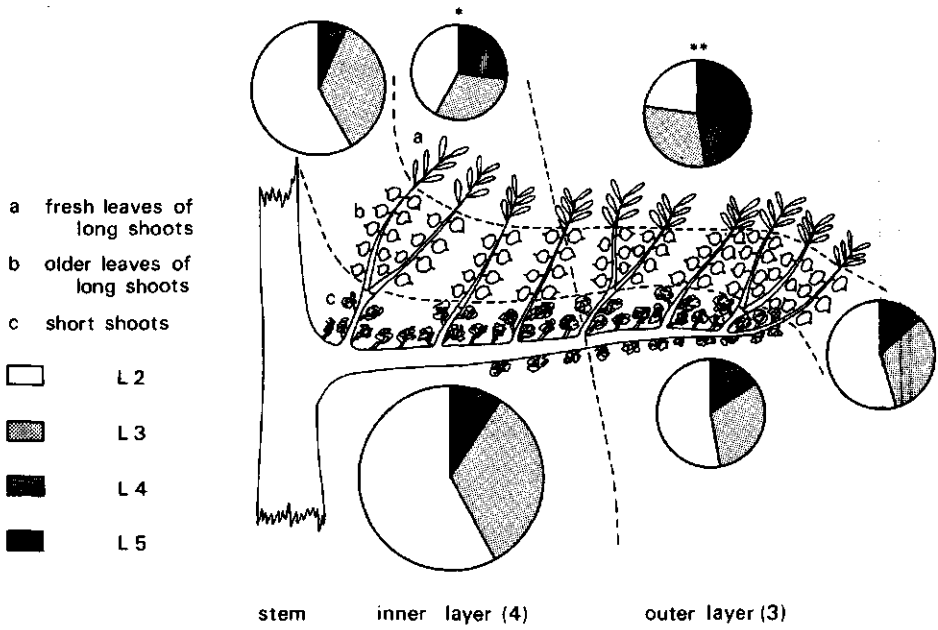

Fig. 4.4 Schematic longitudinal section of the middle part of the canopy. See Fig. 4.3 for numbers in brackets. The circle size shows the relative caterpillar density (caterpillars/leaf), and the sectors show the composition of the caterpillar population on the different leaf types. The caterpillar composition of the circles marked with * and ** differed significantly (χ^2, $\alpha = 0.05$) from those of the other four circles and also from each other.

While it was beyond the scope of this study to investigate causal or functional aspects of host behaviour, the opportunity it offers to a parasitoid such as *C. florus*, which is expected to parasitize caterpillars of fourth and fifth instars only, to adapt its searching behaviour, is very clear. Even in trees in which larger instars were only less than a fifth of the total population (see Table 4.3) the wasp may select those parts of the canopy where half of the caterpillars are either fourth or fifth instars. Also, the caterpillar density in these parts is not much lower than elsewhere, and accessibility to airborne insects is good. If *C. florus* has adapted its searching behaviour, these parts will be visited more frequently than expected on the basis of random searching.

The first experimental support of this preposition came from the experiment on parasitization. The different instars were not compared because of effects of differences in conspicuousness between the host instars. Only the proportions of each instar parasitized in various parts of the canopy were determined (Table 4.4). The outer layer of the canopy as compared with the inner layer, and the young leaves of the long shoots as compared with other leaves contained the highest proportion of parasitized hosts. This cannot be caused by a functional response (type 3) in the

Table 4.4 Number and proportion of hosts parasitized inhabiting various parts of the canopies of trees in the field (experiment V)

	L_3		L_4		L_5	
	number	proportion	number	proportion	number	proportion
Canopy						
inner layer	2	0.01	7	0.11	1	0.07
outer layer	16	0.06	69	0.46	10	0.20
total	18	0.04	76	0.36	11	0.17
Canopy						
short shoots	2	0.01	9	0.12	2	0.11
long shoots						
older leaves	4	0.02	14	0.28	3	0.18
young leaves	12	0.16	53	0.59	6	0.21
total	18	0.04	76	0.36	11	0.17

areas of highest host densities, since the time between introduction of the parasitoids and sampling the trees was too short for more than one host to be met and parasitized per wasp. Moreover, this effect was also shown in the parasitization of third instars, in spite of the fact that their density is highest inside the canopy.

The distance between the point of release and the stem, which was varied, did not appear to affect the distribution of parasitizations in inner and outer layer of the canopy, although release of parasitoids 1 m from the outside of the canopy resulted in parasitizations too few for analysis (Table 4.5). The proportion of parasitized instars from parasitoids released at the three distances could not be compared because they were not applied at the same time. The total proportion of hosts parasitized in experimental trees was 11% as opposed to 2% in control trees.

Table 4.5 Number of parasitized hosts in the inner and outer layer of the canopies of apple trees resulting from three methods of parasitoid release

	Number of parasitized host larvae		
	Method of release*		
	I	II	III
Canopy			
inner layer	1	4	2
outer layer	5	58	19
total	6	62	21

* Method I - release of groups of 100 parasitoids from four points at 1 m from the canopy of one tree only.

Method II - release of groups of 100 parasitoids from opposite ends on the outer layer of the canopies of two trees.

Method III - release of groups of 100 parasitoids from the centre of the canopies of two trees.

4.4 Effects of accessibility and conspicuousness of host larvae on their chance of being parasitized

4.4.1 Introduction

The effects of accessibility and conspicuousness of host larvae were studied separately in experiment VI in which large hosts were offered to the parasitoid in places on the host plant usually inhabited by small hosts, and vice versa. Differences in parasitization of these places may be regarded as being caused by their differential accessibility, whereas differences in parasitization of the host sizes may be attributed to their differential conspicuousness.

4.4.2 Materials and methods

Hosts of third, fourth and fifth instars were reared in the laboratory, and placed separately on leaves of apple trees of the variety James Grieve in the field. They were enclosed as described in Subsection 4.2.2. As the results of experiment V (see Table 4.4) showed that second instar hosts were not parasitized, the number of parasitized second instars, if any, was expected to be too small to obtain significant results about either their accessibility or conspicuousness.

Half of the larvae of each instar was placed on young leaves of long shoots, and half on older leaves at the base of the same long shoots, or where no leaves were available, on short shoots closeby. No more than two larvae were placed on each long shoot, the one at the top and the one at the base being always of the same instar. Successive long shoots alternately received larvae of the three different instars. When no more long shoots were available on a tree, an additional tree was used. The section of the canopy used (part 3) is shown in Fig. 4.3. In this way, groups of equal numbers of the three instars were brought into the field on eight different days from 27 August to 15 September.

After two days, most of the larvae had settled and the enclosures were removed. Each shoot containing settled larvae was labelled in the middle with a white plastic strip (1 x 8 cm) to simplify recollection. Parasitoids were released once at each of three distances from the canopy as described in Subsection 4.3.2, close to three of the eight groups. The other groups of larvae did not receive an extra release of parasitoids, but pressure of parasitism was relatively high because of preceding releases (for experiment V) in the same area.

Three days after parasitoid release, the larvae were recollected and placed in glass vials (23 mm diameter and 97 mm high) and the type of leaf noted. In the laboratory, the larval instar was determined, and whether parasitization by *C. florus* had occurred was noted.

4.4.3 Results and discussion

Of the 410 larvae placed on the leaves of the apple trees, 278 were recollected, the remainder had mainly failed to settle during the period when the enclosures were attached and only a few were lost subsequently. The proportion of parasitized larvae of the three instars feeding at the tops and bases of long shoots for trees with and without parasitoid release is given in Table 4.6. Although the release of parasitoids resulted in a significant increase in total proportion of parasitization from 0.27 to 0.37 (Fisher normal approx., one sided; $p = 0.041$), the distribution of parasitization over the six categories of larvae did not differ between trees with and without parasitoid release (χ^2 2 x 6; $p \cong 0.25$).

Parasitization by *C. florus* was unevenly distributed over the six categories of larvae (Table 4.6). Since host acceptance in the field did not differ in larvae of third, fourth and fifth instars (χ^2 3 x 2; $p > 0.25$), (Table 4.1), this uneven distribution may be explained by differences in encounter probabilities by the parasitoid.

Table 4.6 Proportion of parasitized hosts feeding on the top and at the base of long shoots (Experiment VI)

	Top of long shoots			Base of long shoots			Mean proportion parasitized*
	number recollected	number parasitized	proportion parasitized	number recollected	number parasitized	proportion parasitized	
With parasitoid release**							0.37
L_3	15	2	0.13	18	1	0.06	
L_4	26	13	0.50	21	6	0.29	
L_5	28	24	0.86	24	9	0.38	
Without parasitoid release***							0.27
L_3	26	7	0.27	19	0	0	
L_4	29	12	0.41	18	4	0.22	
L_5	35	21	0.60	19	2	0.11	
Total							
L_3	41	9	0.22	37	1	0.03	0.12
L_4	55	25	0.45	39	10	0.26	0.36
L_5	63	45	0.71	43	11	0.26	0.49
Mean*			0.46			0.18	

* Non-weighted mean.
** Data from 2,8 and 15 September.
*** Data from 27 August, 1, 7, 10 and 15 September.

The proportion of larvae parasitized which fed at the top was always higher than that which fed at the base of long shoots, regardless of the type of instar (Fisher normal approx., one sided; L_3: $p = 0.014$, L_4: $p = 0.041$ and L_5: $p << 0.001$). Thus the chance of a host of a certain instar being encountered by a female parasitoid was considerably higher at the top than at the base of a long shoot or on a nearby short shoot. This indicates that the female parasitoid is attracted to certain parts of the habitat (trees) regardless of whether hosts are present. This has been referred to as host-habitat finding by Doutt, (1959) or host-habitat location by Vinson (1975). Cues mentioned as being important in this step of host selection are mostly chemical stimuli from the host's food or food plants (Doutt, 1959; Vinson, 1975; Vinson, 1976). In the present study, the parts preferred by the parasitoid are located with other parts of the same food plant, but can be distinguished by the fact that new foliage is formed here throughout the growing season. A possible cue may be chemical substances produced by young leaves or by hosts feeding on them, and/or physical stimuli, such as the distinctly lighter green colour of young leaves. The preference of adult parasitoids for places of high light intensity observed in the laboratory could lead to preference in the field for the outer edge of the canopy and avoidance of the darker region inside.

This preference of a female to search places where normally the larger hosts occur caused a bias which is similar for the three instars tested (χ^2 3 x 2; $p \cong 0.25$). On the basis of the combined results of the three instars, the ratio between the accessibility of hosts inhabiting the top of long shoots (a1) and that of hosts in other places in the outer layer of the canopy (a2) can be calculated as follows (see Table 4.6):

$$a1/a2 = (22 + 45 + 71)/(3 + 26 + 26) = 2.5$$

The factor(s) responsible for this ratio already results in differences in the relative HERs with the various instars

because a higher proportion of larger instars were found on the top of long shoots than in other parts of the canopy (Subsection 4.3.3).

The uneven distribution of parasitizations of the six host categories given in Table 4.6, however, results not only from the difference in accessibility of larvae inhabiting the top and base of long shoots but also from differences in parasitization of third, fourth and fifth instars, when corrected for the uneven numbers of each instar inhabiting the top and base of these shoots. All other conditions being equal, the proportion of larvae parasitized are in the ratio L3: L4: L5 = 1:3:4. Thus it would seem that a fourth instar larva is three times, and a fifth instar larva is four times as conspicuous to the parasitoid as a third instar larva. Most probably the size of the host is responsible for this phenomenon because larger larvae create a larger area around themselves in which an arrestment response of the parasitoid is evoked. This may be the result of higher production of kairomones from frass or webbing.

The combined effect of the accessibility and conspicuousness of host larvae on their chances of being encountered was compared with the parasitization data obtained in the field, outlined in Section 4.1. The method used for this field collection is described in detail in Section 5.4. From this extensive collection of hosts in part 3 of the canopy (see Fig. 4.3) the proportion of hosts parasitized was estimated to be 0.0, 0.035, 0.143 and 0.203 for second, third, fourth and fifth instars respectively (Table 4.7). However, in this sample the distribution of the hosts on old and young leaves was not taken into account. These data were compared with the results of experiment VI, showing the combined effect of accessibility and conspicuousness of hosts inhabiting old and young leaves in part 3 of the canopy (Table 4.7).

This comparison is possible, being based on the distribution of hosts over this part of the canopy which was obtained from experiment V (Table 4.7). The number of

Table 4.7 Combined effects of accessibility and conspicuousness of hosts (experiment VI) on the proportion parasitized compared with the proportion parasitized according to field data

	L_3			L_4			L_5		
	old leaves	young leaves	all leaves	old leaves	young leaves	all leaves	old leaves	young leaves	all leaves
Number of hosts in part 3 of the canopy (Table 4.3)	159	39	198	55	52	107	15	14	29
Proportion parasitized according to the results of:									
- experiment VI	0.03	0.22		0.26	0.45		0.26	0.71	
- the field collection			0.035			0.143			0.203
Estimated number of parasitized hosts according to:									
- experiment VI	159 x 0.03 + 39 x 0.22 = 13.4			55 x 0.26 + 52 x 0.45 = 37.7			15 x 0.26 + 14 x 0.71 = 13.8		
- the field collection	198 x 0.035 = 6.9			107 x 0.143 = 15.3			29 x 0.203 = 5.9		

parasitized third, fourth and fifth larval instars in both situations was calculated (Table 4.7), assuming that the distribution of hosts on young and old leaves did not differ in experiment V and the field collection.

The difference in distribution of the parasitized hosts over the three instars in experiment VI and the field collection was not significant (χ^2 2 x 3; $p > 0.9$). Thus the data of the field collection, which showed a strong bias of the parasitoid for fourth and fifth larval instars, can be explained by the combined effect of their better accessibility and higher conspicuousness to the searching female wasp as found in experiment VI.

In Subsection 3.3.2 it was assumed that the HER of the most profitable hosts, the fourth and fifth larval instar, may be so low that less profitable hosts such as the third larval instar should be included in the host range. The results in Table 4.7 show a set of hosts (L_3, L_4 and L_5) accepted for parasitization in a situation where all instars were simultaneously available in the field. When a hundred hosts are parasitized these are distributed thus (using the mean of the values of Table 4.7):

$$L_3: \left(\frac{13.4 + 6.9}{2}\right)100 = 23$$

$$L_4: \left(\frac{37.7 + 15.3}{2}\right)100 = 56$$

$$L_5: \left(\frac{13.8 + 5.9}{2}\right)100 = 21$$

The encountered numbers can be calculated with the use of the proportion in the field (Table 4.1):

$$L_3: 23 \times \frac{1}{0.86} = 27$$

$$L_4: 56 \times \frac{1}{0.94} = 60$$

$$L_5: 21 \times \frac{1}{0.86} = 24$$

Therefore 27 + 60 + 24 = 111 hosts are encountered to result in 100 parasitized hosts. Under the assumption of optimal host choice the HER of a given set of accepted hosts can now be calculated. For the situation where the parasitoid is expected to switch from a host range with to a host range without L_3 it is clear that the profitability (P) of a diet with L_3 is higher than that without $L_3 (P_{5+4+3} > P_{5+4})$. The switching point will lay at $P_{5+4+3} = P_{5+4}$.

Profitability was expressed in number of daughters ($d_i = c_i(1-m_i)(1-r_i)$) per hour, calculated from Table 3.3. The parasitization time t_i can also be found in this table. Rejection time tr is 1.5 hours.

$$P_{5+4+3} = P_{5+4}$$

$$\frac{21\ d_5 + 56\ d_4 + 23\ d_3}{21\ t_5 + 56\ t_4 + 23\ t_3 + (111-100)\ tr + x} =$$

$$= \frac{21\ d_5 + 56\ d_4}{21\ t_5 + 56\ t_4 + (11 + 23)\ tr + x}$$

where x = the travel time necessary to encounter 111 hosts.

For this equation is:

x = 11 987 hours, and HER = $\frac{111}{x} \times 24$ = 0.22 hosts/day

In the laboratory, where the value of d_3 used was measured , the proportion accepted of the third instar was much less than in the field. When the field value of d_3 (which can be calculated from data presented in Chapter 5) is used instead, the equation gives:

x = 1307 hours, and HER = $\frac{111}{x} \times 24$ = 2.0 hosts/day

Since in the field, third instar larvea clearly belonged to the host range (Table 4.1), HER is expected to be less than 2.0 hosts per day. In the laboratory the third instars are not regularly but only partly accepted for parasitization, so HER will be more than but close to 0.22 hosts per day. In this study no attempts were made to determine HER in the field, but in Chapter 5 a second theoretical estimate of HER is made.

5. DETERMINATION OF CLUTCH SIZE BY THE PARASITOID, AND CONSEQUENCES FOR MAXIMIZING REPRODUCTIVITY OF HER OFFSPRING

5.1 Introduction

How and to what extent clutch size is optimized by *Colpoclypeus florus* are discussed in this chapter. In particular the second of three decisions made by a female parasitoid, as listed in Section 1.2 is discussed, that is how many eggs to lay at one parasitization.

Parasitoid larvae are usually confined to the individual host parasitized by their mother, thus the host represents a fixed quantity of food, on which they have to complete their development. When a host is superparasitized, that is more eggs are deposited than can develop to full-grown adults, the subsequent competition for food often results in the death of some larvae. To avoid this disadvantageous intraspecific competition, many parasitoid species discriminate between healthy and parasitized hosts and refrain from ovipositing on or in the latter (for solitary parasitoids see Salt, 1961; Bakker et al., 1967; Van Lenteren, 1976, 1981; for gregarious parasitoids, see Salt, 1961; Jackson, 1966; Wylie, 1970; Klomp, Teerink & Wei Chun Ma, 1980). However, when HER with unparasitized hosts is low, some fitness may be gained when parasitoids lay eggs in hosts already parasitized by other (conspecific) individuals.

In addition to host discrimination, gregarious parasitoids need to evolve behavioural mechanisms to avoid competition within their own group of progeny feeding on the same host. Above a certain clutch size all possible gain of a supernumerary larva is at the cost of brothers or sisters, and no net gain is to be expected for the parent, regardless of how low the HER may be. The ability to vary clutch size with the capacity of the host offers a gregarious parasitoid opportunity for efficient host exploitation without wastage of food. Some gregarious parasitoids are known to adapt the

number of eggs deposited on or in a single host to the size or stage of this host (Salt, 1961, reviews a number of cases; Klomp & Teerink, 1962; Wylie, 1967; Neser, 1973; Gerling & Limon, 1976; Uematsu, 1981).

Competition for food has two effects on competing larvae, and influences their fitness negatively. The most drastic effect often emphasized in literature (e.g. Salt, 1961) is the death of one or more competitors. Although this is the only or the most important effect of contest competition in solitary parasitoids and in gregarious parasitoids parasitizing exceptionally small hosts where it results in scramble competition, there are indications that in larger clutches mortality due to competition for food is less important (Shiga & Nakanishi, 1968). The second effect of competition for food in these larger clutches leads to all larvae completing their development, but results in adults of a less than normal size. Although this may prevent to some extent the death of supernumerary larvae, a decrease in body size may effect the fitness of individual progeny by decreasing for example fecundity or longevity (Klomp & Teerink, 1967; Shiga & Nakanishi, 1968).

The relationship between body size and fitness of the parasitoid determines whether the gain of supernumerary larvae counterbalances the disadvantage for the individual progeny. If not, the parental wasp will try to prevent these effects of food shortage. Pianka (1976) illustrated this with a graphical model, which is modified here (Fig. 5.1) according to Alexander (1982). Body size and fitness are likely to be positively related, but not necessarily in a linear manner. It would be more reasonable to suggest that gains in fitness depend on the proportional increase of body size, and also that there is a minimum size below which fitness is zero (Fig. 5.1, solid line). Assume that a particular body size is the result of the allocation per parasitoid egg of an amount of host material W_h/c (where W_h is host weight and c is clutch size) and that the number of progeny is proportional to c (thus assuming that mortality,

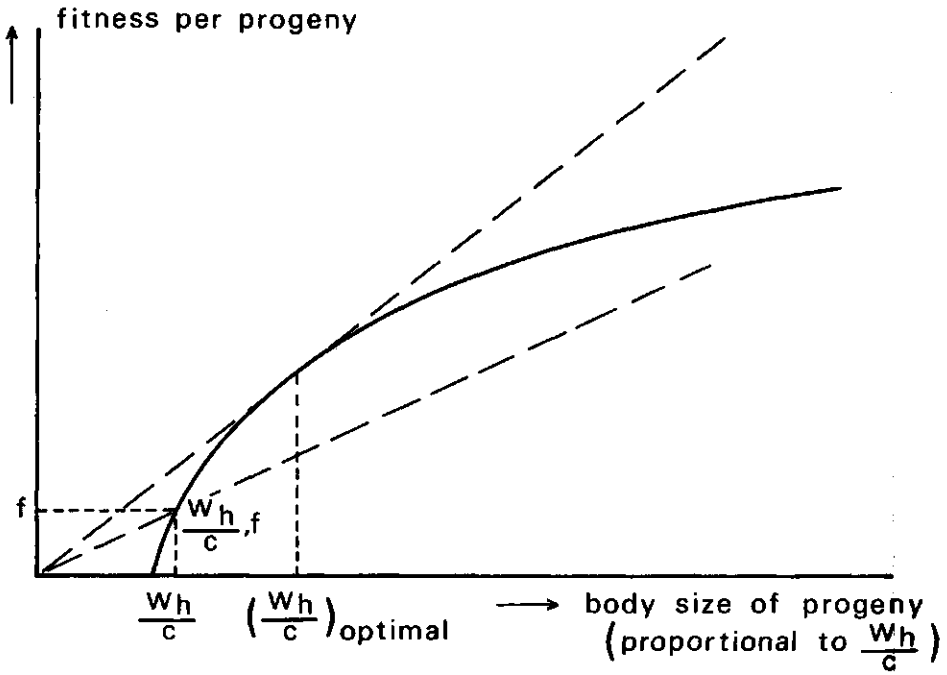

Fig. 5.1 Theoretical relationship between the size of individual progeny, expressed as the ratio of host weight to clutch size (W_h/c), and its fitness (f). Maximum host profitability is indicated by the tangent through the origin, and optimal size of progeny by (W_h/c)optimal (after Pianka, 1976).

if present, is density independent). A parent which deposits c eggs near a host of weight W_h resulting in progeny with individual fitness f, has a host profitability proportional to fc. Consider a point $(W_h/c, f)$ on the graph in Fig. 5.1. The gradient of a straight line through this point and the origin is fc/W_h. If host weight W_h is constant, this gradient is proportional to the host profitability the parent receives. The maximum gradient is found by constructing the tangent through the origin. In this way the optimal clutch size and body size of progeny can be derived easily from point (W_h/c)optimal.

Increasing the number of progeny leading to a diminution in body size by food shortage, although unprofitable for the individual progeny, may sometimes increase the total host profitability for the parent. Hence it may be profitable for a wasp to induce to a certain degree competition for food between her progeny.

Thus it is assumed that the parent wasp tries to maximize the profitability to be derived from one host. When more hosts are parasitized during a lifetime this does not need to be the most optimal strategy. As shown by Klomp & Teerink (1967), it is theoretically more profitable to reduce the clutch size when the host density is so high that the available number of eggs of a female becomes the limiting factor. In such cases, the fitness gain should be maximized per egg laid, instead of maximized per host. Klomp & Teerink (1967) found clutch sizes of *Trichogramma embryophagum* Htg. to be independent of host density. They concluded that constraints in the animal prevented the clutch size being varied according to host density, host size and the parasitoid's fecundity. On the other hand Ikawa & Suzuki (1982) found that clutch sizes of *Apanteles glomeratus* L. gradually decreased with successive ovipositions in unparasitized hosts of the same size. They explained this and another case (*Caraphractus cinctus* Walker; Jackson, 1966) by assuming that the parasitoid perceived an increasing host density and switched from maximizing reproductive success per host to maximizing reproductive success per egg laid. *Colpoclypeus florus* is most probably not able to estimate HER (Section 3.2). Thus it could be expected that if clutch-size optimization occurs, it maximizes the reproductive success per host. Nevertheless; the switching phenomenon described by Ikawa & Suzuki (1982) was investigated for *C. florus*.

After determining the relationship between host weight and clutch size, the causality between the two was analysed. Since it is very unlikely that the parasitoid measures directly the weight of the host, she may use some measure to give a more crude estimate of the capacity of the host. This

may lead to certain constraints in ability to reach optimal clutch size.

Laboratory data on clutch size and pre-adult mortality were compared with field data. Various clutch sizes were tested with respect to the expected reproductive success per host. After manipulation of the number of eggs per host, the results of the parasitizations were measured in terms of the number of daughters and their individual weights. From data on the relationship between the weight of a female and her fertility, the expected reproductive success per host given a certain clutch size was calculated.

5.2 Relationship between host weight and clutch size

5.2.1 Materials and methods

Data on host weight and number of eggs laid near each host from experiment III were used (see Subsection 3.3.1). Since in this experiment only host larvae less than one day after ecdysis were used and since most of the total increase in larval weight occurs during the last instar, complementary data on older fifth instars were collected (experiment VII). At 1-2, 2-3, 3-4 and 4-5 days respectively after ecdysis, a number of fifth instars were weighed, and allowed to be parasitized. The eggs were counted, and the emerging adults counted and sexed, as described in experiment III.

5.2.2 Results and discussion

The results of experiment III are given in Table 3.3 per host instar and in Table 3.5 per host weight. Similar results for experiment VII are presented in Tables 5.1 (host age) and 5.2 (host weight).

The clutch sizes produced on all hosts tested in the two experiments were taken together per host weight class (Fig. 5.2). It can be concluded that host weight and clutch size are positively related. This relationship is present

Table 5.1 Host-age dependent parasitization of *Colpoclypeus florus* on fifth instar hosts in the laboratory

Age of the host (days after ecdysis to L_5)	1 - 2	2 - 3	3 - 4	4 - 5
Number tested	43	40	45	17
Mean weight (mg)	30.3	44.9	47.5	49.1
Proportion accepted by parasitoid	0.98	0.85	0.89	0.88
Mean clutch size of parasitoid	26.9	33.8	28.2	31.5
Proportion of parasitoid eggs becoming adult	0.32	0.29	0.33	0.29
Sex ratio of adults ($\frac{\sigma}{♀ + \sigma}$)	0.16	0.07	0.13	0.09

Table 5.2 Mean clutch size of *Colpoclypeus florus* on fifth instar hosts in the laboratory, classified per host weight

Weight class (mg)	Number tested	Mean clutch size	Weight class (mg)	Number tested	Mean clutch size
14.6 - 17.5	3	31.7	35.6 - 38.5	9	28.3
17.6 - 20.5	1	29	38.6 - 41.5	4	31.3
20.6 - 23.5	4	30.8	41.6 - 44.5	14	29.7
23.6 - 26.5	8	25.4	44.6 - 55.0	19	31.6
26.6 - 29.5	10	27.0	55.1 - 65.0	8	34.3
29.6 - 32.5	10	26.3	65.1 - 75.0	4	35.0
32.6 - 35.5	12	27.2	75.1 - 85.0	4	36.5

within several host instars and thus clutch size is not directly related to host instar. However, the relationship between host weight and clutch size is not linear, which would be expected if the implicit assumption is made that the capacity of a host to provide food for parasitoid larvae is interchangeable with its weight. As a result the number of eggs per unit host weight (expressed by the ratio of clutch size to host weight c/W_h and referred to as intensity by Shiga & Nakanishi, 1968) is not constant but relatively high

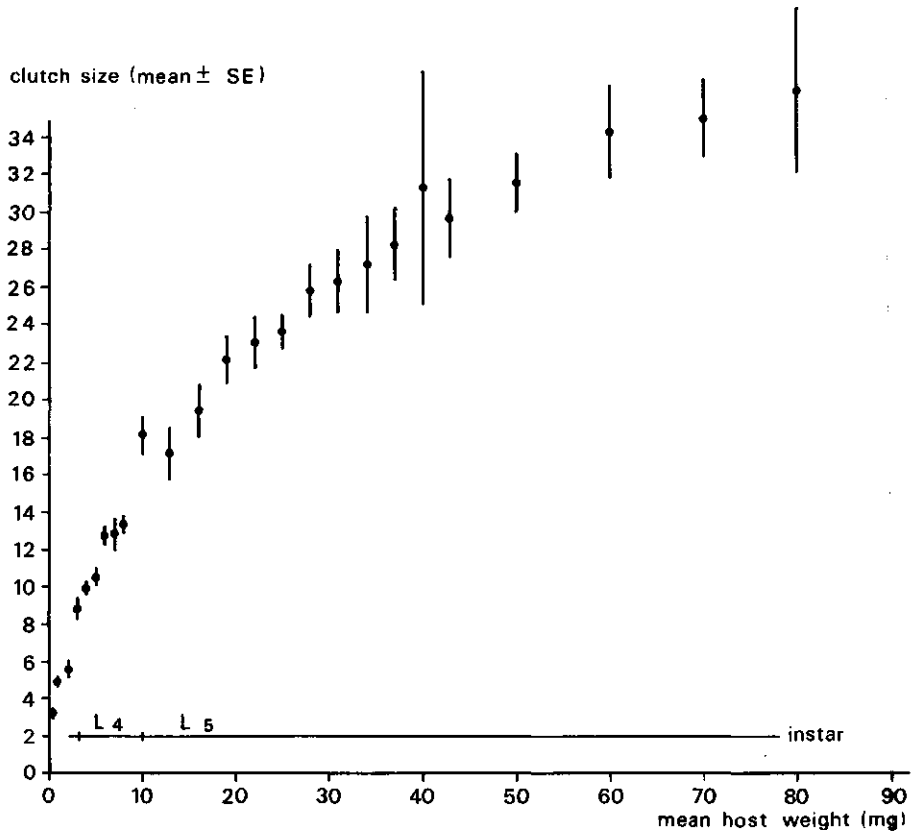

Fig. 5.2 Mean clutch size of *Colpoclypeus florus* for a number of host-weight classes.

in the lower part and relatively low in the upper part of the host-weight range. The following explanations for this phenomenon are put forward:

(i) Differences in intensity are functional because
- either the proportion of biomass consumable by parasitoid larvae is higher in small than in large hosts,
- or the oviposition rate is low and would lead to great age differences between the first and the last egg of large clutches, which would result in unequal allocation of host material and increased mortality due to starvation,

- or the oviposition behaviour of the parasitoid is adapted to the extra growth of the host (which is relatively fast in small hosts) between oviposition and settlement of the parasitoid larvae.

(ii) Differences in intensity are not functional, because
- either, due to the inability of the female parasitoid to measure host weight, they are caused by the nature of the estimate of the host capacity used instead,
- or a female parasitoid has too few eggs available to produce the optimal clutch size on large hosts.

If the differences in intensity are functional, competition for food amongst the parasitoid larvae, if any, should not lead to increased mortality. On the other hand, intensity-dependent mortality may occur if one of the explanations under ii holds. To test this, the clutches were classified according to intensity and mean pre-adult survival calculated (Fig. 5.3). Although overall survival was low (which could be concluded from Tables 3.3 and 5.1), the highest and lowest intensities suffered from increased mortality. In the range of intensities from 0.7 to 2.0 eggs/mg survival levels out, and it is not surprising that with increasing intensity, competition leads to higher mortality. However, it may be expected that this group of high-intensity parasitizations consists of extremes equally distributed throughout the host instars, but it consists almost exclusively of hosts of low weight. The same holds for the group of parasitizations of low intensity, which consists only of older fifth instars. However, in the latter group the reason of the additional mortality is not clear. Pre-adult mortality due to a too small number of eggs per host is mentioned for internal gregarious parasitoids (Taylor, 1937; Salt, 1961). Since the hosts of *C. florus* ultimately die because of the feeding activities of the larvae, they will

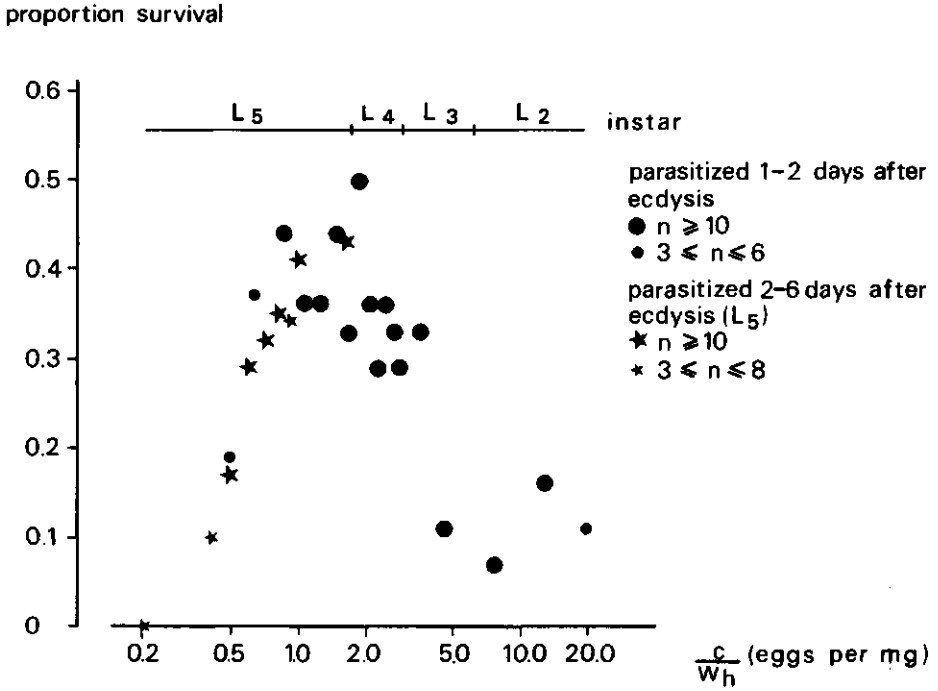

Fig. 5.3 Relationship between intensity of a parasitization, expressed as the ratio of clutch size to host weight (c/W_h), and pre-adult survival of *Colpoclypeus florus*.

live longer when parasitized at relatively low intensity. This may lead to intensity-dependent mortality since the still mobile hosts are able to attack or otherwise disturb and damage the young larvae.

A mortality factor which does not depend on the intensity of the parasitization, may be formed by the physiological changes in older fifth instars due to approaching pupation. But since mortality in this group does not depend on the age of the host (see Table 5.1), and since parasitizing females reject hosts which are close to pupation (J.C. van Veen, pers. comm.) it is more likely that differences in intensity are the main factor determining mortality. Thus apart from the above range of intensities (0.7 to 2.0 egss/mg) in which

survival is constant, the observed differences in intensity cannot be considered to be functional, because they lead to additional mortality in the pre-adult stage of the parasitoid.

With regard to the reasons put forward for differences in intensity being functional, a total of 103 time intervals between oviposition of two successive eggs were determined, most of them in large clutches. The mean time between two ovipositions was 12.1 min, giving an average oviposition rate of 5 eggs/h. Doubling the intensity of a parasitization which originally consisted of 30 eggs would take about 6 h. The difference in age between first and last egg would then be 12 h, which is a short interval compared with the total time prior to hatching (60 h) or with the total feeding time (3.5 days). Therefore in a situation with little or no competition for food this explanation is improbable.

Additional growth of hosts between oviposition and settlement of the parasitoid larvae may be larger in the field than in the laboratory (see Subsection 4.2.2) and the increased mortality in the high-intensity parasitizations may possibly be absent in the field. However, in this case the increased mortality in the low-intensity parasitizations remains to be explained. In Section 5.4, the pre-adult survival of the parasitoid in the field is discussed. Apart from this point, the occurrence of intensity-dependent mortality favours the explanations given for differences in mortality not being functional.

Whereas the explanation of the female parasitoid having too few eggs available only accounts for the fact that large hosts receive too few eggs, the inability of a female parasitoid to measure host weight may explain both the high intensity and the low intensity parasitizations. It may be proposed that the female parasitoid measures the length of the host, while measuring the weight would be functional. She may adapt the number of eggs to the weight of hosts of a particular length, but since weight is a third-power function of length, the weight of a shorter host may be overestimated

and the weight of a longer host underestimated.

In a preliminary experiment on the problem of estimating the capacity of a host, the length of at least four host larvae of each weight class was measured, and the mean clutch size of the parasitoid plotted against the mean length of the hosts of each weight class (Fig. 5.4). The relationship between host length and clutch size was substantially rectilinear, and strongly suggests that some linear measure is used by the parasitoid, which may be host length.

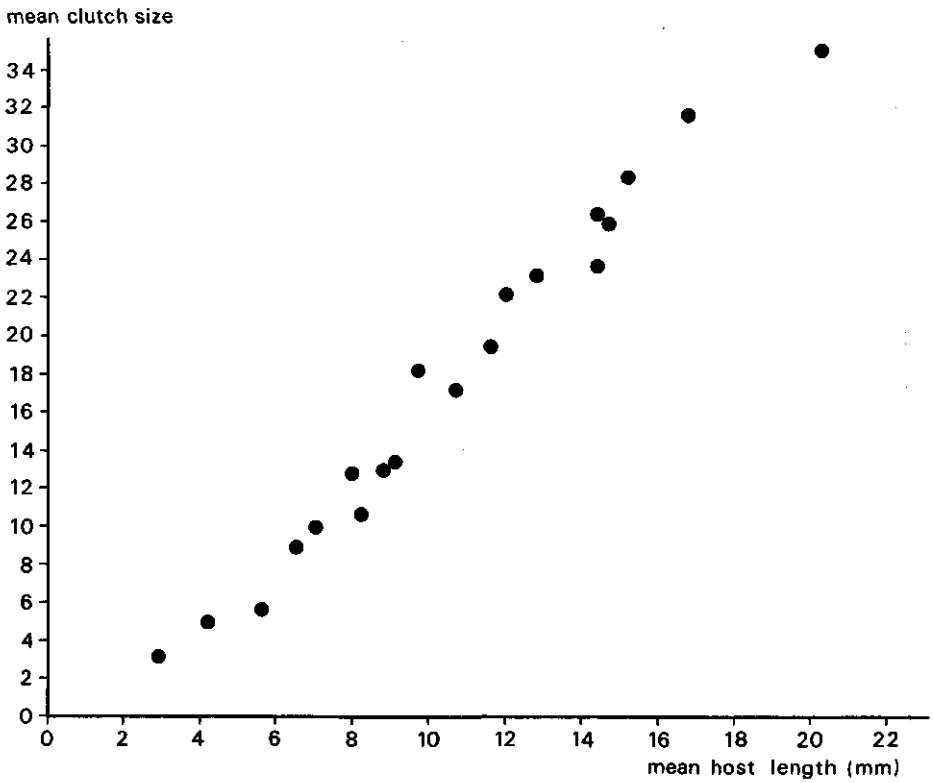

Fig. 5.4 Relationship between mean host length and mean clutch size of *Colpoclypeus florus* for a number of host-weight classes.

5.3 Factor used to determine the clutch size

5.3.1 Introduction

It has been hypothesized above that the parasitoid may determine clutch size by measuring the length of the host rather than its volume. Experiments were designed to distinguish these host characteristics and others which could be used to estimate the capacity of the host, such as the width of the abdomen, the width of the head capsule, the girth of the abdomen or the dimensions of the web.

5.3.2 Materials and methods

Experiment VIII consisted of two host series, each of about 20 larvae of the fourth instar. They were put on leaves in vials following the usual procedure (Subsection 2.3.2). After one day, the host larvae were anaesthetized with CO_2 for a short period and removed from their webs. Each larva of the first series ("short") was shortened by tying a ligature (nylon, ø 0.16 mm) between the second and the third pair of prolegs (Fig. 5.5a), and then removing the caudal one-third part of the body. The larva was weighed, its length measured

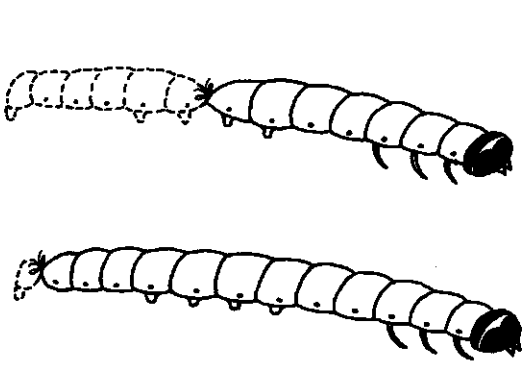

Fig. 5.5 Fourth instar larvae of *Adoxophyes orana* with ligatures used to manipulate the factor length of the host (experiment VIII), a. shortened host ("short"), b. control host ("long").

and then replaced in its web. The larvae of the second series ("long") received the same treatment but the ligature was applied in front of the pair of anal prolegs (Fig. 5.5b) and only these and a very small hind part of the body were removed. After the larvae had recovered from the anaesthesia, standardized female parasitoids were introduced into the vials of both series. The following day the condition of the hosts was checked and the eggs laid near each host were counted.

Experiment IX was designed to test the effect of the dimensions of the host web on determination of clutch size by the parasitoid. About 50 larvae of the third and the fourth instar were placed on leaves in vials as usual. After one day about half of the larvae of each instar were removed from their webs and interchanged. The remaining larvae in each group were also removed but replaced in their own webs. Thus there were four groups of about 25 hosts each: no treatment L_3; L_3 in web of L_4 size; no treatment L_4; and L_4 in web of L_3 size. All hosts were exposed to parasitization as usual and the following day the eggs were counted.

5.3.3 Results and discussion

Preliminary experiments in which the behaviour of short and long hosts was examined showed little difference in liveliness, and both types remained remarkably mobile. Both the short and long hosts could not defaecate and stopped feeding after some time. Therefore the two host series of experiment VIII were considered to differ only in body length, weight and volume (Table 5.3). Both host types were accepted normally (proportion parasitized was about 0.90). The decrease in length and weight of the shortened hosts compared with the control group was considerable (± 35%), but no significant differences were found between the mean clutch sizes. Differences in body-length and -volume of the host did not affect significantly the number of eggs deposited by a female parasitoid. Thus the suggestion made in

Table 5.3 Clutch size of *Colpoclypeus florus* with artificially shortened hosts (short) and with hosts which had a control treatment (long) (experiment VIII)

			Parasitized hosts only		
	Number tested	Number accepted	Length (mm) (mean ± SE)	Weight (mg) (mean ± SE)	Clutch size (mean ± SE)
Long hosts	26	23	7.4 ± 0.2	4.32 ± 0.23	11.6 ± 0.6
Short hosts	20	18	4.8 ± 0.1	2.79 ± 0.17	11.0 ± 0.7
Decrease (%)			35.1	35.4	
t-test, one-sided; $\alpha = 0.05$					NS ($p > 0.25$)

Table 5.4 Clutch size of *Colpoclypeus florus* with hosts in webs of varying size (experiment IX)

Larval instar of host	Size of the web	Number tested	Number accepted	Clutch size (mean ± SE)	t-test, one-sided; $\alpha = 0.05$
L_3	L_3	25	21	4.2 ± 0.3	S ($p = 0.019$)
L_3	L_4	25	19	5.2 ± 0.4	
L_4	L_3	24	22	13.2 ± 0.6	NS ($p > 0.25$)
L_4	L_4	24	20	13.8 ± 0.7	

Subsection 5.2.2 was premature, and the proximate factor used by the parasitoid to determine clutch size is to be found among the other host characteristics listed in Subsection 5.3.1.

During experiment IX it became clear that, although a fourth instar larva could easily be put in the tunnel of L_3 web, the caterpillar began immediately to enlarge it and at the time of introduction of the parasitoid no difference could be seen between these webs and those of L_4. Third instar larvae did not reduce the size of the tunnel, when put in a web of L_4 size. The results of this experiment are given in Table 5.4. The mean clutch size of groups 1 and 2 which

contained third instar larvae was much lower than groups 3 and 4 which contained fourth instar larvae. Although there was also a significant difference in clutch size between groups 1 and 2, the effect of the larger web did not lead to the usual clutch size for the original maker of the web. (There was no difference in clutch size between groups 3 and 4, but since the webs were about the same size at the time of parasitization, these results could not be used). Hence the effect of the dimensions of the web on clutch size, if any, was only very small.

Of the characteristics of the host suggested as being used by the parasitoid as an estimate of the capacity of the host, only the width of the head capsule and the width or the girth of the abdomen remain. No attempts were made to distinguish the last two, instead the effects of these two characteristics were compared with the width of the head capsule, using the natural variation among larvae of the fifth instar. Since the head capsule does not follow the gradual growth of the abdomen, but is enlarged stepwise at the points of ecdysis, it was possible to select a number of hosts (n = 21) of about the same head capsule width but of varying abdominal width. From Fig. 5:6, it can be seen that there is a clear relationship between width of abdomen and clutch size within this group. The reverse selection of hosts was also made, that is a number of hosts of similar abdominal width but of varying head capsule width, which was possible by the variation in head capsules among larvae of the fifth instar. The width of the head capsule did not affect the clutch size and thus is not used as a proximate factor for it (Fig. 5.7).

Although there is a rectilinear relationship between host length and clutch size, no causality was found (see Table 5.3). This may also be the case with host width, but no experiments were carried out in which host width was varied artificially. Nevertheless, from the point of view of the parasitoid, host width may be more easily measured than host volume and even host length. Since both host width and host

Fig. 5.6 Width of abdomen (eye-piece micrometer units) of fifth instar hosts and clutch size of *Colpoclypeus florus*.

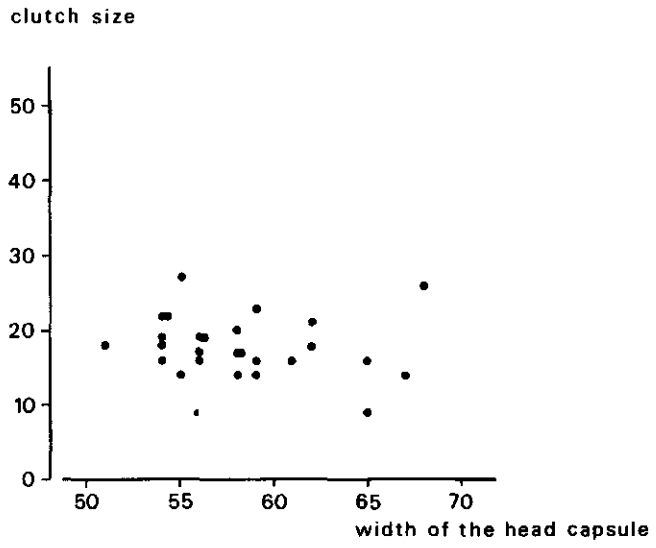

Fig. 5.7 Width of head capsule (eye-piece micrometer units) of fifth instar hosts and clutch size of *Colpoclypeus florus*.

length as linear measures are correlated with clutch size, host width as a proximate factor may explain the rectilinear relationship found between clutch size and host length. If there is yet another host characteristic which is the actual proximate factor used by the parasitoid, it must be related to and with the same dimension as host width.

In general, little research has been carried out on proximate factors used to determine clutch size, although the relationship between host size and clutch size is known for many parasitoids. Sometimes a particular behaviour is observed which it is considered serves to estimate host size. The characteristic "drumming" act of *Trichogramma embryophagum* (Klomp & Teerink, 1962; Uematsu, 1981) has been shown to be connected with the determination of the size of the host (eggs of Lepidoptera). Further investigations on *T. minutum* Riley (Schmidt & Smith, in press) have shown that the mechanism is mechanosensory, and based on the surface area attainable to the drumming performance. The results of Schmidt & Smith (in press) suggest, that the volume of the (spherical) host is first estimated by measuring the curvature, and corrected when only a part of the surface is accessible. This results in fewer progeny in partly concealed hosts, for instance when they are clustered. However, no such behaviour has been observed in *C. flavus*. Also, the area of the host surface, which is accessible to the parasitoid, did not affect the clutch size.

Wylie (1967) studied *Nasonia vitripennis* (Walker) parasitizing on equally sized puparia of *Musca domestica* L. In one group the puparia were exposed for about three-quarters of their length above a plasticine base, in the other group only one-third of their length was exposed. The number of eggs laid in each host did not differ between "exposed" and "buried" hosts. His results for *N. vitripennis* are in agreement with the results of experiment VIII (Table 5.3), and the following explanation is given: "The female parasitoid does not recognize the host's size prior to drilling (....). The progressive chemical and (or) physical

changes in the host caused by piercing develop more rapidly or are perceived more readily by the female in small than in large pupae, thereby leading to earlier rejection of the former than of the latter" (Wylie, 1967). This explanation cannot be put forward for the results of experiment VIII, because hosts of different size ("long" and "short" hosts) appeared to receive the same clutch size. On the contrary, the finding that the parasitoid uses a proximate factor associated with the width of the host explains the results of Wylie (1967), since in both "exposed" and "buried" hosts girth was accessible to the parasitoid. However, if *N. vitripennis* uses the same mechanism as was found for *T. minutum*, the clutch sizes in "exposed" and "buried" hosts would differ.

Some Pimplinae (members of the genera *Pimpla*, *Itoplectis* and *Apechthis*) are also known to use the width of a host (pupae of *Ephestia kuehniella* Z.) as an estimate of its size, but the effect of the length of the host could also be observed (Shaumar, 1966). The use of an estimate associated only with the width of the host, as with *C. florus*, has the disadvantage that the capacity of suitable host species of varying length/width ratios is systematically overestimated or underestimated. This suggests that data on the method the parasitoid uses to estimate the host size may reveal serious constraints in the exploitation of host resources.

5.4. Clutch size and juvenile mortality in the field and laboratory

5.4.1. Introduction

As low survival rates were found for *C. florus*, even at optimum parasitization intensity, juvenile mortality of *C. florus* in the field was determined to ascertain whether artificial mortality had been induced in laboratory experiments.

5.4.2. Materials and methods

Juvenile mortality in the field (experiment XI). A host population in the field was created by artificial introduction, and at regular intervals after potential parasitism, some of these hosts were collected. The first sample, collected just after parasitism served to estimate clutch size in the field. To estimate mortality during pre-adult development from field samples, large numbers of parasitized hosts of a certain stage are required. Therefore, this procedure of introducing a host population in the field and sampling the parasitized hosts later was carried out seven times, at intervals of one week between the beginning of the tests.

In each of the seven tests, 20 trees were infected on the same day each with 10-15 black egg masses. The tests were carried out in the experimental orchard, "De Schuilenburg", between 15 June and 28 July 1981, being the period in which the summer generation of *A. orana* usually starts.

Sampling was done according to the scheme presented in Fig. 5.8. The duration of development of the hosts and of the parasitoids developing on each of four host instars is given. Developmental data originate from laboratory observations at 21°C (see Fig. 2.5). Since the temperature in the field varied (being on the average 18°C), the exact time of sampling was calculated from an extrapolated temperature development table for the larval period of *A. orana*. The developmental rates of the various stages of host and parasitoid in the temperature range was assumed to depend on temperature in the same way. Since each developmental rate has a considerable variation the time of sampling was not critical.

Parasitoids in the following stages of development were sampled (Fig. 5.8): the egg stage (e); the larval stage in the feeding period (l_1) and in the period thereafter (l_2); the pupal stage (p); and the stage of just emerged adult wasps, which have not escaped from the cocoon of their host

(a). These sample points of parasitized hosts of various larval instars (indicated by * in Fig. 5.8) happened to coincide largely with one another. A tree could be sampled only once and from experience was known to yield on average only 5-10 parasitized hosts. Thus by using one tree for each sample point, the seven successive tests could yield about 35-70 parasitized hosts for a sample. Thus the 20 trees of one test were sampled at nine successive times in numbers varying from one to four trees (see Fig. 5.8). In practice, the time interval between samples of one test was about a week. Development in the field at 18°C took about twice as long as in the laboratory at 21°C.

A tree was searched for the occurrence of caterpillars. Leaves with caterpillars were put in glass vials (23 mm diameter and 97 mm high) and sampling was stopped when 30 caterpillars had been found, or after one hour of searching. Sampling was done between 8 July and 14 October.

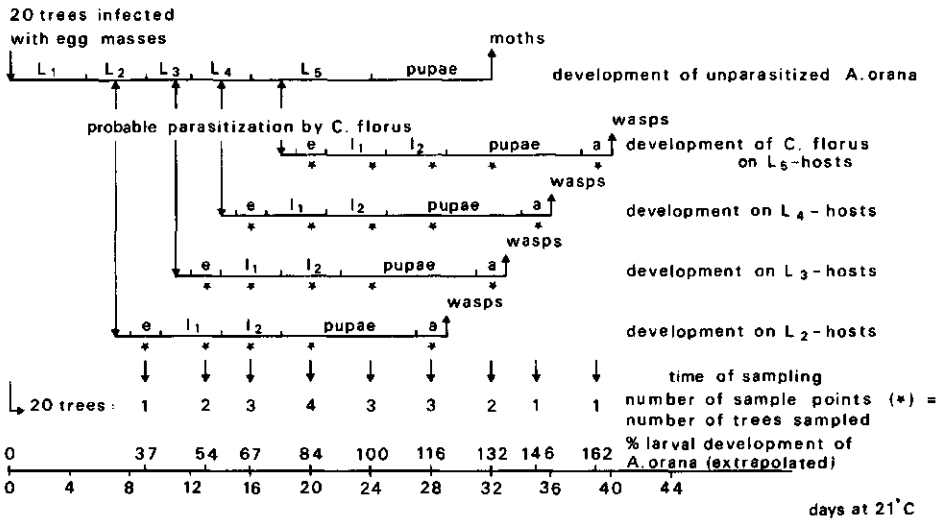

Fig. 5.8 Scheme of a test to determine juvenile mortality of *Colpoclypeus florus* in the field. The experiment (experiment XI) consisted of seven of these tests.

Directly after collection, the caterpillars were examined carefully in the laboratory for the presence of parasitoids. If *C. florus* were present, the number of eggs, feeding larvae, non-feeding larvae, pupae or adult wasps were recorded. Pupae and adult wasps were sexed. Caterpillars with eggs of *C. florus* were weighed, and the head capsules of the parasitized and the unparasitized caterpillars were measured to determine the larval instar.

After examination, all caterpillars parasitized by *C. florus*, or by other ectoparasitoids, were stored with the original leaf in a vial (14 mm diameter and 55 mm high). When necessary, fresh apple leaves were added. The caterpillars without external sign of parasitization were put in identical vials containing diet and stored upside down, which considerably reduced the chance of the diet becoming mouldy. These caterpillars were provided regularly with new diet, and kept at a temperature of 21°C and relative humidity of 55 %. The vials were checked weekly for newly emerged moths and ecto- and endoparasitoids. Parasitoids were identified, counted and sexed.

Clutch size. Clutch size and juvenile mortality of *C. florus* on hosts of various instars resulting from this field experiment were compared with results obtained in the laboratory.

Juvenile mortality in the laboratory (experiment XII). To compare the intermediate mortality of eggs, larvae and pupae of the parasitoid with laboratory data, an additional laboratory experiment was carried out. A total of 244 hosts of the fourth larval instar were offered for parasitization (see Subsection 2.3.2). The number of parasitoid eggs, larvae and pupae in the vials were counted each day, and the vials opened when visual inspection through the glass was not possible. When this was necessary, the parasitoids were disturbed and could not be used again.

5.4.3. Results and discussion

Juvenile mortality in the field (experiment XI). A total of 2424 healthy and parasitized caterpillars were collected (about 17 caterpillars per tree): 126 L_2, 386 L_3, 1095 L_4 and 817 L_5. The last two instars were overrepresented because they were collected in the middle outer part of the canopy (see Fig. 4.4). This effect was unknown at the time of sampling.

Of the 2424 caterpillars, 713 (29 %) were parasitized by *C. florus* and during rearing 241 (10 %) died in the larval or pupal stage without any sign of ectoparasitism. From the 2424 - 241 = 2183 remaining caterpillars, other parasitism could be checked. Of these 462 (21 %) were parasitized (or multiparasitized) by parasitoids other than *C. florus* and 113 (5 %) were multiparasitized by *C. florus* and another parasitoid. Of these multiparasitized hosts, 60 % had some *C. florus* among their adult parasitoid offspring. The mortality of *C. florus* due to multiparasitism can be estimated roughly, as the proportion of hosts which, although parasitized, yielded no adult offspring. This is (113/713) x 40% = 6 %.

Virtually no other host species were present in the 1008 emerging moths (99.5 % *A. orana*) and in the large (n = 494) sample of the parasitized caterpillars (99 % *A. orana*). Identification of parasitized hosts was based on the structure of the head capsules (Evenhuis & Vlug, 1972).

To compare the proportion parasitized by *C. florus* of various larval instars of the host, all the data of experiment XI cannot be used because the host instars were not present simultaneously throughout the sampling period. Only the first sample point of each instar in a test (parasitoid clutches in the egg stage) was used and these data were arranged chronologically. Only samples collected in the same week were used (Table 5.5). Differences in parasitization of the various instars have been discussed in Subsection 4.4.2.

Table 5.5 Proportion of four larval instars of the host parasitized by *Colpoclypeus florus* (experiment XI)*

Larval instar	L_2		L_3		L_4		L_5	
	test No.	proportion of hosts accepted	test No.	proportion of hosts accepted	test No.	proportion of hosts accepted	test No.	proportion of hosts accepted
Week of sampling 4th	4	0	3	0.03	2	0.31	1	0.34
5th	5	0	4	0	3	0.01	2	0.20
6th	6	0	5	0.07	4	0.19	3	0.08
7th	7	0	6	0.04	5	0.06	4	0.19
Mean proportion of hosts accepted		0		0.04		0.14		0.20
Number of hosts accepted		49		79		195		231

* Caterpillars which had been recently parasitized were collected simultaneously in the same part of the orchard so that data for the same week were comparable.

Individuals in almost every possible stage of parasitism were collected. They were grouped in five stages (Table 5.6): e contains the egg stage; l_1 from newly hatched to feeding larvae in various phases of satiation; l_2 larvae whether satiated or not, which had ceased feeding; p the pre-pupae and the pupae; and a the number of adults or, when some or all had escaped from the web, the number of exuviae still present in the web. Parasitized hosts without living parasitoids at the time of sampling were not considered. The number of parasitized hosts of the second larval instar remaining was too small to calculate survival. Parasitoid survival from the egg stage was calculated for the third, fourth and fifth instar of the host and for each stage (s) of parasitism (Table 5.6). Survival in stage s was calculated as: the mean number of survivors in stage s ($\bar{x}_s$) divided by the mean number in the egg stage ($\bar{x}_e$).

In Fig. 5.9 the survival is plotted against the stage of development of the parasitoid. Survival of parasitoids developing on the various host instars follows roughly the same trend: high mortality between hatching and first half of feeding and almost no mortality after feeding has ceased. Survival from egg to adult is about 50 %. In Fig. 5.9 this trend is shown by the line connecting the weighted means at each stage of development.

Before comparing the results of this field experiment with those of the laboratory experiments, two systematic errors need to be discussed. Firstly, the mean number of eggs in the field experiment was estimated from clutches of all ages, therefore half of the mortality in the egg stage, which consists largely of the casual destruction of eggs by the eating host, was not taken into account. This led to underestimation of both mean clutch size and juvenile mortality. Secondly, parasitized hosts on which all the juvenile parasitoids die, are not recognizable as such after a while. The rare cases in which the host still lived were classified in the 10 % not emerged unparasitized hosts since parasitization prevents pupation. However, most of these

Table 5.6 Survival of various stages of *Colpoclypeus florus* in the field on three larval instars of the host (experiment XI)*

Parasitoid survival	on L_3		on L_4		on L_5	
	number ($\bar{x}_s \pm SE$)	proportion ($\bar{x}_s/\bar{x}_e$)	number ($\bar{x}_s \pm SE$)	proportion ($\bar{x}_s/\bar{x}_e$)	number ($\bar{x}_s \pm SE$)	proportion ($\bar{x}_s/\bar{x}_e$)
Developmental stage of parasitoid eggs (e)	7.0 ± 1.5	-	11.2 ± 0.8	-	25.2 ± 2.3	-
newly hatched and feeding larvae (1_1)	3.2 ± 0.8	0.46	8.5 ± 0.9	0.76	17.1 ± 2.0	0.68
non-feeding larvae (1_2)	4.1 ± 0.6	0.58	6.8 ± 0.6	0.61	15.4 ± 1.7	0.61
pre-pupae and pupae (p)	2.5 ± 0.4	0.35	6.4 ± 0.7	0.57	15.6 ± 1.8	0.62
newly emerged adults or their exuviae (a)	3.3 ± 0.3	0.48	6.7 ± 1.1	0.60	9.3 ± 2.0	0.37

* For each stage of parasitism (s) survival was calculated as the mean number in stage s ($\bar{x}_s$) divided by the mean number in the egg stage ($\bar{x}_e$).

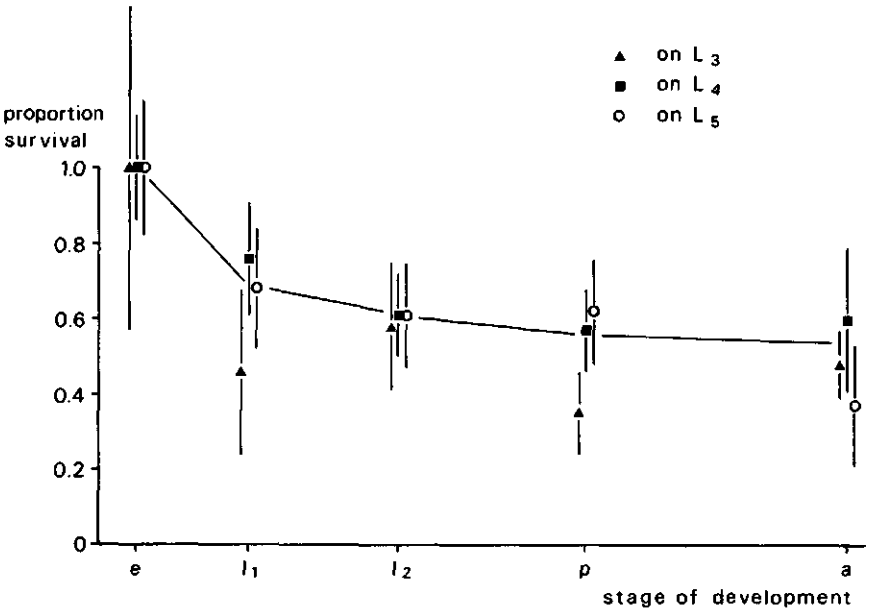

Fig. 5.9 Survival from egg to adult of *Colpoclypeus florus* in the field on three larval instars of the host (L_3, L_4 and L_5). Vertical bars are 95% confidence intervals ($\pm$ 1.96 $(SE)_i/\bar{x}_e$), and the line connects the weighted means at each stage of development of the parasitoid. Stages of development: e = eggs; l_1 = newly hatched and feeding larvae; l_2 = non-feeding larvae; p = pre-pupae and pupae; a = newly emerged adults or their exuviae.

hosts were dead at the time of sampling and have not been included in the results. This also led to underestimation of juvenile mortality. Further, predation, for example by birds whereby the host is taken away, may have been considerable but was not measured by this method.

Juvenile mortality in the laboratory (experiment XII). The results of experiment XII were used to assess the extent of overestimation of survival in the field. In total 218 host larvae (89 %) were parasitized and 122 clutches were counted at 24, 48 and 72 h after parasitoid introduction without the

need to open the vial and disturb the web. From these counts, egg mortality due to destruction by the host was estimated to be 0.42 egg/day. Assuming that this loss was equally distributed throughout the egg stage, total egg destruction by the host during the 60 hours of this stage was 1.04 egg (10 %). Oviposition occurred on average 17 h after parasitoid introduction, thus the error by counting the eggs at 24 h was 0.12 egg and actual clutch size was about 11.0. At day 4, 6 and 7, young larvae could only be counted by taking samples from the whole group, but after day 9 a group of 56 parasitized hosts could be inspected each day through the glass until adult emergence.

In Table 5.7a the mean number of individuals per host are given for each count, excluding those parasitized hosts on which all individuals had died. Survival was calculated in the same way as in the field. Fig. 5.10 shows that juvenile survival on hosts of the fourth instar calculated this way was similar in the laboratory and in the field.

In Table 5.7b the mean number of individuals per host are given, including the unsuccessfully parasitized hosts. Survival was calculated from day 1 onwards, thus correcting for the systematic errors made in calculating juvenile survival in the field. Actual juvenile survival on hosts of the fourth instar appeared to be 12 % less than the uncorrected values (see also Fig. 5.10). Mortality other than as a result of predation occurred before the larvae had reached the non-feeding stage (1_2). Mortality was low between hatching of the eggs and settlement of the young larvae on the host, that is between day 3 and day 4.

Juvenile survival (Table 3.3) was compared with survival based on the same data but excluding unsuccessfully parasitized hosts and 4 % early egg mortality. Egg mortality was assumed to be independent of clutch size. The resulting (uncorrected) values gave an equal survival on hosts of the third, fourth and fifth instar of about 50 %, as was found in the field. However, the real proportion of survival on the third instar was much lower than on the fourth and fifth

Table 5.7 Survival* of juvenile parasitoids in the laboratory on the fourth instar of the host (experiment XII)

Time after introduction of the parasitoids (days)	a. Calculated survival excluding parasitized hosts where all individuals died, and not corrected for early egg mortality			b. Calculated survival including parasitized hosts where all individuals died, and corrected for early egg mortality		
	number of hosts	number of surviving juvenile parasitoids per host ($\bar{x}$ ± SE)	proportion surviving from day 2 onwards	number of hosts	number of surviving juvenile parasitoids per host ($\bar{x}$ ± SE)	proportion surviving from day 1 onwards
1	122	10.9 ± 2.8	-	122	11.0**	1.00
2	122	10.5 ± 3.1	1.00	122	10.5 ± 3.1	0.95
3	122	10.1 ± 3.3		122	10.1 ± 3.3	0.91
4	21	9.9 ± 1.9		21	9.9 ± 1.9	0.90
6	22	7.3 ± 4.3	0.70	22	7.3 ± 4.3	0.66
7	22	6.9 ± 2.9		22	6.9 ± 2.9	0.62
9	49	6.1 ± 3.2	0.58	56	5.3 ± 3.6	0.48
14	49	6.1 ± 3.2	0.58	56	5.3 ± 3.6	0.48
> 21	47	6.2 ± 3.1	0.59	56	5.2 ± 3.7	0.47

* Survival was calculated in the same way as for the field data (a) and corrected for early egg mortality and unsuccessful parasitism (b).

** Actual clutch size was 11.0 because mean oviposition occurred 7 h before the first count, which resulted in an error of 0.12 eggs due to mortality prior to counting.

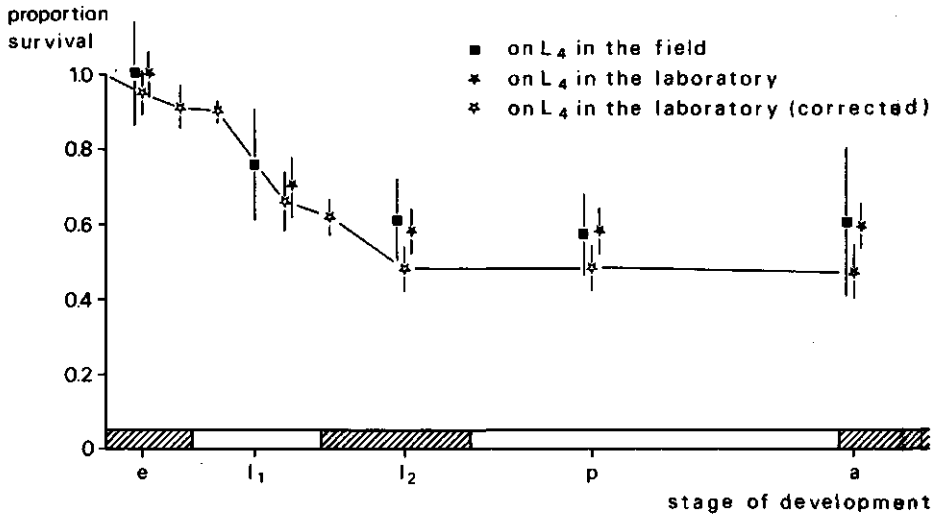

Fig. 5.10 Survival from egg to adult of *Colpoclypeus florus* on the fourth larval instar of the host (L_4). Uncorrected values are given for the field and the laboratory, and values corrected for early egg mortality and unsuccessfully parasitized hosts are given for the laboratory. Vertical bars are 95% confidence intervals (see Fig. 5.9) and the line connects the corrected values. See Fig. 5.9 for stages of development.

Table 5.8 Proportion of eggs of *Colpoclypeus florus* becoming adult in the laboratory on three larval instars of the host*

Larval instar	L_3	L_4	L_5
Proportion of parasitoid eggs becoming adult			
corrected data (see Table 3.3)	0.14	0.41	0.40
uncorrected data	0.50	0.49	0.51

* Original (corrected) data from experiment III were compared with the same data, which were not corrected for unsuccessfully parasitized hosts and early egg mortality.

instar because of the larger number of parasitized hosts which did not yield adult parasitoids (Table 5.8). Thus correction for egg mortality and unsuccessfully parasitized hosts was not of the same magnitude in hosts of the third, fourth and fifth instar.

Clutch size. In 107 parasitized hosts collected during the field sampling, the weight of the host was determined and the number of eggs counted. To compare clutch size per host-weight class in the field with those in the laboratory, the former were corrected for an early egg mortality of 4 %. Clutch sizes in the laboratory (combined results of experiments III and VII) are compared with corrected field values (Table 5.9). Since the variances of the field samples were much higher than those of the laboratory samples, the

Table 5.9 Comparison of laboratory clutch size (experiments III and VII combined) and field clutch size (experiment XI) of *Colpoclypeus florus* for hosts of different weight

Weight class of host (mg)	Clutch size of parasitoid						Welch approximation of t-test (two-sided; $\alpha = 0.05$)
	in the laboratory			in the field (corrected)			
	n	$\bar{x}$	SE	n	$\bar{x}$	SE	
0.0 - 0.5	26	3.2	0.30				
0.6 - 1.5	36	4.9	0.31				
1.6 - 2.5	21	5.6	0.50				
2.6 - 3.5	11	8.8	0.57				
3.6 - 4.5	17	9.9	0.36	2	7.3	-	NS
4.6 - 5.5	20	10.5	0.45	4	8.1	-	NS
5.6 - 6.5	14	12.7	0.53	5	7.9	1.46	p = 0.030
6.6 - 7.5	13	12.8	0.87	2	9.4	-	NS
7.6 - 8.5	7	13.3	0.47	9	10.5	1.31	NS
8.6 - 11.5	12	18.1	1.04	9	13.8	2.25	NS
11.6 - 14.5	13	17.1	1.36	19	14.7	1.74	NS
14.6 - 17.5	25	19.4	1.40	10	31.6	8.36	NS
17.6 - 20.5	19	22.1	1.26	11	34.6	10.13	NS
20.6 - 23.5	18	23.1	1.40	13	23.7	3.41	NS
23.6 - 26.5	13	23.6	0.94	14	26.6	3.86	NS
26.6 - 29.5	12	25.8	1.43	4	24.4	-	NS
29.6 - 32.5	10	26.3	1.72	5	28.3	3.99	NS
32.6 - 35.5	12	27.2	2.57				
35.6 - 38.5	9	28.3	1.89				
38.6 - 41.5	4	31.3	-				
41.6 - 44.5	14	29.7	2.14				
44.6 - 55.0	19	31.6	1.64				
55.1 - 65.0	8	34.3	2.45				
65.1 - 75.0	4	35.0	-				
75.1 - 85.0	4	36.5	-				

Welch approximation of the t-test was used. The clutch size appeared to differ significantly in only one weight class. A source of higher variance in the field may be that hosts in a particular weight class are not necessarily parasitized at the same time after the last ecdysis in the field and in the laboratory. This may result in hosts of the same weight having different dimensions, including the dimension used by the parasitoid to determine clutch size. Another source of variance may be the possible occurrence of superparasitism, although this did not lead to significantly larger clutch sizes.

Thus it may be concluded that the low survival rates of juvenile parasitoids observed in laboratory experiments were not due to circumstances less favourable than those in the field. Apart from those kinds of predators which removed whole hosts and their parasitoids from the sampled space, survival in the field was comparable to laboratory survival both in mean rate and in change in rate during development. Clutch size in the field differed significantly from laboratory clutch size in only one out of 13 host-weight classes. Since the lightest and heaviest hosts were little parasitized in the field (compare for instance the two columns of Table 5.9) most of the clutches (90 out of 107) were in the parasitization-intensity range between 0.7 and 2.0 eggs/mg, where survival was optimal (see Fig. 5.3).

The high juvenile mortality is apparently not artificially induced, because it also occurred in the field. The size of the clutches in both the field and the laboratory minimized the effect of intraspecific competition of parasitoid larvae on their mortality. Most mortality occurred shortly after the first settlement on the host on day 4 to 6. Hosts on which parasitoid larvae have died early were covered with many small black scars indicating that parasitoid larvae have tried repeatedly (and failed?) to make a hole in the integument for feeding. Since in general the host lives until day 5 or 6, this phenomenon and much of the early larval mortality may be due to failure of the parasitoid to overcome

the defense system of the host. Other larvae may succeed in sucking haemolymph out of the host and die of starvation in a later stage.

5.5 Optimal clutch size

5.5.1 Introduction

The intensity of parasitization has a range, from 0.7 to 2.0 eggs/mg, where juvenile survival is at a maximum and about 40 % (Subsection 5.2.2). Both this wide intensity range and the level of juvenile survival do not differ in the laboratory and in the field (Subsection 5.4.2). When the intensity of parasitization varies and survival is constant the number of adults emerging from a certain amount of host material will differ. If the host material is limited, it will lead to differences in body weight of the parasitoid progeny and thus will affect their fitness. Preliminary investigations showed that these types of competition effects are very likely to occur.

Variations in individual body weight of the progeny, as a result of the intensity of parasitization, should be evaluated in terms of differential reproductive success. (For males see Chapter 6). For females the following fitness characteristics may be distinguished. In a situation of high HER the fecundity and the skill to parasitize hosts are of special importance. These were studied by measuring the proportion of total fecundity actually achieved (fertility) in such a situation. In a situation of low HER, longevity and skill to search for hosts are important. Only longevity was measured. Behavioural characteristics, such as host selection, host discrimination and oviposition behaviour, and the chance to be inseminated, are also important characteristics determining the reproductive success of a female. However, these were assumed not to be affected by her body weight. Data on body weight of parasitoids used in this study supported this assumption.

To investigate the effect of the intensity of parasitization on body weight of individual female offspring, the natural variation in intensity which was used to study the relationship between intensity and mortality, was not used. The effects of intensity on body weight were expected to be less obvious than on mortality, thus the intensity of parasitization was varied by changing the clutch size artificially from 0.7 to 2.0 eggs/mg.

5.5.2 Materials and methods

Larvae of the fourth instar were used as host because they are easily accepted and the clutches produced on them are easier to count and to manipulate.

The use of host material (experiment XIII). The remains of 107 parasitized hosts of which clutch size and number of emerged adults were known, were carefully collected and dried at a temperature of 110°C for 24 h, and then weighed (W_{dr}). These remains included those of parasitoid larvae that had died. The fresh weight of the host before parasitization (0-1 day after ecdysis) was also known (W_h). From 38 unparasitized hosts at 0-1 day after ecdysis, both the fresh weight (W_h) and the dry weight (W_{dh}) was determined and the regression of W_{dh} on W_h calculated. The original dry weight W_{dh} of the parasitized hosts was calculated using this regression, and the dry weight of consumed host material W_{dc} was calculated ($W_{dh} - W_{dr} = W_{dc}$).

Pupal weight and fertility (experiment XIV). Parasitoid pupae were collected from the laboratory stock by opening the web of fifth instar hosts at 17 or 18 days after introduction of the parental wasp. The pupae were sexed and weighed and each transferred to a small glass vial (21°C, 95% relative humidity) which contained a small piece of felt. The felt served as a hold during emergence of the adult wasp. On the first day after emergence each female was mated with one male. The females were provided with a host every second day until they died and the total number of deposited eggs

during their lifetime (fertility) was determined. Drops of honey and water were available as a food source in each host vial (21°C, 95% relative humidity), because otherwise longevity may have been limited (see Table 2.3).

Pupal weight and longevity (experiment XV). Pupae and emerged females were prepared in the same way as for experiment XIV. The females were transferred to a small glass vial and provided with a drop of water (21°C, 95% relative humidity) on the second day after emergence. The period of time until the death of each female was recorded.

Clutch size and fitness gain (experiment XVI). Four series of host larvae were parasitized. After oviposition the eggs were counted. In the first series each first clutch was left unchanged (no treatment) and half of the eggs in each second clutch were removed with a thin hooked needle (group ½c). In the second series, alternately a clutch was left unchanged (no treatment), a clutch received an additional number of eggs equal to half the clutch size (group 1½c) and a clutch received an additional number of eggs equal to the clutch size (group 2c). The additional eggs were of the same age as the other eggs, and were placed close by those under the first web layer (see Fig. 2.4). In the third series, a no treatment group, a group of ¾c and a group of 1¼c were formed in the same way. In the fourth series a no treatment group was compared with a group in which the clutch size on each host remained unchanged but the eggs all had been transferred from one host web to another.

After experimental treatment the series were kept under normal rearing conditions and on day•17-18 after introduction of the parental wasp the pupae were collected, counted, sexed and weighed individually. Each pupa was transferred to a glass vial (21°C, 95% relative humidity) which contained a piece of felt, and the emergence of the adults recorded.

5.5.3 Results and discussion

The use of host material (experiment XIII). The regression of the dry weight on the fresh weight (both in mg) of fourth instar larvae of the host can be described by a straight line:

$W_{dh} = 0.152\ W_h + 0.153$ ($r = 0.95$, $P << 0.001$).

The percentage of consumed host material expressed as $100\ W_{dc}/W_{dh} = 100\ (W_{dh} - W_{dr})/W_{dh}$ varied from 9 to 94% within the group of 107 hosts of the fourth larval instar.

The absence of a correlation ($r = -0.06$, NS) between the ratios c/W_{dh} (number of eggs per mg dry weight of host) and a/c (proportion of eggs becoming adult) supports the independence of parasitization intensity and juvenile survival. The ratio c/W_{dh} is not the same as, but only comparable with parasitization intensity c/W_h, since $W_{dh} = 0.152\ W_h + 0.153$ does not include the origin. It is not surprising that there is no correlation ($r = 0.05$, NS) between the ratios c/W_{dh} and $100\ W_{dc}/W_{dh}$ (percentage of consumed host material). Therefore instead of c/W_{dh} the ratio a/W_{dh} (number of emerged adults per mg dry weight of host) was used to test whether host material is exhausted and competition for food leads to reduced individual weight of the progeny.

For each class of parasitized hosts grouped according to the ratio a/W_{dh} the mean percentage of consumed host material was calculated and also its 95% confidence limits (Fig. 5.11). The total percentage of consumed host material increased with increasing number of adults per mg dry weight of the host and was almost constant above 4 adults per mg. It was concluded that for more than four adults emerging per mg dry weight (most of these parasitized hosts) food resources were limited and a decreasing amount of host material was available for each of the nascent adults. The same conclusion was reached by Shiga & Nakanishi (1968) for a similar situation. When fewer than four adults emerged per mg dry weight, the limitation of food resources depended on the

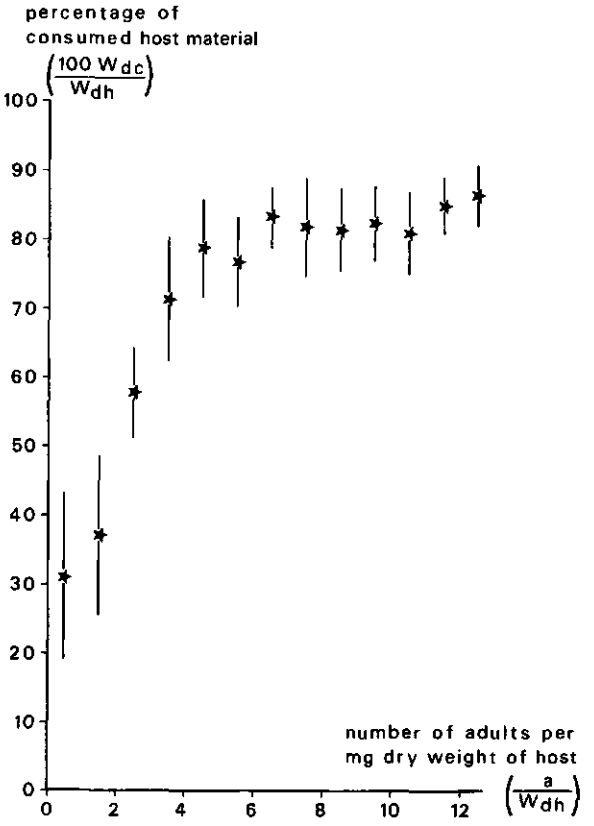

Fig. 5.11 Relationship between the number of emerged adult *Colpoclypeus florus* per mg dry weight of the host and the percentage of consumed host material. Bars are 95% confidence intervals.

extent to which host material could not be reached because it was incorporated in the remains of parasitoid larvae which had subsequently died. Shiga & Nakanishi (1968) found that the total biomass of the adults decreased in the range of food exhaustion with an increasing number of adults, apparently by increase in total loss by respiration. They found that the mean number of adults emerging from a unit of host weight coincided with the point of maximum biomass production. This phenomenon was also observed in the present study. The mean number of adults divided by the mean dry

weight of host is (see Fig. 5.11):

$$\bar{a}/\bar{W}_{dh} = 4.5/0.96 = 4.69$$

However, by placing too much emphasis on this point, it may be reasoned that the total biomass of the progeny is interchangeable with its total fitness, but as shown in Section 5.1 this is not necessarily the case.

Pupal weight and fertility (experiment XIV). The pupal weight (W_p) of a sample of 94 female parasitoids varied from 102 to 451 µg ($\bar{W}_p$ = 238 µg). On average a female was offered eight hosts and oviposition was recorded on five of these. Six females did not oviposit at all. Fertility (F) of the other females varied from 9 to 83 eggs per female and $\bar{F}$ = 40.6 eggs per female (n = 94).

The clutch size gradually decreased from on average 11.0 on the first host offered to 4.7 on the last host parasitized; the mean being 8.1 eggs per host. Initially this seemed to support the idea of Ikawa & Suzuki (1982) that some parasitoids can switch from large to small clutches when perceiving high HERs. However, the phenomenon appeared to be the result of the experimental design, where the parasitoids were forced from one host to another every 48 h. In a smaller scale experiment, in which glass funnels (Subsection 3.3.1) were used, this phenomenon of gradually decreasing clutch sizes was not observed. Some parasitoids had a parasitization time exceeding 48 h from the second host onwards. However, fertility did not differ from the earlier experiment, since fewer hosts were found with eggs.

The relationship between pupal weight and fertility is shown in Fig. 5.12. The shape of the curve resembles that in the graphical model discussed in Section 5.1 (Fig. 5.1). An optimal pupal weight of the progeny with respect to maximization of total expected fertility of the progeny (W_p optimal) is easy to construct and is about 200 µg (see Fig. 5.12). The sample of pupae used for this experiment was not collected at random to enlarge the variation of pupal weight. This may explain why $\bar{W}_p$ (= 238 µg) did not correspond to the value of (W_p)optimal.

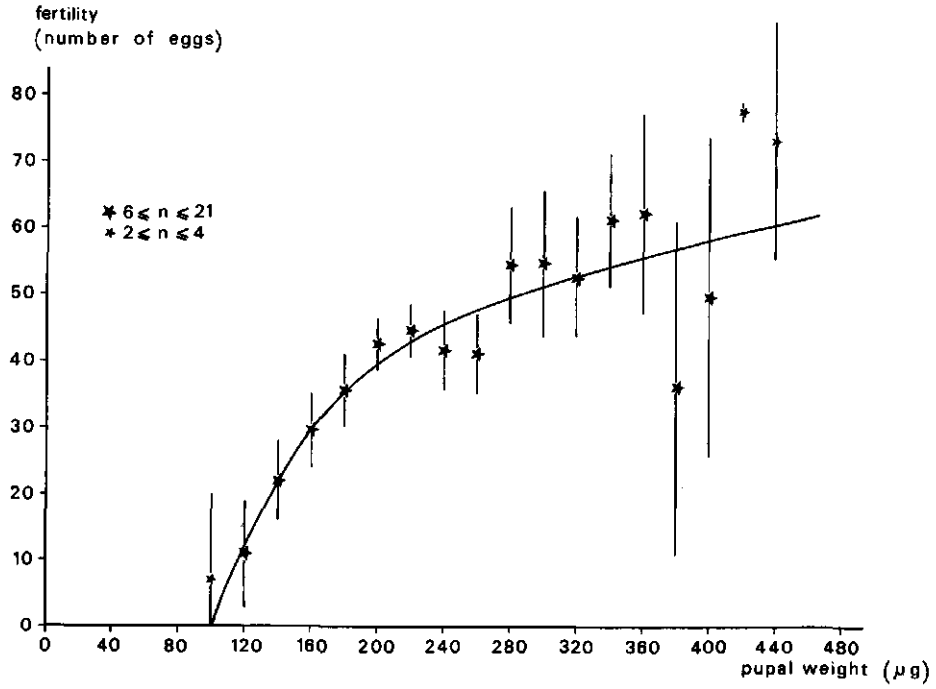

Fig. 5.12 Relationship between pupal weight of female *Colpoclypeus florus* and her fertility. The running mean and 95% confidence interval for class widths of 40 µg are given each 20 µg. The curve was fitted by eye.

If it is assumed that the parent wasp tries to maximize the total fertility of its progeny (most likely in situations where hosts are not the limiting factor), should it then parasitize hosts at such an intensity that the mean pupal weight of the resulting progeny is 200 µg as predicted by Fig. 5.12? This is not plausible. Firstly, there is considerable variation in the $\bar{W}_p$ achieved as a result of external factors, such as mortality. Mean pupal weights higher than (W_p) optimal may not have the same negative effect on total fertility as pupal weights lower than (W_p)optimal because of the shape of the relationship between W_p and F. In theory this would shift (W_p)optimal to the smallest (negative) effect. Secondly, it is assumed in

Section 5.1 that the only loss of host biomass during the conversion to pupal biomass is intensity-independent. This may be the case for loss due to mortality (dead larvae) and due to respiration. Shiga & Nakanishi (1968) suggested an intensity-dependent loss of biomass due to respiration in the case of *Gregopimpla himalayensis* (Hym., Ichneumonidae), but this may also be explained by a loss of water. However, a number of clutches within the intensity range considered, suffered high mortality so that food exhaustion did not occur. There are strong indications that this phenomenon, as well, is not bound to a particular part of the intensity range, since no correlation could be found between the ratios c/W_{dh} and $100\ W_{dc}/W_{dh}$. However, it was felt necessary to implicate the progeny resulting from these clutches in the discussion about the optimal clutch size. Therefore no conclusion is drawn from Fig. 5.12 about the optimal clutch size.

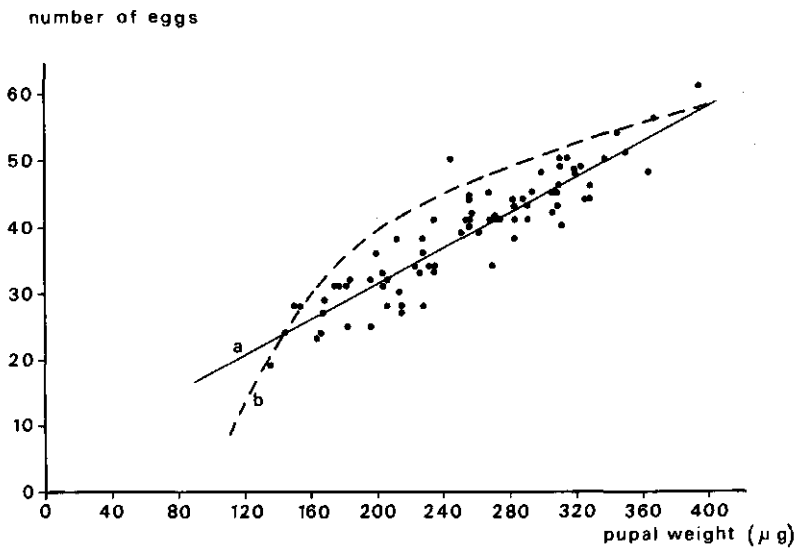

Fig. 5.13 Relationship between pupal weight of female *Colpoclypeus florus* and a. fecundity estimated by dissection (Subsection 3.2.2), and b. fertility, as given in Fig. 5.12.

Finally, fertility is compared with the fecundity estimates obtained from dissected seven-day old adult females (Subsection 3.2.2). Pupal weights were determined in the same way in both experiments. Fig. 5.13 shows that the fecundity estimates from dissections had no curvi-linear relationship with pupal weight. Also, fecundity measured in this way was lower than fertility, therefore the additional eggs mature only when the female oviposits and/or some eggs are resorbed when no hosts are available.

Pupal weight and longevity (experiment XV). The pupal weight (W_p) of a sample of 169 female parasitoids varied from 104 to 455 µg ($\bar{W}_p$ = 231 µg), and longevity (L) from 4 to 17 days ($\bar{L}$ = 11.3 days).

The relationship between pupal weight and longevity shown in Fig. 5.14 can be described by the line $y = 6.1 + 0.023\ x$ ($r = 0.76$, $P < 0.001$), and is not curvilinear as that in the model (Fig. 5.1). This implies

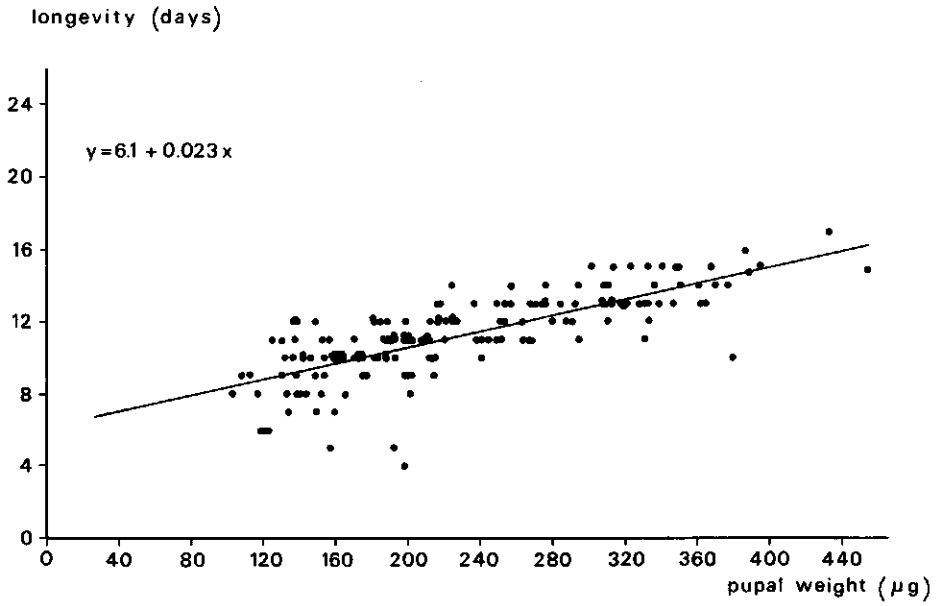

Fig. 5.14 Relationship between pupal weight of female *Colpoclypeus florus* and longevity of the adult, when only water is available.

that to maximize total longevity of the progeny in the circumstances given, in theory W_p should be as low as possible. No viable female could be obtained which weighed less than 100 µg and it is likely that mortality increases with decreasing W_p in this area. In addition to the arguments presented in experiment XIV, this is further argument for not drawing a conclusion from Fig. 5.14 about optimal clutch size in a situation of low HER.

Since lack of food may affect the longevity of adult parasitoids, the data on longevity from experiment XV (without honey) and from experiment XIV (honey available) were compared (Fig. 5.15). Although the latter data appeared

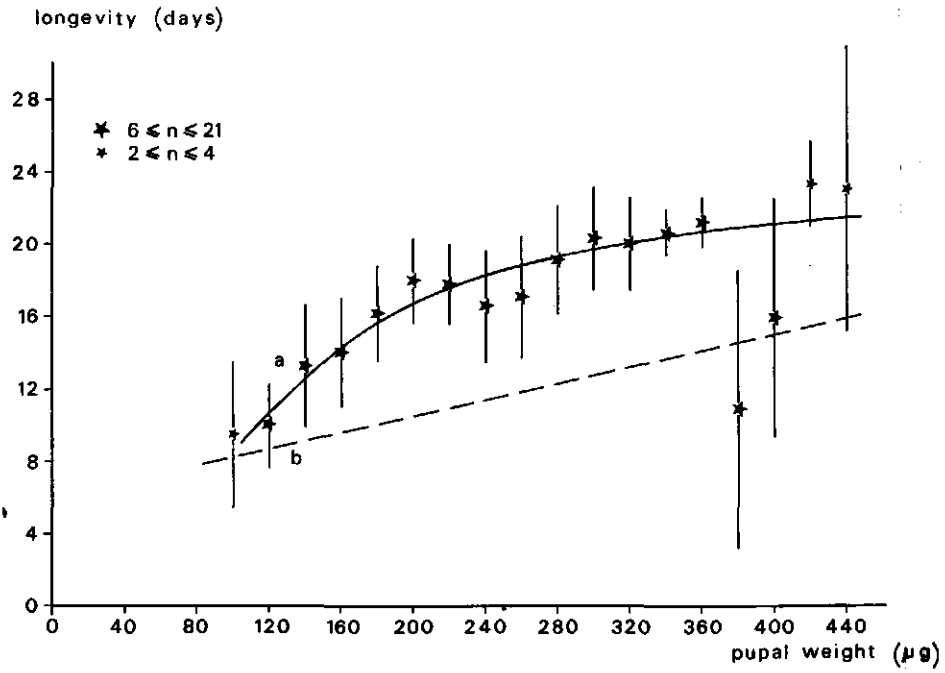

Fig. 5.15 Relationship between pupal weight of female *Colpoclypeus florus* and longevity of the adult a. when honey is available, and b. without honey as given in Fig. 5.14. For a. the running mean and 95% confidence interval for class widths of 40 µg are given each 20 µg. The curve was fitted by eye.

to have a curvi-linear regression of L on W_p, construction of (W_p)optimal did not produce a different result. The increase of longevity is more obvious under these circumstances. The availability of honey may have accounted for this and not the presence of hosts since host feeding was not observed.

Clutch size and fitness gain (experiment XVI). In the fourth series to test the effect of the transfer of eggs from one host web to another, no significant difference was found between the proportion of eggs becoming adult in the treatment (all the eggs of a clutch transferred) and with no treatment (Table 5.10). Thus mortality was not increased by the experimental treatment.

Tabel 5.10 Effect of the transfer of parasitoid eggs from one host web to another on the proportion of eggs becoming adult (experiment XVI)

	Number of clutches tested	Clutch size (mean ± SE)	Proportion of eggs becoming adult (mean ± SE)
Treatment (eggs transferred)	51	11.7 ± 0.4	0.23 ± 0.03
No treatment	54	12.2 ± 0.4	0.27 ± 0.03
t-test, one-sided; $\alpha = 0.05$			NS ($p = 0.19$)

The results of the first, second and third experimental series are given in the Tables 5.11, 5.12 and 5.13 respectively. The host weights in the groups of one series did not differ significantly, whereas the clutch sizes in the groups of one series all differed significantly because of the different treatments. The resulting mean parasitization intensities of the groups ranged from 0.98 eggs per mg (½c) to 3.82 eggs per mg (2c). As can be seen from Fig. 5.3, some intensity-dependent mortality can be expected in the upper part of this intensity range. The only series with significant differences in juvenile survival was that in which the highest intensities were tested (Table 5.12). The no treatment group in this series had a significantly higher

Table 5.11 Comparison of parasitoid development of normal clutch sizes (c) and clutches of half the normal size (experiment XVI)

	No treatment group (c)	Treatment group (½c)	Test of significance ($\alpha = 0.05$)
Number of clutches	71	72	
Host weight (mg; mean ± SE)	5.6 ± 0.2	5.2 ± 0.2	NS (t-test)
Original clutch size	10.9	10.1	
Final clutch size (mean ± SE)	10.9 ± 0.3	5.1 ± 0.2	$p << 0.001$ (t-test)
Juvenile survival (mean ± SE)	0.56 ± 0.03	0.60 ± 0.04	NS (t-test)
Sex ratio ($\frac{♂}{♂ + ♀}$)	0.14	0.14	
Pupal weight of ♀♀ (μg; mean ± SE)	221 ± 4	300 ± 7	$p << 0.001$ (t-test)
Calculated offspring fertility			
per egg laid (mean ± 95 % CL)	18.6 ± 2.8	25.1 ± 5.7	
per parasitization (mean ± 95 % CL)	202.0 ± 34.6	127.5 ± 28.9	
Calculated offspring longevity (♀♀ only)			
per egg laid (mean ± 95 % CL)	5.33 ± 0.29	6.75 ± 0.37	
per parasitization (mean ± 95 % CL)	57.8 ± 3.1	34.3 ± 1.9	

CL = confidence limit.

Table 5.12 Comparison of parasitoid development of normal clutch sizes (c) and clutch sizes 1½c and 2c (experiment XVI)

	No treatment group (c)	Treatment groups		Test of significance ($\alpha = 0.05$)
		1½c	2c	
Number of clutches	48	37	37	
Host weight (mg; mean ± SE)	5.8 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	NS (F-test)
Original clutch size	11.1	11.4	10.2	
Final clutch size (mean ± SE)	11.1 ± 0.3	17.1 ± 0.7	20.5 ± 1.0	$p << 0.001$ (F-test)*
Juvenile survival (mean ± SE)	0.59 ± 0.04	0.44 ± 0.04	0.46 ± 0.02	$p \simeq 0.01$ (F-test)**
Sex ratio ($\frac{♂}{♂ + ♀}$)	0.17	0.15	0.17	NS (χ^2 3x2)
Pupal weight of ♀♀ (µg; mean ± SE)	229 ± 5	191 ± 4	171 ± 4	$p << 0.01$ (F-test)***
Calculated offspring fertility				
per egg laid (mean ± 95 % CL)	19.5 ± 3.2	12.6 ± 2.3	11.1 ± 2.0	
per parasitization (mean ± 95 % CL)	217.2 ± 36.1	215.8 ± 39.1	227.7 ± 41.7	
Calculated offspring longevity (♀♀ only)				
per egg laid (mean ± 95 % CL)	5.48 ± 0.30	3.97 ± 0.25	3.72 ± 0.25	
per parasitization (mean ± 95 % CL)	61.1 ± 3.3	67.9 ± 4.2	76.1 ± 5.0	

* Statistical comparison by F-test followed by Tukey's test: all differences between the groups are significant.
** Statistical comparison by F-test followed by Tukey's test: juvenile survival differs significantly only between the no treatment group and both the 1½c and the 2c group.
*** Statistical comparison by F-test followed by Tukey's test: all differences between the groups are significant.
CL = confidence limit.

Table 5.13 Comparison of parasitoid development of normal clutch size (c) and clutch sizes ¾c and 1¼c (experiment XVI)

	No treatment group (c)	Treatment groups		Test of significance ($\alpha = 0.05$)
		¾c	1¼c	
Number of parasitizations	37	39	38	
Host weight (mg; mean ± SE)	5.8 ± 0.2	5.9 ± 0.2	6.1 ± 0.3	NS (F-test)
Original clutch size	11.6	11.4	11.2	
Final clutch size (mean ± SE)	11.6 ± 0.4	8.5 ± 0.3	13.9 ± 0.5	$p << 0.001$ (F-test)*
Juvenile survival (mean ± SE)	0.31 ± 0.05	0.39 ± 0.05	0.39 ± 0.04	NS (F-test)
Sex ratio ($\frac{\sigma}{\sigma + \varphi}$)	0.19	0.17	0.15	NS (χ^2 3x2)
Pupal weight of ♀♀ (µg; mean SE)	284 ± 10	339 ± 9	267 ± 7	$p << 0.01$ (F-test)**
Calculated offspring fertility				
per egg laid (mean ± 95 % CL)	12.2 ± 2.6	16.5 ± 4.4	14.9 ± 3.1	
per parasitization (mean ± 95 % CL)	141.8 ± 30.7	139.8 ± 37.0	207.6 ± 42.9	
Calculated offspring longevity (♀♀ only)				
per egg laid (mean ± 95 % CL)	3.32 ± 0.12	4.38 ± 0.22	4.05 ± 0.21	
per parasitization (mean ± 95 % CL)	38.5 ± 1.4	37.2 ± 1.8	56.5 ± 3.0	

* Statistical comparison by F-test followed by Tukey's test: all differences between the groups are significant.
** Statistical comparison by F-test followed by Tukey's test: pupal weight of ♀♀ differs significantly only between the ¾c group and both the no treatment and the 1¼c group.
CL = confidence limit.

juvenile survival than both the 1½c and the 2c group.

The different treatments did not affect the sex ratio of the emerged adults, so each egg had an equal chance of being transferred, irrespective of sex. As a result of this the sex ratios of the altered clutches may not have been as optimal as the sex ratio of the original clutch, unless each additional female egg in a clutch would need the same increase of investment in males in a clutch. This problem is discussed in Chapter 6.

The mean pupal weight of the emerged females differed significantly between the groups in a series. In the almost complete absence of intensity-dependent mortality, the increased intensity resulted in a decrease in the mean individual weight of the offspring. In the intensity range studied, this was by far the most important effect of a change in the number of eggs laid on a host. Whereas the clutch size does not affect the proportion of consumed host material as had been observed previously, the parent parasitoid can increase the number and meanwhile decrease the individual weight of her offspring or vice versa by changing the number of eggs in a clutch. Before discussing whether the clutch size normally produced (c) can be considered to be an optimal solution to this phenomenon, differences in results between the three experimental series are discussed.

The juvenile survival of the three series are compared in Fig. 5.16. The no treatment group in the third series had a lower juvenile survival than those in the other two series. This difference is significant (F-test, $\alpha = 0.05$; $p << 0.001$) and also leads to the no treatment group in the third series having a higher pupal weight of females (Fig. 5.17). The reason for the higher mortality in this series is unknown. The fourth series (no treatment versus transfer treatment) also had a low juvenile survival (Table 5.10). However, both series were set up in the same period and may have been influenced by technical problems resulting in lower relative humidity in the rearing room for a short period during the experiment. Although the three groups of the third series can

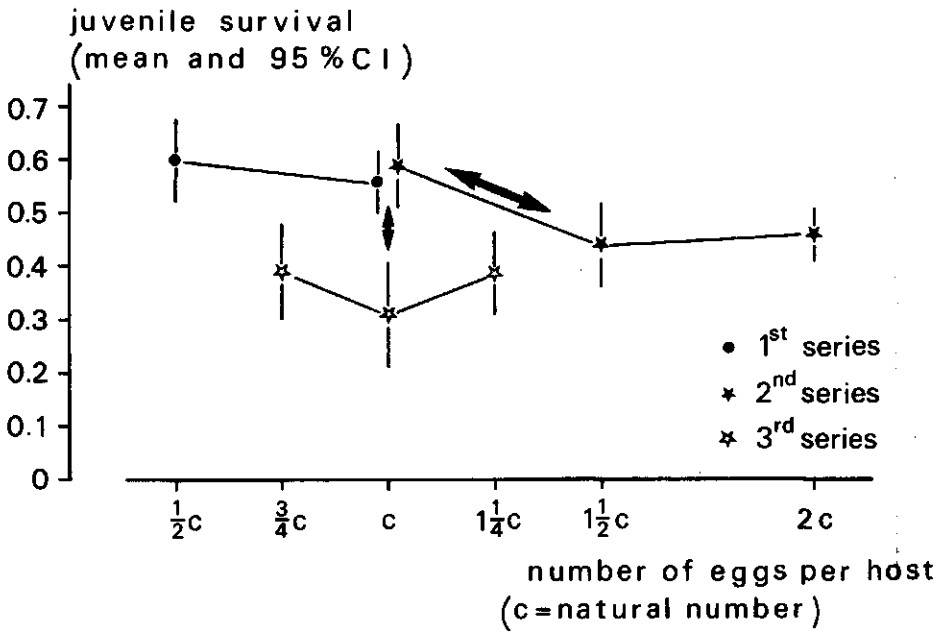

Fig. 5.16 Juvenile survival of *Colpoclypeus florus* in eight groups of clutches of natural (c) and artificially obtained ½c, ¾c, 1¼c, 1½c, 2c) size. Groups connected by a line are of the same experimental series. Arrows indicate significant differences between groups.

be compared, the three experimental series can only be compared when correction is made of abnormal mortality in the third series.

The correction used is based on the assumption that in the three experimental series mortality is equal. If this is the case, then the mean number of adult daughters is proportional to the original clutch size and can be used as the new abscissa instead (sex ratios in the three no treatment groups of the series did not differ significantly: χ^2 3x2, $\alpha = 0.05$; $p \approx 0.43$). The values obtained experimentally for the mean clutch size in each group (now represented by the mean number of adult daughters) are necessarily less evenly distributed along the abscissa. To

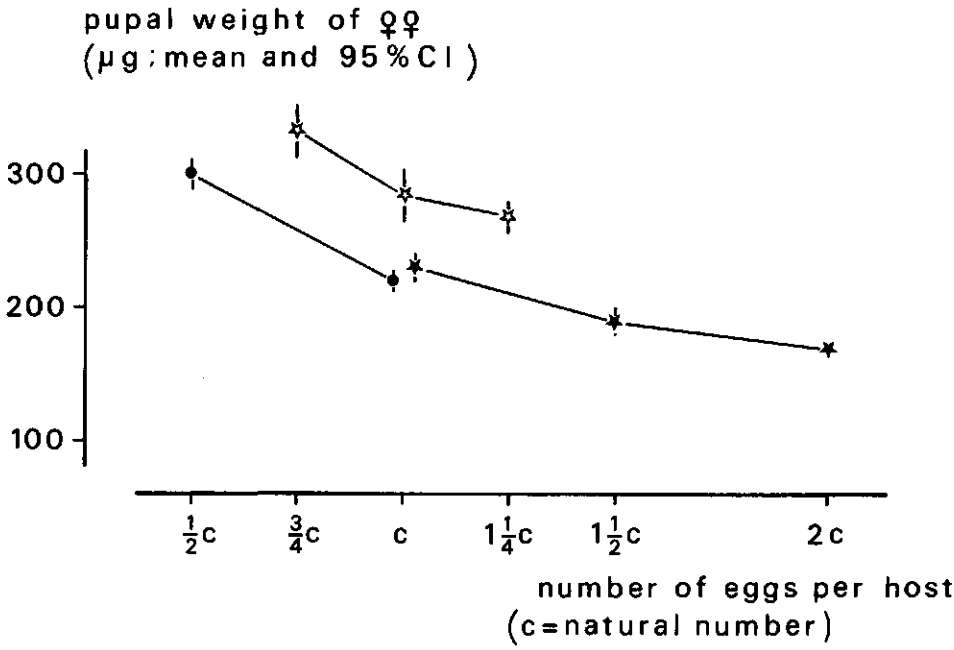

Fig. 5.17 Relationship between artificially obtained clutch sizes and pupal weight of female *Colpoclypeus florus*. For symbols see Fig. 5.16.

show the effect of these corrections, both the corrected and the uncorrected values were depicted in separate figures (Figs 5.18 and 5.19, and Figs 5.20 and 5.21).

Two fitness measures for the parent wasp were calculated to evaluate the clutch size produced (Tables 5.11, 5.12 and 5.13):

(i) In a situation of high HER, eggs will become the limiting factor. Each egg of the parent should produce offspring with a total fertility as high as possible. Therefore maximizing the expected offspring fertility per egg laid by the parent will guarantee the highest offspring reproductivity in this case.

The expected offspring fertility per egg laid was calculated as follows. Frequency distributions of the pupal weights of the females in n weight classes

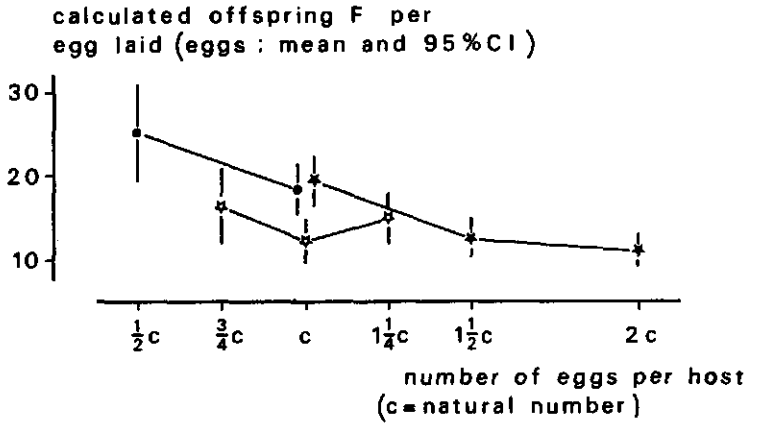

Fig. 5.18 Relationship between artificially obtained clutch sizes and the calculated offspring fertility per egg laid. For symbols see Fig. 5.16.

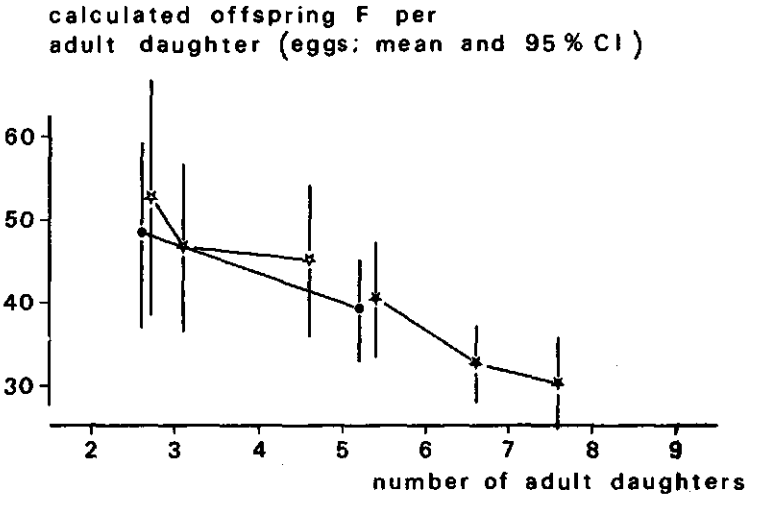

Fig. 5.19 Relationship between the number of adult daughters and the calculated fertility per daughter in artificially obtained clutch sizes. For symbols see Fig. 5.16.

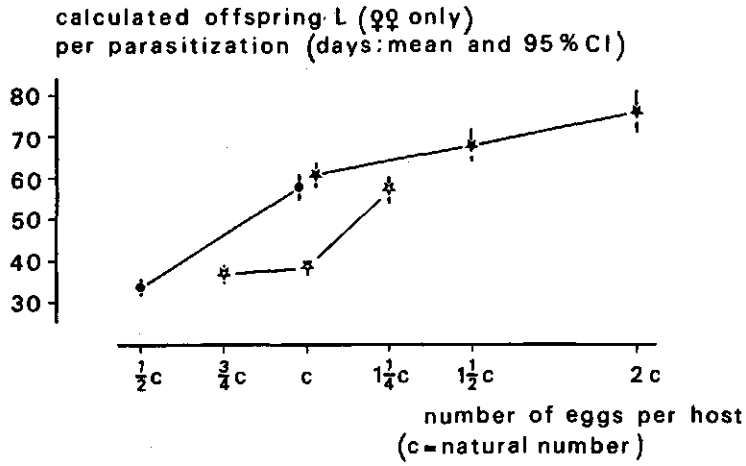

Fig. 5.20 Relationship between artificially obtained clutch sizes and the calculated offspring longevity per parasitization. For symbols see Fig. 5.16.

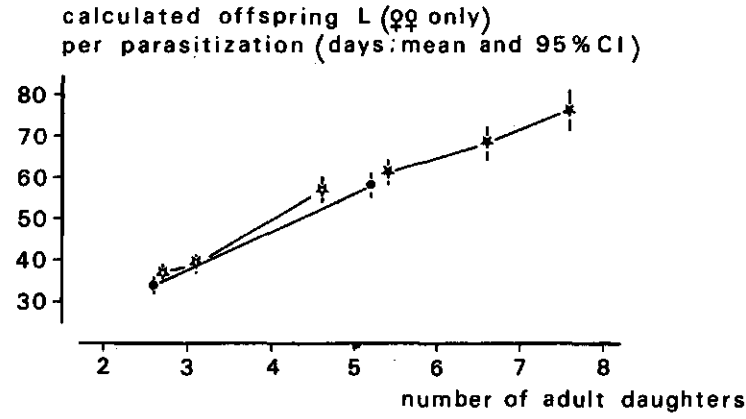

Fig. 5.21 Relationship between the number of adult daughters and the calculated offspring longevity per parasitization in artificially obtained clutch sizes. For symbols see Fig. 5.16.

(class width 20 μg) were made for each group in a series. For each weight class i with frequency f_i a value F_i was constructed using the curvilinear regression of F on pupal weight (Fig. 5.12).
The total offspring fertility within a treatment group ($\Sigma_{i=1}^{n} f_i \times F_i$) was then divided by the total number of eggs laid to produce this offspring.
In Fig. 5.18 the calculated offspring fertility per egg laid is given for each group. In Fig. 5.19 in the same way the calculated offspring fertility per adult daughter is given, to correct for different mortality rates. Here the total offspring fertility within a treatment group was divided by the total number of adult daughters in this offspring $\Sigma_{i=1}^{n} f_i$, thus the ordinate simply gives $\bar{F}$ of the daughters. In general, about two eggs were necessary to obtain one adult daughter, hence the ordinate values of Fig. 5.19 are about twice as high as those of Fig. 5.18 (with the exception of those of the third series).
In both figures there is a trend to decrease the number of eggs laid per host, when maximizing offspring fertility per egg laid is the strategy to increase fitness. The decrease in clutch size is profitable until the increasing body size of the individual offspring may have negative effects on function, that is skill to parasitize hosts, which negate the positive effect of fecundity gain and reduce individual fertility. Unfertilized eggs, necessary to produce males and inseminate daughters, will also limit the decrease in clutch size (see Chapter 6). The normally produced clutch size (c) cannot be explained in terms of this fitness measure. If the parent parasitoid produces clutches smaller than normal (c) she produces offspring with at least equal and may be higher fertility per egg laid, and in addition saves some of her own

fertility for following hosts.

(ii) In a situation of low HER, the total number of eggs laid by the parent parasitoid only comes close to F when the wasp has enough time available to search for the required number of hosts, hence the longevity (L) of the females is the limiting factor. In this situation each parasitized host represents the investment of a certain amount of longevity to find and parasitize it, and should produce female offspring with longevity as high as possible. Therefore in this case, maximizing female offspring longevity per parasitization is the best strategy to increase offspring reproductivity.

Total female offspring longevity was calculated in analogy to that of total offspring fertility, with the use of the rectilinear regression of longevity on pupal weight (Fig. 5.14). Total female offspring longevity within a treatment group ($\Sigma_{i=1}^{n} f_i \times L_i$) was then divided by the total number of parasitizations which produced this offspring.

In Figs 5.20 and 5.21 the calculated female offspring longevity per parasitization is given for each group. In both figures a clear trend is visible which indicates that when female offspring longevity per parasitization has to be maximized, the clutch size should increase to at least twice the normal size. Further increase may be profitable until the decreasing body size of the individual female has negative effects on their function, that is skill to search for hosts, which negate the positive effect of total longevity gain. However, also in this situation of low HER the normally produced clutch size (c) cannot be explained by using this fitness measure.

However, between the two extreme values of HER discussed above it is likely that both fertility and longevity are

limiting factors. Consider a certain value HER = x. A parasitoid which is adapted to this value of HER will produce daughters which need more total fertility and have some longevity left to search for and parasitize hosts when HER > x, and which need more total longevity and have some fertility left when HER < x. When HER = x the offspring of this parental wasp will expend all fertility and longevity available. Thus at this value of HER the number of daughters produced according to the "L strategy" (low HER) will be equal to the number of daughters produced according to the "F strategy" (high HER). The number of hosts parasitized multiplied by the number of daughters per host will be equal to the number of eggs laid multiplied by the number of daughters per egg:

$$L \times HPR \times c(1-m)(1-r) = F \times (1-m)(1-r), \text{ and}$$

$$HPR = \frac{F}{Lxc} \qquad (1)$$

where HPR (host parasitizing rate) is a measure of the number of hosts parasitized per unit of time and depends on HER, the proportion accepted (acc) and on the time lost by rejecting a host (tr) and by parasitizing a host i (t_i).

HER as a measure for the number of hosts encountered per unit travel time (tt) depends on availability, accessibility and conspicuousness of hosts:

$$HPR = \frac{HER \times tt \times acc}{tt + HER \times tt \times acc \times t_i + HER \times tt \times (1-acc) \times tr}$$

$$HPR = \frac{HER \times acc}{1 + HER \times acc \times t_i + HER \times (1-acc) \times tr} \qquad (2)$$

When equation (1) and (2) are combined

$$\frac{F}{L \times c} = \frac{HER \times acc}{1 + HER \times acc \times t_i + HER \times (1-acc) \times tr} \qquad (3)$$

With this equation, the HER of a diet with third, fourth and fifth instar hosts, for which a measured set of clutch size, fertility and longevity is optimal, was calculated. The clutch size was based on the data in Table 5.6. With the use of the mean number of parasitized hosts of each of the larval instars in the field (Table 4.7), mean clutch size was calculated for a situation where all instars were present simultaneously:

$$\bar{c} = \frac{7.0(\frac{13.4 + 6.9}{2}) + 11.2(\frac{37.7 + 15.3}{2}) + 25.2\ (\frac{13.8 + 5.9}{2})}{(\frac{13.4 + 6.9}{2}) + (\frac{37.7 + 15.3}{2}) + (\frac{13.8 + 5.9}{2})}$$

$$= 13.2 \text{ eggs}$$

For the calculations the longevity must be reduced by the period that the females remain in the cocoon of the host after emergence. This period was 2.3 days (see Chapter 6). The same fertility values were used. Values of HPR and HER were calculated from each set of values of clutch size, fertility and longevity of experiment XVI. The results are given in Table 5.14. HPR and HER for which the natural size of a clutch is optimal are 0.34 and 0.53 hosts/day respectively.

In Subsection 4.4.3, HER was estimated on the basis of the diet chosen by the parasitoid. Here tt of 111 hosts was expressed as x. HER was expected to be close to the lower limit of the range 0.22 to 2.0 hosts/day. HPR ranged from 0.17 to 0.71 hosts/day according to these data and equation 2. The value of HER estimated on the basis of the natural size of a clutch confirms this expectation. When longevity is higher, for instance if a carbohydrate food source is available regularly in the field, the value of HER will be even closer to the lower limit of the range. However, the values of HER estimated on the basis of the increased clutches, although different, also are within the lower part of the range. This confirms the HER estimated in Subsection 4.4.3, but it is not clear whether the natural

Table 5.14 Values of HPR and HER calculated from clutch size (c = 13.2 eggs), fertility and longevity as obtained in experiment XVI

Clutch size	First series		Second series			Third series		
	c	½c	c	1½c	2c	c	¾c	1¼c
Fertility per adult daughter (eggs; mean ± 95 % CL)	39.0 ± 5.9	48.3 ± 10.9	40.4 ± 6.7	32.6 ± 5.9	30.1 ± 5.5	46.4 ± 10.1	52.4 ± 13.9	45.1 ± 9.3
Longevity per adult daughter (days; mean ± 95 % CL)	11.16 ± 0.60	13.01 ± 0.72	11.37 ± 0.61	10.25 ± 0.64	10.06 ± 0.66	12.62 ± 0.46	13.94 ± 0.69	12.26 ± 0.65
HPR (hosts per day)	0.33	0.68	0.34	0.20	0.15	0.34	0.46	0.27
HER (hosts per day)	0.51	1.82	0.53	0.27	0.19	0.53	0.84	0.39

CL = confidence limit; HPR = host parasitizing rate; HER = host encounter rate.

clutch size or a larger clutch size is optimal. To solve this problem an estimation of the mean HER in the field should be made. Figure 5.22 shows the relationship between HPR and the optimal clutch size.

Adaptation to change in host density, which means adaptation of the clutch size to the new HER, is considered to occur by differential reproductivity and not (within one generation) by the estimation of HER by the female parasitoid. Thus the number of hosts parasitized by one female will not increase with increasing host density, but the number of hosts normally parasitized will be parasitized in a shorter period and fecundity will be the limiting factor. At a decreasing host density longevity will be the limiting factor and as a result fewer hosts will be parasitized and some of the eggs will not be laid.

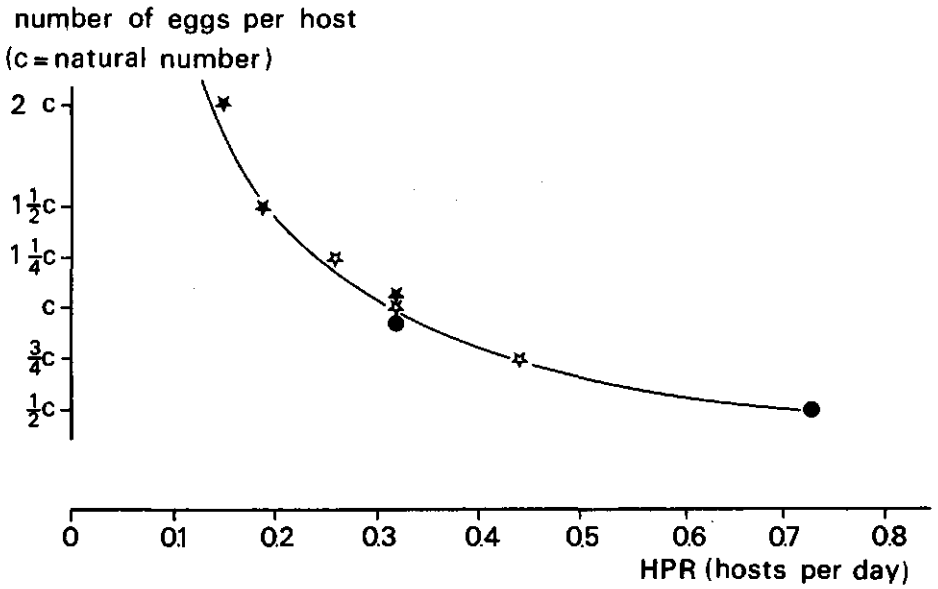

Fig. 5.22 Relationship between host parasitizing rate (HPR) and optimal clutch size of *Colpoclypeus florus*, as predicted by c = F/L x HPR. For symbols see Fig. 5.16.

6. SEX ALLOCATION

6.1. Introduction

Fisher (1930) was the first to explain why the two sexes are usually produced in approximately equal numbers. His theory predicts equal investment in the sexes for random mating populations, implying a sex ratio ($\sigma/(\sigma + \varphi)$) of ½ when a male and a female represent equal amounts of investment. His argument is not affected by the occurrence, in most hymenopterous insects, of arrhenotokous reproduction and possible control of the progeny sex ratio by the mother (Hamilton, 1967). However, where sons of a small group of females compete with each other for mates, a phenomenon called local mate competition, Fisher's assumption of unrestricted competition for mates does not hold. When n is the number of mothers of a random mating group of offspring, a female-biased sex ratio of $(n - 1)(2n - 1)/n(4n - 1)$ is predicted for a haplodiploid genetic system (Hamilton, 1979; Taylor & Bulmer, 1980). In a situation of complete outbreeding where n is very large, the sex ratio will equal Fisher's ½. Females of completely inbred lines (n = 1) will produce a sex ratio of almost zero, i.e. will produce just enough sons to fertilize their daughters. In a number of hymenopterous parasitoids such a female-biased sex ratio as a result of local mate competition is found (Hamilton, 1967; Hamilton, 1979). For a quantitative test of Hamilton's model the degree of inbreeding needs to be known, hence these kind of tests are scarce. Waage (1982a) showed that the sex ratios of solitary scelionid wasps vary with the specific levels of sib-mating as predicted by Hamilton's model. Werren (1980) extended the model to include situations where, in gregarious parasitoids, the local mating population consists of clutches of unequal size. When females of the gregarious parasitoid *Nasonia vitripennis* (Walker) are the second ones to attack a host, they adjust the proportion of sons to the ratio between the first and the second clutch as predicted by the model

(Werren, 1980).

This situation of parasitism on a host that has been previously parasitized is also considered by Suzuki & Iwasa (1980). Their model differs from Werren's model in that (a) the sex ratios produced by both the first and second female of a gregarious parasitoid are predicted as a result of a non-cooperative game between them; and (b) not only the clutch size ratio between the two females is incorporated in the model but also the probability of double parasitism, the ratio of males to females in contribution to resource competition, the effect of crowding on female reproductive success and the degree of inbreeding. The predictions of their model that concern the sex ratio produced by the first female are that this sex ratio should increase when:

1) the probability of double parasitism increases.
2) the clutch size ratio c_2/c_1 increases (c_1 is the clutch size of the first and c_2 is the clutch size of the second female). For this prediction some experimental evidence is presented (Suzuki & Iwasa, 1980) using data on *Nasonia vitripennis* from Holmes (1972) and Wylie (1965) but the difference with a fixed sex ratio is not very marked.
3) the crowding effect on female reproductive success, that is the effect which causes the reproductive success of a female egg to be lower at high parasitization intensity, increases.
4) the degree of inbreeding decreases. Since the inbreeding coefficient is a reflection of the mating structure of foregoing generations, it does not depend on the (local) probability of double parasitism.
5) the ratio of male to female contribution to resource competition decreases.

It can be expected that the sex ratio produced by the first female should also be adjusted to a probability of double parasitism (p). According to Suzuki & Iwasa (1980) we may distinguish parasitoid species which are able to assess p

at an oviposition site, and those who produce genetically fixed sex ratios that reflect the overall mean p through previous generations.

Is it necessary for an understanding of the sex ratios produced by *C. florus* to have information about p in the field? A model which could explain the various sex ratios of *C. florus* was constructed without using a variable or constant p in it. This means: if *C. florus* acts like the model predicts, it behaves optimally in situations without double parasitism, but suboptimally in situations with a certain probability of double parasitism. If *C. florus* does not act like the model predicts, this may be due to adjustment of its sex ratio to a value $p > 0$.

We can assume, that the chance of double parasitism with *C. florus* equals zero, and the validity of the following assumptions (which will be tested later in this chapter):

- all insemination occurs exclusively from brothers to sisters of the same clutch,
- the ratio mean weight of a male/mean weight of a female is as low as possible,
- one male of mean weight is able to inseminate all the females of a clutch in such a way that no lack of sperm occurs in these females during their reproductive period,
- pre-adult mortality does not differ between males and females.

This situation resembles the "biofacies of extreme inbreeding and arrhenotoky" (Hamilton, 1967) where it is expected that it will lead to extreme economy in the production of males as is known for a number of other species (insects and mites listed by Hamilton, 1967). What sex ratio should result in the case of *C. florus*?

The chance that at least one adult male emerges from a clutch will be $1 - m^x$, where m is the fraction pre-adult mortality of males (and females) and x is the number of unfertilized eggs ($x > 0$). Pre-adult mortality is high and variable (Subsection 5.4.2), so it considerably decreases the chance of an egg to become adult. It is assumed that the

behaviour of the mother is adapted to the mean mortality and that she cannot estimate the mortality that her own clutch will undergo.

The expected number of females reaching maturity on a host is $(1 - m)\ (1 - r)c$, where r is the sex ratio ($\sigma/(\sigma + \varphi)$) of adults as well as eggs and c is the clutch size. Since $x = r.c$ this can also be written as $(1 - m)\ (c - x)$.

Using the number of inseminated daughters as a measure of fitness, the fitness F of a female producing x unfertilized eggs per clutch can be represented by

$$F = (1 - m^x)\ (1 - m)\ (c - x).$$

A maximum of F occurs when $F' = 0$ and $F'' < 0$. When $(1 - m^x) = A$ and $(1 - m)\ (c - x) = B$ then

$$A' = -m^x \ln m \text{ (positive, since } 0 < m < 1),$$

$$A'' = -(\ln m)^2 m^x \text{ (negative)}$$

$$B' = m - 1 \text{ (negative) and } B'' = 0$$

$$F' = A'B + AB' = (m - 1)\ [m^x\ (c - x)\ \ln m - m^x + 1]$$

$$F'' = A''B + A'B' + A'B' + AB'' \text{ and } F'' < 0$$

So F has a maximum when $F' = 0$

$$m^x\ (c - x)\ \ln m - m^x + 1 = 0$$

By substituting the appropriate values of m and c the optimal value of x can be calculated for each situation. For instance with $c = 10$ and $m = 0.5$ the optimal value of x is 2.6 ($r = 0.26$) and $F = 3.09$. With $c = 20$ and $m = 0.5$ the optimal value of x is 3.6 ($r = 0.18$) and $F = 7.52$. With $c = 10$ and $m = 0.1$ the optimal value of x is 1.3 ($r = 0.13$) and $F = 7.44$.

The model is identical to the one formulated as the "precise sex ratio strategy" by Green et al. (1982). They showed theoretically that a selective advantage exists for highly inbred parasitoids to produce a precise sex ratio instead of a binomial sex ratio. However, this advantage becomes smaller when clutch size is greater and egg survival is lower. Fitness gain is about ten percent when $c = 15$ and $m = 0.5$ (Green et al. 1982). If sex ratios are not

binomially distributed, there must be some non-random pattern in the production of the sexes as they are laid within a clutch. This was investigated. The various assumptions necessary for the model were also studied for *C. florus*.

The sex ratio results of laboratory and field experiments were compared and used to test the model.

6.2. Insemination capacity of males

6.2.1. Introduction

Experiments were conducted to determine whether one male is able to inseminate all the females of a clutch without these females running out of sperm during their reproductive period. First the period that males and females stayed in a host cocoon was recorded. This was necessary to analyse the opportunities of an individual male to use its insemination capacity within the same clutch, and also to estimate the chances of an insemination between non-siblings.

Insemination capacity was expected to depend on the size of the male, necessitating the use of males of different size. A male is considered to be of optimal size, when it combines the necessary insemination capacity with the allocation of the smallest amount of host material.

The insemination capacity a male needs within the clutch depends on the sex ratio of adults emerging from that clutch. From former experiments it is known (Table 3.5) that on large hosts this ratio sometimes is as low as 0.07 (approximately one male for fourteen females). The insemination capacity of a male should therefore be tested in a group of about fifteen females. The percentage of females that are inseminated and can produce female offspring during their reproductive period is a measure of the insemination capacity of a male.

6.2.2. Materials and methods

The time between emergence of males, emergence of females and the moment of escape from the host cocoon (experiment XVII).

Twenty-four hosts of the fourth larval instar were parasitized according to the method described in Subsection 2.3.2. Parasitoid development was studied by looking through the glass of the vial at places where the cocoon of the host was thin enough. After seventeen days of parasitoid development a funnel was fitted to each vial and the number of individuals of each sex that had emerged and/or left the cocoon of the host was counted each eight hours. All the clutches which were used contained both males and females.

Insemination capacity of males of different weight (experiment XVIII)

Fourteen male pupae of very different weight were collected from the laboratory stock. After weighing they were put each in a glass vial provided with a piece of felt to facilitate emergence. Two days after emergence the adult male was transferred to a vial (14 mm diameter) together with five virgin females which had emerged within the previous 24 h. The vial could be closed with a plunger covered with felt. The plunger was used to keep the volume available for the wasps during the experiment comparable to that of a host cocoon (approximately 0.2 cm^3). After four hours five identical females were added and four hours later the number of females was brought to fifteen. Thus the successive emergence of females was imitated. The wasps were kept together for a confrontation period equal to the mean contact period measured in experiment XVII. Additionally a group of ten males was tested in a slightly different way, to investigate whether time is limiting to inseminate fifteen females. The females were introduced in two groups of seven and eight females respectively and mean confrontation period

was nearly half of that previously used.

Insemination of the females was checked by dissecting each female in a 6% NaCl-solution. The spermatheca was transferred to a microscope slide and by pressing the cover glass carefully the contents were liberated and could be studied under the microscope (magnification 150x, phase-contrast). The presence of sperm was scored.

Eleven males which had undergone this experiment were afterwards offered a total number of 23 virgin females (1-5 females/male). This was to check the reliability of the dissecting method and the quality of the sperm. It was expected that a number of males would have run out of sperm and consequently that some of the females could only produce male eggs. The females were therefore enabled to produce offspring on a host of the fifth larval instar and were dissected afterwards as described above. The offspring was reared and sexed.

6.2.3. Results and discussion

The time between emergence of males, emergence of females and the moment of escape from the host cocoon (experiment XVII.)

The twenty-four clutches had a mean number of seven parasitoids per host and a sex ratio of 0.17. Time was set at zero for the last observation where none of the pupae had yet emerged. After each period of eight hours the percentage of individuals of each sex that had emerged and/or left the cocoon of the host was calculated. Three females did not emerge. After all males had left the cocoon the observations were stopped. At this time twenty-three females, although emerged, had not left the cocoon. The results are given in Figure 6.1. Males emerged earlier and left the cocoon earlier than females, so the opportunity to inseminate sisters is offered between female emergence and male departure. For each emerged female (n = 142) this period was determined and mean contact period was 45 h. In experiment XVIII this mean

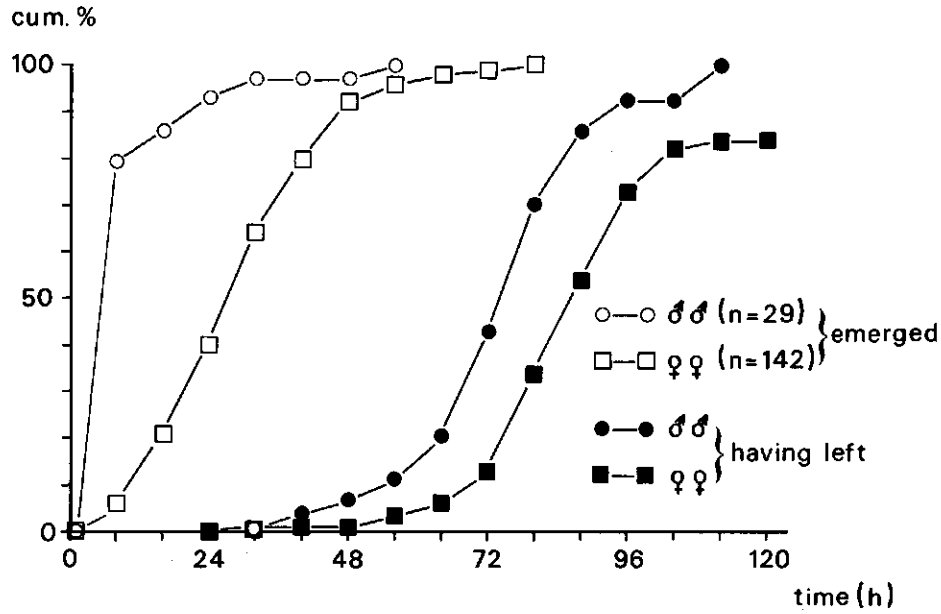

Fig. 6.1 Emergence of males and females of *Colpoclypeus florus* and their departure from the host cocoon, as a cumulative percentage over 24 clutches. At time = 0 no adults had yet emerged.

contact period was applied. No male left before the last female of that clutch had emerged. The first adult that emerged was in 80% of the cases a male, whereas only in 50% of the cases the first adult that left the cocoon was a male.

The mean stay in the cocoon as an adult female, used in Subsection 5.5.3 (2.3 days), was also determined from the results of this experiment.

Insemination capacity of males of different weight (experiment XVIII).

The insemination capacity of the first 14 males which each had a mean confrontation period with the 15 females of 45 h is given in Table 6.1. Seven females died or escaped during the experiment, or the spermatheca was lost during dissection. Insemination capacity of the male is expressed as

Table 6.1. Insemination capacity of males of different weight, defined as the percentage which is inseminated in a group of 15 females

Weight of male (mg x 10^{-3})	77	81	83	100	100	106	107	122	140	145	150	197	263	287
Number of females checked on the presence of sperm	14	15	15	15	15	15	14	12	13	15	14	15	14	15
Number of females which contained sperm	0	8	7	2	10	7	6	4	13	15	14	15	13	13
Percentage of females inseminated	0	53	47	13	67	47	43	33	100	100	100	100	93	87

the percentage of females which appeared to contain sperm of the total number of females checked for the presence of sperm. This insemination capacity was positively correlated with the weight of the male (Spearman-rankcorrelation, one-sided; $p < 0.006$). The ten males tested using a confrontation period of 21 h produced results which are very alike (Fig. 6.2). Obviously under normal conditions time is not limiting to inseminate 15 females in a clutch.

The 23 virgin females offered at the end of the experiment were dissected after they each had produced a large clutch. In 15 females no sperm could be found and all but one of these produced male offspring only. Apparently the sperm was overlooked in one of the females. The results obtained by this method may therefore give a small underestimation of the insemination capacity. There are no indications that the quality of the sperm is inferior, since the 8 females where sperm was found all produced at least some female offspring. The quantity of sperm in the spermatheca was not investigated systematically. Only occasionally, namely in females inseminated by very small males or in females known to be the last of a series of successfully inseminated females, the sperm did not fill the whole spermatheca. It is assumed that normally the contents of the spermatheca is sufficient to fertilize all the eggs of a female. Wilkes (1965) found that in the eulophid *Dahlbominus fuscipennis* (Zett.) sperm is transferred to the female in sperm bundles, and the spermatheca was found to contain a remarkably constant number of some 150 sperm as a

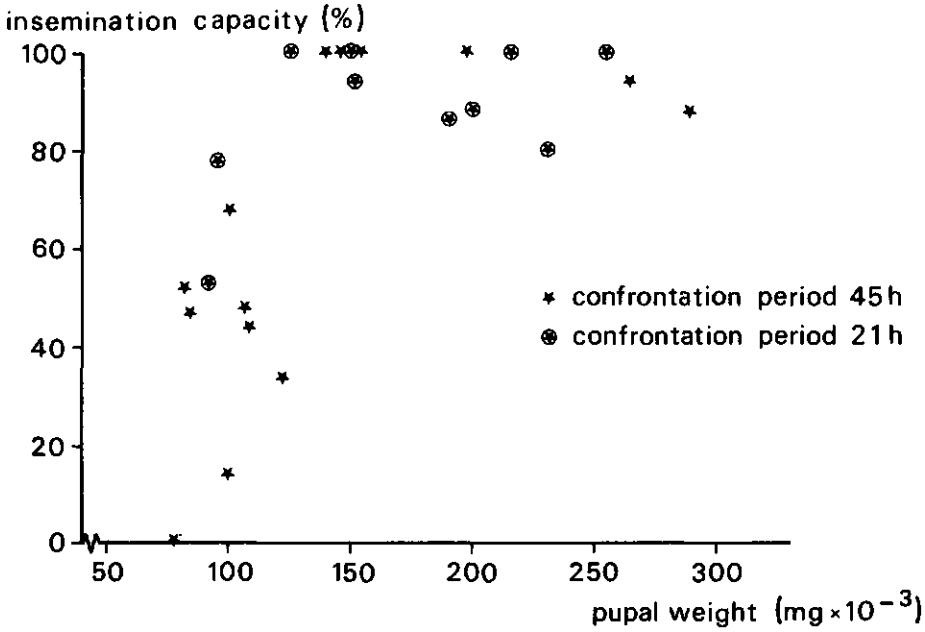

Fig. 6.2 Insemination capacity of males of *Colpoclypeus florus* of different weight, expressed as the percentage inseminated of a group of 15 females.

result of one insemination. The average number of progeny of females of this eulophid is 90, and evidence is very strong that no more than one sperm is used for each egg (Wilkes, 1965). This economy in the use of the sperm is also found in other parasitoid Hymenoptera (Speicher, 1936) but females of the chalcidoid *Nasonia vitripennis* may exhaust their sperm supply during their reproductive period (Cousin, 1933).

The low insemination capacity of small males may be caused by a low production of sperm. Those cases where the sperm did not fill the whole spermatheca point to this explanation. There are no indications that the courting behaviour of small males is insufficient to make females receptive for copulation, or that their dimensions prevent them copulating.

In other observations a small male was only seen to fail

to copulate with a female, where a larger male was also unsuccessful. The differences in the duration of the courting and copulation behaviour were very small compared with the total time (45 h) available for inseminations in the cocoon. Moreover, as was shown above time is far from the limiting factor.

In Fig. 6.2 insemination capacity of the 24 males tested is plotted against pupal weight. Only males with a pupal weight of more than 0.12 mg had the capacity to inseminate 15 females under these circumstances. This capacity may not be needed in most of the clutches, since mean sex ratio on hosts of the fifth larval instar is 0.12 (approximately 1 male for 8 females) and on hosts of the fourth larval instar it is 0.23 (approximately 1 male for 4 females) (Table 3.5) Males with a pupal weight of about 0.09 mg can do as well as the heavier males in these cases.

To test whether some optimization of mean pupal weight of males takes place, male pupae were collected from hosts parasitized in the laboratory as usual (Subsection 2.3.2) and weighed. Mean pupal weight was 0.154 mg, whereas 70% weighed more than 0.12 mg and 95% more than 0.09 mg (Fig. 6.3) Pupae collected from hosts of the fifth larval instar

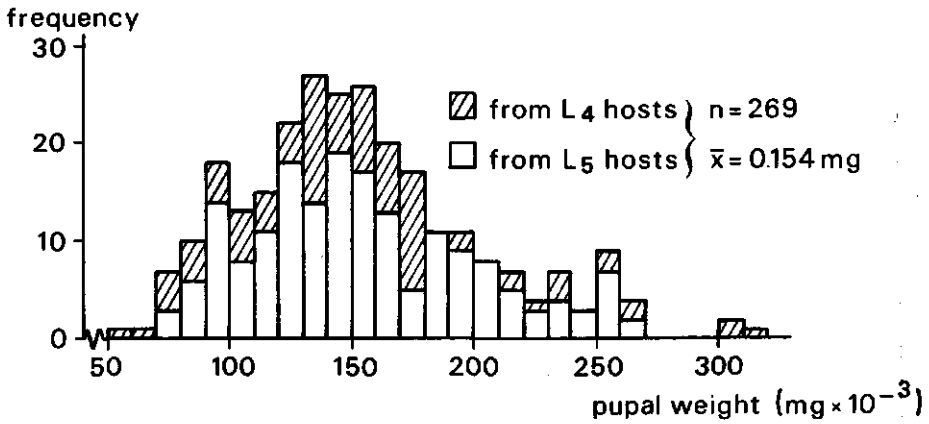

Fig. 6.3 Frequency distribution of the pupal weight of male *Colpoclypeus florus*.

(n = 180, $\bar{x}$ = 0.154 mg), however, were not heavier than those collected from hosts of the fourth larval instar (n = 89, $\bar{x}$ = 0.153 mg). This was expected by the higher insemination capacity needed here. On the other hand it is clear that host material is saved, since the weight-classes above 0.16 mg are underrepresented in spite of their equally high insemination capacity.

6.3. Sequence in which male and female eggs are laid

6.3.1. Introduction

In the model (Section 6.1) no distinction is made between the pre-adult mortality of males and females. To justify this simplification it must be shown that the primary and secondary sex ratios do not differ substantially, despite the high preadult mortality common in this species. Once this is established for the laboratory, it should also be determined for field situations. As mortality in the laboratory and in the field are equal, it is sufficient to show that this mortality affects the sex ratio in the field in the same way as the sex ratio in the laboratory.

One of the methods used to investigate pre-adult mortality of the sexes offered the opportunity to determine the sequence in which male and female eggs are deposited within one clutch. Green et al. (1982) suggested to look for a non-random pattern in this sequence, which would be evidence for a precise instead of a binomially distributed sex ratio. A non-random pattern would therefore agree with the prediction of the model. Research on different gregarious parasitoids (Flanders, 1935; Abdelrahman, 1974; Mertins, 1980; Feijen & Schulten, 1981; Waage, 1982b; Suzuki et al., 1984; Waage & Ng Sook Ming, 1984; Putters & Van den Assem, 1985) suggests that for various species different non-random sequences exist. A mechanism in which the male eggs are laid at the beginning of oviposition and/or at regular intervals between the female ones is recorded by a

number of authors (Feijen & Schulten, 1981; Waage, 1982a; Suzuki et al., 1984; Waage & Ng Sook Ming, 1984; Putters & Van den Assem, 1985). Such a process is efficient to ensure the production of the optimal sex ratio irrespective of the size of the clutch. Each clutch is provided with the necessary males (not more) and completed with as many females as host resources allow. Species where males have a fairly low insemination capacity are expected to produce male eggs at regular intervals.

6.3.2. Materials and methods

The following method (experiment XIX) was used to determine if the sex ratio is affected by mortality in the same way in the field and in the laboratory. A field sample (see Subsection 5.4.1) was split into two groups. Parasitized hosts collected with eggs of the parasitoid, which were assumed to have had little or no mortality, were reared to the pupal stage in the laboratory under laboratory mortality conditions, and were sexed. Parasitized hosts collected with pupae of the parasitoid, under field mortality conditions until that stage, were likewise sexed. Then these two sex ratios were compared.

Differences in the oviposition behaviour of the female wasp while depositing a fertilized or an unfertilized egg, such as was found for *Trichogramma chilonis* Ishii (Suzuki et al., 1984), was not observed. To test the difference between the primary and the secondary sex ratio it was necessary to determine the sex of individual eggs cytologically (experiment XX). Female wasps were observed while producing a clutch of eggs and the place, time and sequence of egg deposition was noted by means of schematic drawings. The eggs were incubated for 24 h, transferred to microscope slides and placed into a drop of 2% lacto acetic orcein. The lacto acetic orcein solution was made according to Van Heemert (1974) and used as a medium for fixing and staining. A cover slide was used to gently press the contents

of the egg outside the chorion. After staining for about 24 h at 20°C, the embryonic tissue was squashed and analysed cytologically (magnification 1000x). Other parasitoid clutches on hosts of the same size and age were reared to adult wasps under normal laboratory conditions. The sex ratio of these adults was compared with the sex ratio of the clutches determined with the cytological method.

6.3.3. Results and discussion

In Table 6.2 the secondary sex ratios as a result of laboratory and field mortality factors respectively (and based on field primary sex ratios) were compared. They do not differ significantly.

Table 6.2 Secondary sex ratio of field clutches which have suffered from mortality in the field and in the laboratory respectively (experiment XIX)*

Host stage	L_3 (n)	L_4 (n)	L_5 (n)
Sex ratio after mortality suffered in the field	0.35 (9)	0.21 (51)	0.23 (21)
Sex ratio after mortality suffered in the laboratory	0.30 (3)	0.20 (41)	0.23 (50)
χ^2 2x2 (two-sided; α = 0.05)	p = 0.94	p = 0.95	p = 1

* Number of clutches in parentheses

Whether there is any difference between the primary and secondary sex ratio due to differential mortality of the sexes, can be determined from the results of the cytological experiment. Most of the temporary preparations of the eggs contained some cells in meta- or anaphase, where single chromosomes could be observed. The precise chromosome number (n = 6) could not be determined in each preparation, but the difference between a haploid (male) and diploid (female) set was not difficult to see. In this way from a total number of 68 clutches of various sizes all the eggs could be sexed (Table 6.3). Only one of the 56 clutches with more than two

Table 6.3 Primary sex ratio of eggs sexed cytologically, compared with secondary sex ratio of clutches reared to adult stage in the laboratory (experiment XX)*

Host stage	L_2 (n)	L_3 (n)	L_4 (n)	L_5 (n)
Sex ratio of eggs	0.28 (29)	0.26 (19)	0.25 (15)	0.13 (5)
Sex ratio of adults	0.29 (12)	0.29 (15)	0.18 (15)	0.11 (20)
Mortality	0.86	0.75	0.47	0.48
$P = P_o$ (two-sided; $\alpha = 0.05$)	$p = 0.89$	$p = 0.93$	$p = 0.19$	$p = 0.39$

* Number of clutches in parentheses

eggs contained no males. Clutches of one or two eggs mostly contained no males. Mortality of the clutches reared to the adult stage in the laboratory again was high. Nevertheless the sex ratio of the emerging adults did not differ significantly from the sex ratio determined cytologically in the egg stage (Table 6.3). Mortality in the pre-adult stages therefore does not affect the sex ratio of emerging adults.

The sequence of male and female eggs within the clutch could be determined for most of the clutches (Fig. 6.4). Observations were carried out at short intervals, hence occasionally more than one egg was laid between two observations. This only devaluated the results when those eggs appeared to belong to different sexes. These cases are indicated in Fig 6.4. Usually the last few eggs of a clutch are male. Only occasionally one or two male eggs are laid in between the female eggs. The deposition of male eggs at the end of a sequence indicates that the allocation of males to a clutch is not a random process. The advantage of an early placement of males, mentioned by Waage (1982a) and Waage & Ng Sook Ming (1984) for egg parasitoids (see Subsection 6.3.1) is not present here. If egg deposition of *C. florus* has to be stopped prematurely, there is the disadvantage that the emerging female offspring has a large probability to be left uninseminated. However, a parasitoid of egg masses which are parasitized egg by egg will start without knowing the ultimate number of eggs needed. Before each following egg is

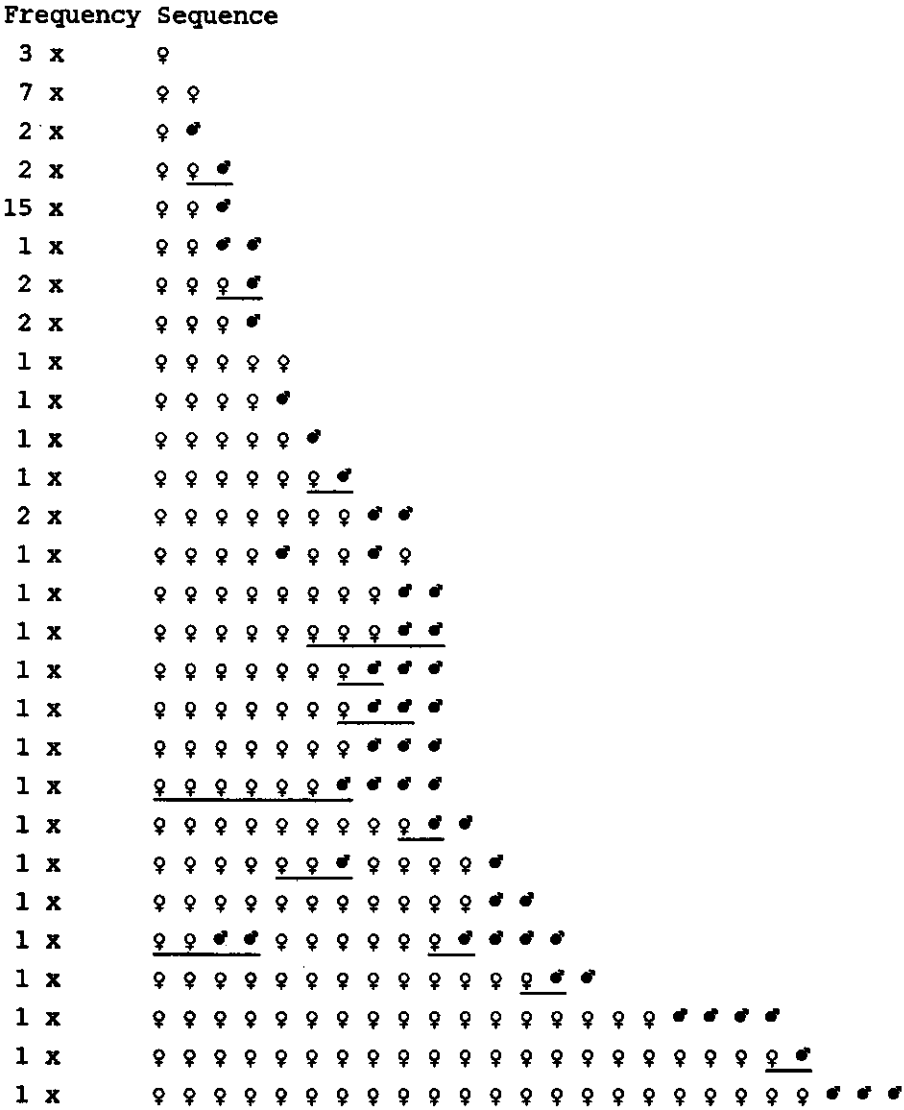

Fig. 6.4 Sequence of deposition of male and female eggs in different-sized clutches (experiment XX). The sequence of underlined males and females is not certain. Frequency of each sequence is given, and total number of clutches observed is 55.

deposited she will make a decision on the basis of the presence of still unparasitized host eggs (Waage & Ng Sook Ming, 1984). Meanwhile the chance of being disturbed during egg deposition is considerable. A larval parasitoid like *C. florus* probably has more knowledge of the total resource of its host at the start of egg laying, and does not constantly have to compare the "parasitized" and the "unparasitized" part of its host before each egg deposition. An indication of this was found in the results of Chapter 5.3. Evidence was collected with an additional experiment (experiment XXI). During parasitization of hosts of the fourth larval instar, eggs were taken away. This treatment was always executed between the fifth and the ninth egg laid, and without much disturbance. In this way a number of one to five eggs was taken away twice from each clutch, leaving four eggs maximally. Final clutch sizes were compared with clutches with a comparable disturbance, but where no eggs were removed. It was shown that eggs which were taken away during parasitization were not replaced by the wasp (Table 6.4). If somehow the information about the number of eggs to lay is stored in the animal in advance, and the chance of being disturbed during egg deposition is small, then the sequence of males and females offers no special problem. An explanation of the sequences met with *C. florus* may be that this information is stored as a motivational state which is proportional to the size of the host encountered and decreases after each egg laid (Fig. 6.5). Two

Table 6.4 Comparison of total number of eggs laid and number of eggs present at parasitoid departure between clutches where eggs have been taken away during parasitization and control clutches (experiment XXI)

	n tested	Total number of eggs laid (mean ± SE)	Number of eggs present at parasitoid departure (mean ± SE)
Treatment (between 5th and 9th egg laid with host, twice a number of 1-5 eggs was taken away leaving 4 eggs)	44	12.86 ± 0.33	5.16 ± 0.43
Control treatment (disturbance comparable to treatment but no eggs removed)	58	12.12 ± 0.38	12.12 ± 0.38
t-test; one-sided; $\alpha = 0.05$		NS ($p \simeq 0.08$)	S ($p << 0.001$)

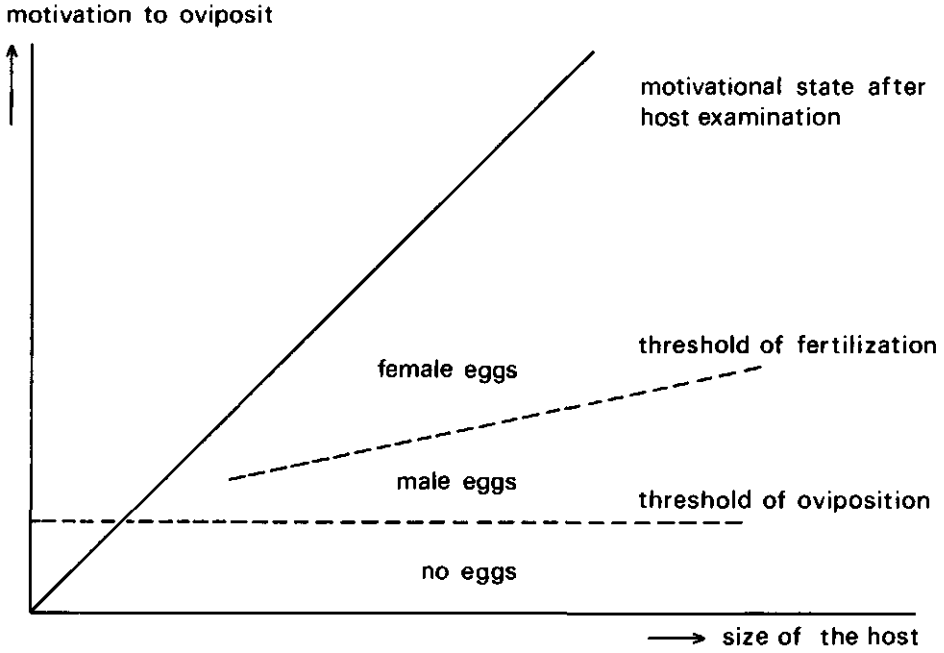

Fig. 6.5 Preliminary explanation of the sequence of male and female eggs in a clutch of *Colpoclypeus florus*. The time axis (during oviposition) is perpendicular to the abscissa.

thresholds could be passed during egg laying, namely a fixed threshold where egg deposition stops and, just above this threshold another one where fertilization of the eggs stops. The results of experiment XX suggest that the latter threshold may be not a fixed one but in some way depends on the clutch size. The number of male eggs on the host instars L_2 and L_3 differs significantly from that on the host instars L_4 and L_5 (F-test, $p << 0.001$, followed by Tukey's test).

6.4. The sex ratio: model predictions and reality

6.4.1. Introduction

Pre-adult mortality of *C. florus* does not differ between males and females (Section 6.3) and is equally high both in

the field and in the laboratory (Section 5.4). If in addition comparable field and laboratory clutches show to have the same secondary sex ratios it can be assumed that the same primary sex ratio decisions underlie these results. If not, then circumstances other than mortality must cause different primary sex ratios.

Optimal values predicted by the model (Section 6.1) can be used, assuming that no double parasitism occurs. The assumptions that one male is sufficient for the insemination of the females of a clutch and that juvenile mortality is independent of sex have proved to be true. Although not conclusively shown, it is very probable that insemination occurs exclusively from brothers to sisters of the same clutch and that male contribution to resource competition is minimized.

6.4.2. Materials and methods

Secondary sex ratios were obtained from the results of laboratory experiment III and field experiment XI. Field data were collected from the pupal stage of the parasitoid. Two tests were made. The sex ratios of clutches on the same host instar were compared between laboratory and field. The number of males in a clutch was compared between the different host instar groups of both laboratory and field.

Optimal numbers of unfertilized eggs in a clutch (x_i*) were calculated for each host instar in the laboratory and the field situation apart by substituting mortality and clutch size in $(1 - m_i{}^{x}i)\ (c_i - x_i)\ (1 - m_i)$. The substituted mortality values are those based on mortality within the successful clutches only. This was done because the sex ratio of clutches with 100% mortality is not relevant. If the parasitoid compensated for this mortality as well, the number of males in successful clutches would be unnecessarily high. Hence field mortality was only corrected for that part of egg mortality which is not measured in the field.

Laboratory mortality, however, was calculated for this

purpose using only those clutches resulting in at least one adult. It was assumed that having success, i.e. producing at least one adult, does not depend on clutch size or sex ratio.

The number of eggs in a clutch actually unfertilized is calculated by multiplying clutch size and sex ratio ($c_i . r_i = x_i$).

6.4.3. Results and discussion

The sex ratio of clutches laid on hosts of the third larval instar did not differ between laboratory and field hosts. The same holds for hosts of the fourth larval instar. A significant difference, however, was found between laboratory and field sex ratio on hosts of the fifth larval instar (Table 6.5). It must be concluded that this difference was already present in the primary sex ratio, since it was shown that mortality is equal in the field and the laboratory and independent of sex. The difference cannot be attributed to the accidental fact that clutch size in the tested field hosts was larger than in the laboratory hosts due to their higher mean weight (see Table 5.9). A larger clutch size may cause an increase of the absolute number of unfertilized eggs since male insemination capacity may become insufficient. The sex ratio which is a relative figure should, however, remain constant in this case. Double parasitism as a possible cause of higher sex ratios (Werren, 1980; Suzuki & Iwasa, 1980) may explain part of the higher sex ratio on fifth instar larvae in the field. It is not common enough, to cause a

Table 6.5 Secondary sex ratio of field (experiment XI) and laboratory clutches (experiment III)*

Host stage	L_3 (n)	L_4 (n)	L_5 (n)
Sex ratio in the field	0.35 (9)	0.21 (51)	0.23 (21)
Sex ratio in the laboratory	0.34 (56)	0.23 (82)	0.12 (90)
χ^2 2x2 (two-sided; $\alpha = 0.05$)	$p = 0.81$	$p = 0.50$	$p << 0.001$

* Number of clutches in parentheses

significantly higher clutch size (Table 5.9). The other cases mentioned by Suzuki & Iwasa (1980) in which the sex ratio is thought to increase (see Section 6.1) do not apply here. The main cause for this difference therefore remains uncertain.

Clutch size is strongly correlated to the size of the host. By taking the absolute number of adult males in a clutch we were able to compare the sex ratio decisions made with different host instars (Table 6.6). It was not

Table 6.6 Numbers of adult males in a clutch

Laboratory hosts	$\bar{x}$	SE	Field hosts	$\bar{x}$	SE
L_2	0.56	0.18			
L_3	0.78	0.24	L_3	0.67	0.24
L_4	1.15	0.16	L_4	1.25	0.21
L_5	1.03	0.19	L_5	3.19	0.87*

* F-test ($p << 0.01$) was followed by Tukey's test: only the number of adult males with L_5-hosts in the field differs significantly from the other values.

surprising that the hosts of the fifth larval instar from the field showed a significantly higher number of adult males in their clutch than the other hosts. This appeared to be the only significant difference within the seven host types considered. These results seem to contrast with those of experiment XX, which showed an increasing number of unfertilized eggs between hosts of the third and hosts of the fourth larval instar. However, intermediate mortality may have caused higher variances within the samples, thus obscuring the differences between them. A variable fertilization threshold therefore remains a possibility, but the necessity of it will be discussed at the end of this chapter.

In order to test if the model can explain the sex ratio decisions made by *C. florus*, the mean number of unfertilized eggs (x_i) was calculated for each of the seven cases listed in Table 6.7. Mean clutch size (c_i) and mean adult sex ratio (r_i) were multiplied for each case. Also the optimal values

Table 6.7 Values of mortality (m_i), clutch size (c_i) and sex ratio of adults (r_i) used to calculate the number of unfertilized eggs (x_i) and its optimal value (x_i^*) with respect to a maximization of the number of inseminated daughters (f_i)

Host		m_i	c_i	r_i	$x_i = c_i \cdot r_i$	$f_i = (1-m_i^{x_i})d_i$	x_i^*	$f_{max} = (1-m_i^{x_i^*})d_i$	$\frac{f_i}{f_{max}}$
Laboratory	L2	0.69	3.2	0.56	1.792	0.212	1.4	0.226	0.94
	L3	0.55	5.1	0.34	1.734	0.978	1.8	0.979	1.00
	L4	0.56	11.1	0.23	2.553	2.905	3.0	2.938	0.99
	L5	0.54	19.3	0.12	2.316	5.938	3.8	6.444	0.92
Field	L3	0.67	7.4	0.35	2.590	1.025	2.6	1.025	1.00
	L4	0.46	11.8	0.21	2.478	4.299	2.7	4.310	0.97
	L5	0.41	26.5	0.23	6.095	11.986	3.4	12.971	0.92

for x_i (x_i^*) were calculated by substituting c_i, r_i and mean mortality within the successful clutches (m_i) in the expression

$$f_i = (1 - m_i^{x_i}) (c_i - x_i) (1 - m_i)$$

and by searching for the value of x_i which maximizes the number of inseminated daughters (f_i). The values of m_i, c_i and r_i used are tabulated in Table 6.7. For each pair of values of x_i and its x_i^* the values of f_i and f_{max} were calculated by substitution. The degree of agreement is expressed by the ratio f_i/f_{max}, for it is not the difference between x_i and its optimum value which natural selection acts upon, but the resulting loss of a part of the maximally attainable number of inseminated daughters.

From the results tabulated in Table 6.7 it can be seen that, although the fit of x_i with x_i^* is often very poor, in all cases the expected number of inseminated granddaughters is close to the maximum value predicted by the model. In three cases the difference is less than one percent while the largest difference is eight percent.

The chance that these differences are not statistically significant is considerable due to a high variance caused by mortality. Apart from this it should be realized how the advantage of an even more precise sex ratio (a fitness gain of 0 - 8%) could be achieved. From Fig. 6.6 it can be seen that a result of 90 percent of the maximum can be attained by a wide range of male eggs. The maximum value can only be reached, of course, by one specific number of male eggs. In

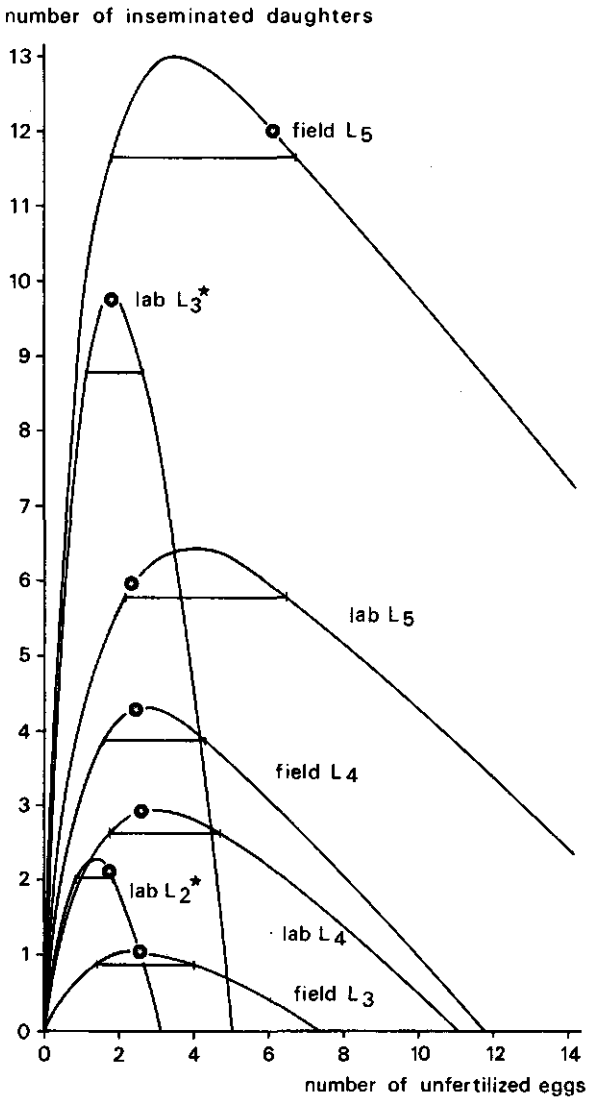

Fig. 6.6 **Relationship between the number of unfertilized eggs laid in a clutch and the expected number of inseminated daughters for different host larval instars as predicted by the model. The realized numbers of unfertilized eggs and the 90%-range are indicated. The ordinate values should be divided by ten for two curves indicated with *.**

this connection it is striking that these sub-optimal intervals show a considerable overlap between the different host instars. This means that here the parasitoid has the opportunity to be adapted to different host sizes without changing its x. Gaining the last 10 percent, however, requires a total change in strategy, because each host size has its own optimal value of x_i. A fixed x, for instance as a result of the fixed fertilization threshold postulated in Section 6.3, should result in two types of sub-optimal values of x. With small hosts and a low value of x_i* the realized value of x should be higher than optimal, whereas with large hosts and a higher value of x_i* the realized value of x should be lower than optimal. The realized value of x should have the best fit with intermediate values of x_i*. This effect can indeed be observed in Fig. 6.6. The only exception is the value of field hosts of the fifth larval instar, which is otherwise also aberrant.

7. CONCLUSIONS AND GENERAL DISCUSSION

7.1 The process of host selection

Doutt (1964) divided the process leading to successful parasitism into four stages: (a) host-habitat location, (b) host location, (c) host acceptance, and (d) host suitability. The first three can be combined as aspects of the host selection process (Vinson, 1976). In the present study the three stages of host selection by which larvae of *Adoxophyes orana* are selected as hosts for *Colpoclypeus florus* were analysed.

Once it is in the canopy of an apple tree, *C. florus* preferentially searches for hosts at the tops of long shoots. As a result of this preference these hosts, irrespective of size, are two and a half times more likely to be encountered by the parasitoid than hosts in other places in the outer layer of the canopy. As nearly all the tops of long shoots belong to the outer layer of the canopy, this preference may cause a still higher bias for hosts at the tops of long shoots when the whole canopy is considered. When *C. florus* parasitizes *A. orana*, more hosts of the fourth and fifth larval instar than younger larvae are encountered, since these largest instars of *A. orana* also have a preference for the tops of long shoots.

This element of host searching behaviour means that *C. florus* is adapted to hosts which in the summer have the same preference as *A. orana*. The preference for the tops of long shoots is not so marked for older larvae of other tortricids (De Jong & Beeke, 1982). This may explain why larvae of *Clepsis spectrana*, which is also bivoltine and has a high acceptance in the laboratory (Gruys & Vaal, unpubl.), are hardly ever parasitized in the field.

The proximate factors which cause the preference of *C. florus* for the tops of long shoots are not known. Knowledge of these factors, however, may help to explain why release experiments in spring (Gruys & Vaal, 1984) have

failed. Host-plant odour and light intensity distribution are likely to be different in spring and in summer and have shown to be important cues in host-habitat location for other parasitoids (Vinson, 1975; Vinson, 1976, for a review). It can be asked how the parasitoids manage with these changes in their habit with respect to host searching and host size selection. Weseloh (1982) showed that tree microhabitat preference of a parasitoid is not always adapted to the occurrence of profitable hosts.

The effect of conspicuousness of the host also causes differences in the encounter probabilities between hosts of different instars. Hosts of the fifth larval instar are four times and hosts of the fourth larval instar are three times as conspicuous to the parasitoid as hosts of the third larval instar. This causes another bias towards an encounter with more profitable host larvae.

In the laboratory as well as in the field the proportion of hosts accepted for oviposition by *C. florus* is affected by the weight of the host. However, the relative profitability is low where the maximum level of acceptance already is attained. In the laboratory, this results in acceptance of hosts of very different profitability. This weak preference for host of high profitability is typical for predators and parasitoids foraging at a low density of the prey or host (MacArthur & Pianka, 1966). In the field, however, the combined effect of host-habitat location and host location results in relatively few small hosts being encountered. Consequently the "diet" of the parasitoid is restricted to the larger hosts. However, within these large hosts the realized profitability can be very different. This is caused by the variation of clutch size and above all mortality of juvenile parasitoids. No proximate factor is known for this parasitoid to predict the profitability of individual hosts in detail: probably the parasitoid has to work with the mean profitability of hosts of a certain dimension.

Field data showed a strong bias of the parasitoid to parasitization of fourth and fifth larval instars of the

host. This is in agreement with a "diet" calculated with the use of the known variables for accessibility, conspicuousness and acceptance of host of different instars. Under the assumption of optimal host choice such a diet should result in a host parasitizing rate of 0.17 to 0.71 hosts per day depending on the proportion of third instar larvae included in the diet.

It is not expected that a change in absolute host density causes a change in the diet. To adapt the diet to a new host density, the parasitoid has to be able to estimate the density of the host. No evidence for this ability is found in *C. florus* and this may explain the low number of hosts parasitized during a lifetime (about two or three). Differences in host density in the same group of trees will be found between host generations whereas differences in host density within a host generation will be found between different parts of the tree. During the host-habitat search of *C. florus*, the densities experienced by the individual parasitoid are not very different. This results from the facts that (a) individual parasitoids are only active during one generation of the host and (b) once a cluster of hosts is found all the eggs of one female can be deposited. Preference for host instars is therefore expected to be genetically fixed and adapted to the lowest densities that occur, since these are decisive for the survival of the parasitoid and its offspring (Stairs, 1983). This does not mean that the "diet" is invariable. Preference will not change, but the probabilities of encountering different host instars will alter as a result of changes in their relative densities: e.g. at the end of one generation of *A. orana* more hosts of the fifth larval instar than of the fourth larval instar will be found and parasitized. A similar stable preference at changing relative densities of host instars is given by Heong (1981).

It can be asked what the real host densities are which relate to the values of the host encounter rate and the host parasitizing rate estimated in this study. A suggestion for

further research with respect to this question is to investigate the physical and chemical cues that are used and the distances at which they operate. Another, more difficult way to tackle this problem is to follow individual wasps when they search for hosts in natural habitats.

7.2 Exploitation of selected hosts

Once a host is selected for oviposition, the investment of the parasitoid in this host can be expressed in time and number of eggs. Female wasps have a limited longevity and fecundity. One of these may be limiting in certain situations. At high host densities the fecundity is likely to become limiting. In this situation each egg of the parent should produce offspring with the highest possible total fertility (F). In the present study this is called the "F-strategy". At low host densities the time to search for hosts may become limiting. In this case each host parasitized requires the investment of an amount of time and should produce female offspring with the highest possible total longevity (L). This is called the 'L-strategy".

The following results were obtained:

(1) Longevity and fecundity of a female are positively related to her body weight.
(2) Body weight of a female is determined by and negatively related to the number of eggs laid by her mother per unit host weight.
(3) *C. florus* adapts her clutch size to the weight of the host, using a proximate factor probably the host width.
(4) As a result of (3) the variation in the number of eggs laid per unit host weight is small as is the variation in the body weight of females.
(5) The normally produced clutch sizes induce competition for food among the juveniles, which means that the resulting body weight of females is not that maximally attainable.
(6) As a result of (3) density dependent mortality of

juveniles is, however, prevented.

In *C. florus* the "F-strategy" requires a low number of eggs per unit host weight, while the "L-strategy" requires a high number of eggs per unit host weight. The clutch sizes produced by *C. florus* on hosts of the fourth larval instar could neither be explained by the "F-strategy" nor by the "L-strategy". However, if the clutch size is adapted to the current host density, a female parasitoid will have a body weight which will allow her to invest most of her eggs during her lifetime. To test this hypothesis the measurement of the host encounter rate and its variation is necessary. In the present study a calculation was only made of the host encounter rate and host parasitizing rate to which the clutch size of *C. florus* is thought to be adapted. Further research is clearly necessary.

A considerable change in host density would require a newly adapted clutch size. A decreasing clutch size was found in *C. florus*, when successive hosts were offered at short intervals. However, this was shown to be an artifact, caused by the long parasitization time of this parasitoid. When a proper experimental design was used, the clutch size remained constant. A decreasing clutch size as observed by Gerling & Limon (1976) and Parkman et al. (1981) in *Euplectrus laphygmae* Ferrière and *E. plathypenae* Howard (Hym., Eulphidae) respectively, may also be caused in this way, but they do not mention parasitization times. Other parasitoids may have the skill to adapt their clutch size in this way (Ikawa & Suzuki, 1982). However, in *C. florus* it is thought that the behaviour which determines the clutch size is genetically fixed and changes by means of differential reproductivity of the genotypes. Once a population is adapted to the current host density range, the maximum number of hosts found in a lifetime is reached at this host density. Does this mean that a functional response is not expected at higher host densities than the range to which the parasitoid is adapted? The same can be asked for the kind of numerical response which acts by means of higher reproduction at places

with high host densities, since maximum fertility is already attained. It would be premature to try to answer these questions here.

It is interesting that in *C. florus* longevity is less sensitive than fertility to changes in body weight. This means that the fertility/longevity ratio of offspring increases when the number of eggs laid per unit of host weight is decreased. The number of eggs per unit of host weight may be decreased after an increase in the host density, as a response of the individual parasitoid or as a result of natural selection. The increase of host density thus not only results in smaller clutches but also in a higher fertility/longevity ratio. In the progeny total fecundity will be much higher at the same or nearly the same longevity. This may be functional at higher host densities, and thus be an important ultimate factor not only for *C. florus* but also for those parasitoids which directly respond to changes in host density.

Further research on host exploitation, as on host selection must focus on the field values of the host parasitizing rate in *C. florus*. The rate of 0.34 hosts/day at which the current clutch size is thought to be optimal has to be verified. It should be realized that this value is calculated for the situation where hosts of the third, fourth and fifth larval instar are simultaneaously available.

The precise nature of the proximate factor used to determine host weight was not elucidated. Knowledge of it would increase the understanding of constraints which may operate in the parasitization of host species having a different shape. For *A. orana* this constraint causes overestimation of the smallest larvae and underestimation of the largest larvae. This is thought to give rise to the higher pre-adult mortality existing in both cases. An experiment where the extremely high, respectively low clutch size to host weight ratio of these hosts are brought to the mean level may demonstrate whether mortality decreases in this way.

7.3 Sex allocation

Colpoclypeus florus has the capacity to control the sex ratio of her offspring, by means of the haplodiploid sex determination system. In gregarious parasitoids this usually leads to clutches in which females predominate. *C. florus* showed to be arrhenotokous and not thelytokous: fertilized eggs produce diploid females and unfertilized eggs produce haploid males. In thelytoky were only females are produced the host resources are only spent on females. This may be advantageous and that is why (in Chapter 1) arrhenotoky was considered to be a constraint in *C. florus*. However, arrhenotoky as opposed to thelytoky enables outbreeding and the formation of new genotypes. Since outbreeding can be considered as in conflict with a female (or male) biased sex ratio (Fisher, 1930; Hamilton, 1967), an arrhenotokous parasitoid should decide between the number of offspring and its genetic variability. This decision is not discussed in this study and in Chapter 6 outbreeding is considered to be rare. Besides the fact that there are good arguments to consider inbreeding as a rule, it is very difficult, if not impossible, to give a quantitative estimate of the advantage of the genetic variability gained by outbreeding. This advantage can be considerable even when outbreeding is rare. Nevertheless partial outbreeding may result in the sex ratio (proportion males) being higher than the optimal sex ratio in the case of total inbreeding.

In the field it was repeatedly found that the proportion of clutches consisting of only females, which in the case of total inbreeding would result in uninseminated females, was higher than the proportion of clutches which were totally male. These latter clutches are produced by uninseminated females. So a proportion of the females might be inseminated later and may have produced mixed clutches. Unfortunately these data were from the same generation of parasitoids. Similar data in future studies collected from subsequent generations could provide an estimate for the occurrence of

inseminations outside the original clutch. The chance that these kinds of inseminations take place will increase with decreasing sex ratio, since then more females leave a clutch uninseminated. The resulting degree of outbreeding, however, will increase the sex ratio and an equilibrium is predicted.

The chance of inseminated females being inseminated a second time should also be investigated. Females of *C. florus* show a period of non-receptivity after insemination (in fact: after having performed a receptivity display). The duration of this period and the way sperm competition acts subsequently should be determined.

The insemination capacity of males of *C. florus* is size-dependent: large males have a higher insemination capacity than small ones. However, the number of females that a male of mean weight is able to inseminate is sufficient for the largest clutches. This puts a functional limit to the mean size of males; apparently it is not important to inseminate more females than are present in their own clutch. Although the insemination capacity of a male is size-dependent, above a certain size fitness is virtually not increased by a higher insemination capacity, because females outside the clutch of origin are hardly ever encountered. As a result the optimal weight of a male is low compared to the optimal weight of a female (Charnov et al., 1981).

It was demonstrated that the primary and secondary sex ratios are the same, and that male eggs are laid at the end of an ovipositional sequence. Determination of the sequence in which eggs are laid can be easily established for *C. florus*, since eggs are laid outside the host. The cytological technique that was used may also be used in the study of sex ratios in other gregarious parasitoids to test the hypothesis that male eggs are laid at the end of a clutch when host size is clearly limited, and is estimated by the parasitoid before oviposition. It is important to note here that gregarious parasitoids which are now known to lay male eggs at the beginning of a sequence and/or at regular intervals are parasitoids of egg-masses (Feijen &

Schulten, 1981; Waage, 1982a; Suzuki et al., 1984; Waage & Ng Sook Ming, 1984), since the size of eggs-masses probably is not estimated by the parasitoid in advance.

Clutches of one or two eggs usually contain no males. Pre-adult mortality is so high that not more than one adult will survive in these clutches. Females alone may have a higher progeny expectance than males alone, when the chance to find a mate outside the clutch is low.

When egg survival is low, the advantage of a precise sex ratio versus a binomial one is also less (Green et al., 1982). In the present study pre-adult mortality is about 50 per cent. Nevertheless the allocation of males to a clutch is not a random process and the number of males can be explained by a model assuming a precise sex ratio mechanism. Thus the evolutionary advantage of arrhenotoky which makes precise sex ratios possible by the ability of controlling the sex of the progeny, is also utilized in a situation where this advantage is less due to high mortality.

High mortality may have obscured a relationship between the clutch size and the number of adult males that arises. More research is needed to decide if there is a variable or a fixed fertilization threshold. The sex ratio of *C. florus* can be explained without assuming a certain probability of double parasitism. *C. florus* behaves optimally in situations where no double parasitism occurs, but suboptimally in situations with a certain probability of double parasitism. The difficulty for *C. florus* to assess the probability of double parasitism in the field may be the same as that to estimate the host encounter rate (HER): a low number of host encounters during lifetime. Further investigations should attempt to estimate the probability of double parasitism in the field and the probable fitness loss of a genetically fixed sex ratio strategy.

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References

Abdelrahman, I., 1974. Studies in ovipositional behaviour and control of sex in *Aphytis melinus* DeBach, a parasite of California red scale, *Aonidiella aurantii* (Mask.). Australian Journal of Zoology, 22: 231-247.

Albajes, R., E. Bordas & J.M. Vives, 1979. Contribucion al conocimiento de la biocenosis de *Epichoristodes acerbella* Walk. en El Maresme. Anales del Instituto Nacional de Investigaciones Agrarias, Serie Protección Vegetal, 11: 103-108.

Alexander, R.McN., 1982. Optima for animals. Edward Arnold, London.

Ankersmit, G.W., 1968. The photoperiod as a control agent against *Adoxophyes reticulana* (Lepidoptera; Tortricidae). Entomologia Experimentalis et Applicata, 11: 231-240.

Assem, J. van den, 1976. Male courtship behaviour, female receptivity signal, and size differences between the sexes in Pteromalinae (Hym., Chalcidoidea Pteromalidae), and comparative notes on other Chalcidoids. Netherlands Journal of Zoology, 26: 535-548.

Auersch, O., 1960. Weitere Beobachtungen zum Auftreten des Fruchtschalenwicklers (Apfelschalenwicklers) *Adoxophyes reticulana* Hb. Anzeiger für Schädlingskunde, 33: 83-87.

Bakker, K., S.N. Bagchee, W.R. van Zwet & E. Meelis, 1967. Host discrimination in *Pseudeucoila bochei* (Hymenoptera: Cynipidae). Entomologia Experimentalis et Applicata, 10: 295-311.

Barel, C.J.A., 1973. Studies on dispersal of *Adoxophyes orana* F. v. R. in relation to the population sterilization technique. Mededelingen Landbouwhogeschool Wageningen, 73-7: 1-107.

Bouček, Z., 1959. A study of Central European Eulophidae, part I: Eulophinae, and part II: *Diaulinopsis* and *Cirrospilus*. Acta Entomologica Musei Nationalis Pragae, 33: 117-194.

Bouček, Z. & R.R. Askew, 1968. Index of Palearctic Eulophidae (excl. Tetrastichinae). In: V. Delucchi & G. Remaudière (eds). Index of entomophagous Insects. Le François, Paris, p. 1-254.

Čapek, M., 1963. Verzeichnis der Parasiten, die aus schädlichen Insecten an Vúlh in Banska Štiavnica erzogen wurden. Teil III: Wirte von Ichneumonidae, Proctotrupoidea, Chalcidoidea und Bethylidae (Hym.). Vedecké Práce Výskumného Ústavu Lesného Hospodárstva v Banskej Štiavnici, 4: 239-272. (in Slovak; German summary).

Carl, K.P., 1976. Beobachtungen über Parasiten und Räuber von *Adoxophyes orana* F. v. R. in den Jahren 1963 bis 1965. In: Nützlinge in Apfelanlagen. Broschüre OILB/SROP nr. 3. p. 49-52.

Carl, K.P. & H. Zwölfer, 1965. Untersuchungen zur biologischen Bekämpfung einiger Unkräuter und Schädlinge in Landwirtschaft und Obstbau. 2. Bericht über die Arbeiten der Europäischen Station des Commonwealth Institute of Biological Control, Delémont, Schweiz. I. Teil. Anzeiger für Schädlingskunde, 38: 81-87.

Chapman, P.J., 1973. Bionomics of the apple-feeding Tortricidae. Annual Review of Entomology, 18: 73-96.

Charnov, E.L.; R.L. Los-den Hartogh; W.T. Jones & J.van den Assem, 1981. Sex ratio evolution in a variable environment. Nature, 289: 27-33.

C.I.L.B., 1960. Liste d'Identification no. 3. Entomophaga, 5: 337-353.

Cousin, G., 1933. Etude biologique d'un Chalcidien: *Mormoniella vitripennis*. Bulletin Biol. Fr. et Belg., 67: 371-400.

Delucchi, V., 1962. Hyménoptères Chalcidiens du Maroc III. Eulophidae. Al Awamia, 5: 53-66.

Domenichini, G., 1963. Ricerche sulle biocenosi del melo nell' Italia settentrionale. Bollettino di Zoologia Agraria e di Bachicoltura, 5: 1-23. (English summary).

Doutt, R.L., 1959. The biology of parasitic Hymenoptera.

Annual Review of Entomology, 4: 161-182.

Doutt, R.L., 1964. Biological characteristics of entomophagous adults. In: P. DeBach (ed.). Biological control of insect pests and weeds. Chapman & Hall, London, p. 145-167.

Erdös, J., 1951. Eulophidae novae. Acta Biologica Hungarica, 2: 169-237.

Erdös, J., 1956. Additamenta ad cognitionem faunae Chalcidoidarum in Hungaria et regionibus finitimis. VI. 19. Eulophidae. Folia Entomologica Hungarica, 9: 1-64.

Evenhuis, H.H., 1974. *Colpoclypeus florus* (Hymenoptera, Eulophidae), an important potential parasite of *Adoxophyes orana* (Lepidoptera, Tortricidae) in apple orchards. Mededelingen Fakulteit Landbouwwetenschappen Gent, 39: 769-775.

Evenhuis, H.H. & J. Vlug, 1972. Morphologische Unterschiede zwischen den Mandibeln der am Apfel lebenden Raupen der Blattwicklerarten. Zeitschrift für angewandte Entomologie, 71: 152-155.

Feijen, H.R. & G.G.M. Schulten, 1981. Egg parasitoids (Hymenoptera; Trichogrammatidae) of *Diopsis macrophtalma* (Diptera; Diopsidae) in Malawi. Netherlands Journal of Zoology, 31: 381-417.

Fenili, G.A., 1977. Ricerche sul compartemento biologico della *Epichoristodes* (= *Tubula*) *acerbella* (Walker) Diakonoff vivente in ambiente diversi. Redia, 60: 387-421. (English summary).

Fisher, R.A., 1930. The genetical theory of natural selection. Oxford University Press, Oxford.

Flanders, S.E., 1935. Host influence on the prolificacy and size of *Trichogramma*. Pan-Pacific Entomologist, 11: 175-177.

Galis, F. & J.J.M. van Alphen, 1981. Patch time allocation and search intensity of *Asobara tabida* Nees (Braconidae), a larval parasitoid of *Drosophila*. Netherlands Journal of Zoology, 31: 596-611.

Gerling, D. & S. Limon, 1976. A biological review of the genus *Euplectrus* (Hym.: Eulophidae) with special emphasis on *E. laphygmae* as a parasite of *Spodoptera littoralis* (Lep.: Noctuidae). Entomophaga, 21: 179-187.

Graham, M.W.R. de V., 1959. Keys to the British genera and species of Elachertinae, Eulophinae, Entedontinae and Euderinae (Hym., Chalcidoidea). Transactions of the Society for British Entomology, 13: 169-204.

Green, R.F., G. Gordh & B.A. Hawkins, 1982. Precise sex ratios in highly inbred parasitic wasps. The American Naturalist, 120: 653-665.

Gruys, P., 1982. Hits and misses. The ecological approach to pest control in orchards. Entomologia Experimentalis et Applicata, 31: 70-87.

Gruys, P. & F. Vaal, 1984. *Colpoclypeus florus*, an eulophid parasite of tortricids in orchards: rearing, biology and use in biological control. Entomologia Experimentalis et Applicata, 36: 31-35.

Hamilton, W.D., 1967. Extraordinary sex ratios. Science, 156: 477-488.

Hamilton, W.D., 1979. Wingless and fighting males in fig wasps and other insects. In: M.S. Blum & N.A. Blum (eds). Sexual selection and reproductive competition in insects. Academic Press, New York, p. 167-220.

Heemert, C. van, 1974. Meiotic disjunction, sex-determination and embryonic lethality in an X-linked "simple" translocation of the onion fly. Chromosoma, 47: 45-60.

Heong, K.L., 1981. Searching preference of the parasitoid *Anisopteromalus calandrae* (Howard) for different stages of the host, *Callosobruchus maculatus* (F.) in the laboratory. Researches on Population Ecology, 23: 177-191.

Holmes, H.B., 1972. Genetic evidence for fewer progeny and a higher percent males when *Nasonia vitripennis* oviposits in previously parasitized hosts. Entomophaga, 17: 79-88.

Hubbard, S.F. & R.M. Cook, 1978. Optimal foraging by parasitoid wasps. Journal of Animal Ecology, 47: 593-604.

Ikawa, T. & Y. Suzuki, 1982. Ovipositional experience of the gregarious parasitoid *Apanteles glomeratus* (Hym: Braconidae) influencing her discrimination of the host larvae, *Pieris rapae crucivora*. Applied Entomology and Zoology, 17: 119-126.

Ivancich Gambaro, P., 1962. Osservazioni sulla biologia di *Argyrotaenia pulchellana* Hw. e di *Adoxophyes reticulana* Hb. nel Veronese. Bollettino di Zoologia Agraria e di Bachicoltura, 4: 1-20. (English summary).

Ivancich Gambaro, P., 1968. L'incidenza dei nemici naturali sullo sviluppo delle popolazioni di tortricidi (Lepidoptera) dannosi alla fragola. Frustula Entomologica, 7: 1-8.

Jackson, D.J., 1966. Observations on the biology of *Caraphractus cinctus* Walker (Hymenoptera: Mymaridae), a parasitoid of the eggs of Dytiscidae (Coleoptera). III. The adult life and sex ratio. Transactions of the Royal Entomological Society of London, 118: 23-49.

Janssen, M., 1958. Über Biologie, Massenwechsel und Bekämpfung von *Adoxophyes orana* Fischer von Roeslerstamm (Lepidoptera: Tortricidae). Beiträge zur Entomologie, 8: 291-324.

Janssen, M., 1959. Tortriciden in Rheinischen Obstanlagen. Anzeiger für Schädlingskunde, 32: 6-8.

Jansson, A., 1954. Studier över svenska chalcidider. 4. Kläckta arter. 2. Entomologisk Tidskrift, 75: 255-265.

Jong, D.J. de, 1980. Monitoring techniques, forecasting systems and extension problems in relation to the summer fruit tortricid *Adoxophyes orana* (F. v. R.). EPPO Bulletin, 19: 213-221.

Jong, D.J. de & A.K. Minks, 1981. Studies on *Adoxophyes orana*, the major leaf-roller pest in apple orchards in the Netherlands. Mitteilungen der Schweizerischen Entomologischen Gesellschaft, 54: 205-214.

Jong, D.J. de & H. Beeke, 1982. Bladrollers in appel- en pereboomgaarden. Mededeling Proefstation voor de Fruitteelt Wilhelminadorp, 19: 1-218.

Karczewski, J., 1962. Die Bedeutung der Heidelbeere (*Vaccinium myrtillus* L.) für die Waldentomozönose. Folia Forestalia Polonica, A 9: 3-201. (in Polish; German summary).

Klomp, H. & B.J. Teerink, 1962. Host selection and number of eggs per oviposition in the egg parasite *Trichogramma embryophagum* Htg. Nature, 195: 1020-1021.

Klomp, H. & B.J. Teerink, 1967. The significance of oviposition rates in the egg parasite *Trichogramma embryophagum* Htg. Archives Néerlandaises de Zoologie, 17: 350-375.

Klomp, H., B.J. Teerink & Wei Chun Ma, 1980. Discrimination between parasitized and unparasitized hosts in the egg parasite *Trichogramma embryophagum* (Hymenoptera, Trichogrammatidae): a matter of learning and forgetting. Netherlands Journal of Zoology, 30: 254-277.

Kochetova, N.I., 1974. Factors determining the sex ratio in some entomophagous hymenoptera. Entomological Review, 2: 1-5.

Krebs, J.R., 1978. Optimal foraging: decision rules for predators. In: J.R. Krebs & N.B. Davies (eds). Behavioural ecology: an evolutionary approach. Blackwell Scientific Publications, Oxford, p. 23-63.

Lempke, B.J., 1976. Naamlijst van de Nederlandse Lepidoptera. Koninklijke Nederlandse Natuurhistorische Vereniging, Hoogwoud.

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

Lenteren, J.C. van, 1981. Host discrimination in parasitoids. In: D.A. Nordlund, R.L. Jones & W.J. Lewis (eds). Semiochemicals: their role in pest control. Wiley & Sons, New York, p. 153-179.

Limon de la Oliva, F. & J. Blasco Pascual, 1973. Preliminaries for the natural control and measures to use against pests of *Citrus*. Bolétin Informativo de

Plagas, 109: 69-86. (in Spanish; English summary).

Lucchese, E., 1941. Contributi alla conoscenza dei Lepidotteri del melo. III. *Acroclita naevana* Hb. Bollettino del Laboratorio di Entomologia Agraria di Portici, 5: 1-60.

MacArthur, R.H. & E.R. Pianka, 1966. On the optimal use of a patchy environment. American Naturalist, 100: 603-609.

Maynard Smith, J., 1978. The evolution of sex. Cambridge University Press, Cambridge.

Mertins, J.W., 1980. Life history and behaviour of *Laelius paedatus*, a gregarious bethylid ectoparasitoid of *Anthrenus verbasci*. Annals of the Entomological Society of America, 73: 686-693.

Montà, L.P. dalla & P. Ivancich Gambaro, 1973. Osservationi sulla biologia e l'etologia del *Colpoclypeus florus* Wlk. (Hymenoptera, Chalcidoidea, Eulophidae) in laboratorio e in pieno campo. Redia, 54: 243-259.

Neser, S., 1973. Biology and behaviour of *Euplectrus* species near *laphygmae* Ferrière (Hymenoptera: Eulophidae). Entomology Memoir, Department of Agricultural Technical Services, Republic of South Africa, 32: 1-31.

Nikolova, V., 1972. The plantain-leaf sawfly: a new pest of oil-bearing rose. Rastitelna Zaschita, 20: 28-31. (in Bulgarian).

Nikolova, V. & V. Natskova, 1966. Studies on the morphology and bionomics of *Pardia cynosbatella* L. and *Notocelia roborana* Illg. (Lepidoptera, Tortricidae). Rastenievudni nauki, 3: 135-146. (in Bulgarian; English summary).

Parkman, P., M. Shepard & J. Powell, 1981. Oviposition biology and population dynamics of *Euplectrus plathypenae* Howard (Hym.; Eulophidae). Journal of the Georgia Entomological Society, 16: 336-341.

Pianka, E.R., 1976. Natural selection of optimal reproductive tactics. American Zoologist, 16: 775-784.

Putters, F.A. & J. van den Assem, 1985. Precise sex ratio in a parasitic wasp: the result of counting eggs. Behavioral Ecology and Sociobiology, 17: 265-270.

Pyke, G.H., H.R. Pulliam & E.L. Charnov, 1977. Optimal foraging: a selective review of theory and tests. The Quarterly Review of Biology, 52: 137-154.

Pykhova, V.T., 1968. The control of the grape moth. Zashchita Rastenii, 13: 48-49 (in Russian).

Quednau, F.W., 1967. Notes on mating behaviour and oviposition of *Chrysocharis laricinellae* (Hymenoptera: Eulophidae), a parasite of the larch casebearer (*Coleophora laricella*). Canadian Entomologist, 99: 326-331

Russo, L.F., 1966. Lepidotteri tortricidi dannosi al nocciuolo in Campania. Nota preliminare. Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri, 24: 317-322. (English summary).

Salt, G., 1961. Competition among insect parasitoids. Symposium of the Society of Experimental Biology, 15: 96-119.

Scaramozzino, P.L. & A. Ugolino, 1979. *Pandemis heparana* (Den. & Schiff.), un tortricide dannoso al pesco in Piemonte. Informatore Fitopatologico, 29: 3-6. (English summary).

Schmidt, J.M. & J.J.B. Smith, in press. Host volume measurement by the parasitoid wasp *Trichogramma minutum*: the roles of curvature and surface area. Entomologia Experimentalis et Applicata.

Schoener, T.W., 1971. Theory of feeding strategies. Annual Review of Ecology and Systematics, 2: 369-404.

Shaumar, N., 1966. Anatomie du système nerveux et analyse des facteurs externes pouvant intervenir dans le déterminisme du sexe chez les Ichneumonidae Pimplinae. Annales des Sciences Naturelles, Zoologie, 12: 391-493.

Shiga, M. & A. Nakanishi, 1968. Intraspecies competition in a field population of *Gregopimpla himalayensis* (Hym: Ichneumonidae) parasitic on *Malacosoma neustria testacea* (Lep: Lasiocampidae). Researches on Population Ecology, 10: 69-86.

Soenarjo, E., 1979. Effect of labelling with the radioisotope

zinc-65 on the performance of the eulophid, *Colpoclypeus florus*, a parasite of Tortricidae. Entomologia Experimentalis et Applicata, 25: 304-310.

Speicher, B.R., 1936. Oogenesis, fertilization and early cleavage in *Habrobracon*. Journal of Morphology, 59: 401-421.

Stairs, G.R., 1983. Oviposition by *Glypta fumiferanae* (Hym., Ichneumonidae) in *Choristoneura fumiferana* (Lep., Tortricidae) larvae and the effect of honey on their fecundity and survival. Canadian Entomologist, 115: 689-692.

Stamp, N.E., 1982. Behavioural interactions of parasitoids and Baltimore checkerspot caterpillars (*Euphydryas phaeton*). Environmental Entomology, 11: 100-104.

Suzuki, Y. & Y. Iwasa, 1980. A sex ratio theory of gregarious parasitoids. Researches on Population Ecology, 22: 366-382.

Suzuki, Y., H. Tsuji & M. Sasakawa, 1984. Sex allocation and effects of superparasitism on secondary sex ratios in the gregarious parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Animal Behaviour, 32: 478-484.

Talitzki, V.J., 1961. Parasitic hymenoptera and tachinids, parasites of orchard pests in Moldavia. Trudy Moldav. Nauchno-issled. Inst. Sadov. Vinogr. Vinod., 7: 119-154. (in Russian).

Talitzki, V.J., 1966. Entomophagous insects associated with insect pests in orchards in the Moldavian S.S.R. Trudy Moldav. Nauchno-issled. Inst. Sadov. Vinogr. Vinod., 13: 149-189. (in Russian).

Taylor, T.H.C., 1937. The biological control of an insect in Fiji: an account of the coconut leaf-mining beetle and its parasite complex. The Imperial Institute of Entomology, London.

Taylor, P.D. & M.G. Bulmer, 1980. Local mate competition and the sex ratio. Journal of Theoretical Biology, 86: 409-419.

Uematsu, H., 1981. The ovipositional behaviour in *Euplectrus kuwanae* Crawford (Hym.; Eulophidae), a parasitoid of *Argyrogramma albostriata* (Bremer et Grey) (Lep.; Noctuidae). Applied Entomology and Zoology, 16: 443-450.

Vinson, S.B., 1975. Biochemical coevolution between parasitoids and their hosts. In: P.W. Price (ed.). Evolutionary strategies of parasitic insects and mites. Plenum Press, New York, p. 14-38.

Vinson, S.B., 1976. Host selection by insect parasitoids. Annual Review of Entomology, 21: 109-133.

Waage, J.K., 1979. Foraging for patchily-distributed hosts by the parasitoid *Nemeritis canescens*. Journal of Animal Ecology, 48: 353-371.

Waage, J.K., 1982a. Sib-mating and sex ratio strategies in scelionid wasps. Ecological Entomology, 7: 103-112.

Waage, J.K., 1982b. Sex ratio and population dynamics of natural enemies: some possible interactions. Annals of Applied Biology, 101: 159-164.

Waage, J.K. & Ng. Sook Ming, 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, F., 1839. Monographia Chalciditum I. London.

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.

Weseloh, R.M., 1982. Implications of tree microhabitat preferences of *Compsilura concinnata* (Dipt., Tachinidae) for its effectiveness as a gypsy moth parasitoid. Canadian Entomologist, 114: 617-622.

Wilbert, H., 1965. Die Größenvariabilität von *Aphelinus semiflavus* Howard (Hym., Aphelinidae) und ihre Ursachen. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 72: 670-684.

Wilkes, A., 1965. Sperm transfer and utilization by the arrhenotokous wasp *Dahlbominus fuscipennis* (Zett.) (Hymenoptera: Eulophidae). Canadian Entomologist, 97: 647-657.

Wylie, H.G., 1965. Effects of superparasitism on *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae). Canadian Entomologist, 97: 326-331.

Wylie, H.G., 1967. Some effects of host size on *Nasonia vitripennis* and *Muscidifurax raptor* (Hym: Pteromalidae). Canadian Entomologist, 99: 742-748.

Wylie, H.G., 1970. Oviposition restraint of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) on hosts parasitized by other hymenopterous species. Canadian Entomologist, 102: 886-894.

Wylie, H.G., 1973. Control of egg fertilization by *Nasonia vitripennis* (Hymenoptera: Pteromalidae) on hosts parasitized by other hymenopterous species. Canadian Entomologist, 105: 709-718.

Inleiding en samenvatting

Evolutie is een centraal begrip binnen de biologie. Het houdt de veronderstelling in dat soorten in de loop van de tijd kunnen veranderen en dat door deze geleidelijke verandering nieuwe soorten kunnen ontstaan. De drijvende kracht hierachter is de natuurlijke selectie, die uit de door het toeval ontstane natuurlijke variatie binnen een soort gemiddeld genomen steeds die individuen laat overleven en voortplanten, welke het best zijn aangepast aan de omstandigheden waarin zij leven. Hierbij is uiteindelijk het zich voortplanten van een individu beslissend voor zijn bijdrage aan de evolutie van de soort; immers overleven alleen is niet voldoende om erfelijke eigenschappen aan de volgende generatie door te geven. Natuurlijke selectie beïnvloedt dus de samenstelling van de volgende generaties, doordat zij verschillen veroorzaakt in het voortplantingssucces van soortgenoten. Wanneer de levensomstandigheden steeds gelijk blijven, is de verwachting dat het evolutieproces uiteindelijk leidt tot individuen waarvan het voortplantingssucces niet kan worden verbeterd. Het voortplantingssucces is dan maximaal, en het resultaat van een organisme, dat wat betreft het produceren van nakomelingen optimaal aan de omgeving is aangepast.

Nu betekent dit niet dat van een dier alle eigenschappen van de bouw, het inwendig functioneren en het gedrag perfect geoptimaliseerd zijn. Sommige eigenschappen staan onder een minder zware selectiedruk, doordat ze weinig of geen invloed hebben op het voortplantingssucces. Andere eigenschappen zijn erfenissen uit het verleden van de soort, bijvoorbeeld aanpassingen aan omstandigheden in het verleden. Als zo'n eigenschap weinig variabel is, zijn er geen alternatieven voorhanden waar de natuurlijke selectie op kan aangrijpen. Wanneer men het gedrag van een dier wil leren begrijpen door aan te nemen dat dit het aantal nakomelingen maximaliseert, en tevens de bovengenoemde bedenkingen wil omzeilen, heeft

men de meeste kans op resultaat bij gedrag dat 1) duidelijk in verband staat met het produceren van nakomelingen en 2) bestaat uit een aantal praktische uitvoerbare alternatieven waaruit het dier ogenschijnlijk kan kiezen.

Vaak beslaat het voortplantingsgedrag, zelfs in ruimere zin (het zoeken van een partner, nestbouw, balts, paring, het leggen van eieren of werpen van jongen, broedzorg) slechts een klein deel van het totale leven van een dier. Het zoeken van voedsel, inclusief dat voor de jongen, vergt veel meer tijd en inspanning.

In studies die het voedselzoekgedrag tot onderwerp hebben wordt, impliciet of expliciet, aangenomen dat het voortplantingssucces wordt bepaald door de mate waarin het dier zijn netto energie-opname weet te maximaliseren. Er zijn goede argumenten aan te voeren voor deze aanname, en uit een aantal studies is gebleken dat dieren inderdaad hun voedselzoekgedrag in deze zin optimaliseren.

Bij sluipwespen bestaat er echter een zeer direct verband tussen het gedrag van de volwassen wesp en het aantal nakomelingen dat een wespevrouwtje weet te realiseren; zozeer zelfs dat dit gedrag evengoed voortplantingsgedrag als voedselzoekgedrag genoemd kan worden. Dit is een belangrijke reden om sluipwespen als object te kiezen bij de studie naar optimalisatie van gedrag. Een andere reden om aandacht te schenken aan het gedrag van sluipwespen is de betekenis die zij kunnen hebben bij de biologische bestrijding van insektenplagen. Doordat sluipwespen parasiteren op andere insekten en hun gastheer tenslotte doden kunnen zij in sommige gevallen het plaaginsekt op een aantalsniveau brengen of houden, dat onder de economische schadedrempel ligt. Fundamenteel oecologisch onderzoek kan bijdragen aan het oplossen van de vraag waardoor de bestrijding van plaaginsekten met behulp van sluipwespen soms wel en soms geen succes heeft.

In dit proefschrift worden de resultaten van een onderzoek beschreven aan het gedrag van de sluipwesp *Colpoclypeus florus*. Na een inleiding over de zojuist behandelde onderwerpen (Hoofdstuk 1) volgt een beschijving van de sluipwesp en zijn in Nederland belangrijkste gastheer (Hoofdstuk 2). Ook worden de kweekmethoden en de omstandigheden, waaronder de experimenten zijn uitgevoerd, beschreven.

Colpoclypeus florus is een wespje van 2 mm lang, dat parasiteert op rupsen van een bepaalde vlinderfamilie, de bladrollers. Het komt in Europa zeer verspreid voor en is zeldzaam in natuurlijke en semi-natuurlijke gebieden. Vaak en in grote aantallen wordt het echter gevonden in intensief gecultiveerde omgevingen, zoals hazelnotenplantages of aardbeienvelden in Italië, sinaasappelboomgaarden in Spanje, wijngaarden in de Oekraïne of appelboomgaarden in Duitsland. In Nederland wordt *Colpoclypeus florus* vooral aangetroffen in appelboomgaarden, wanneer daar een aantasting plaats vindt door de vruchtbladroller (*Adoxophyes orana*).

De rups van dit kleine vlindertje leeft in een zelfgemaakt spinsel op het appelblad waarvan het vreet. Het vertoont vijf, in grootte toenemende vervellingsstadia voordat verpopping plaats vindt. Het wespevrouwtje zoekt rupsen van de grotere stadia (1-2 cm lang) op en steekt ze aan met haar legboor. Als gevolg hiervan wordt de rups enkele uren apathisch, waardoor het wespje vrij in het spinsel kan rondlopen. Zij legt nu vrij snel achter elkaar tien tot twintig eieren rond de rups in het spinsel en vertrekt. De totale duur van dit parasiteringsgedrag bedraagt bij één gastheer ruim een dag. In vergelijking met andere sluipwespesoorten is dit erg lang. De rups herstelt na het vertrek van de wesp snel en begint zelfs weer te eten. Na enkele dagen komen echter de wespeeieren uit en de larfjes zuigen zich aan de buitenkant van de rups vast. Ze beginnen nu de rups leeg te zuigen en deze sterft dientengevolge.

Wanneer de rups vrijwel is leeggezogen verpoppen de larven zich naast hun gastheer. Een week nadien komen de volwassen wespen uit de pophuid en banen zich een weg door het spinsel naar buiten. De totale ontwikkeling van ei tot volwassen wesp duurt nog geen drie weken. De levensduur van een vrouwelijke wesp is ongeveer twee weken.

Door de extreem lange duur van het parasiteringsgedrag rees het vermoeden dat een *C. florus* vrouwtje in haar leven maar weinig gastheren kan parasiteren. Het leek zinvol om het onderzoek te richten op de vraag of de weinige gastheren die geparasiteerd worden optimaal worden uitgebuit. De investering van een flink aantal eieren en een aanzienlijk deel van de totaal beschikbare tijd moet immers optimaal renderen. Drie stadia in het parasiteringsgedrag werden onderzocht en hierbij werden de volgende vragen gesteld:

1) Moet het vrouwtje elke gastheer die ze vindt accepteren, of moet ze bijvoorbeeld de kleine gastheren verwerpen?
2) Hoeveel eieren moet ze leggen bij een gastheer van een bepaalde grootte?
3) Hoeveel zonen en hoeveel dochters moet ze laten opgroeien op één gastheer?

Bij deze laatste vraag is het van belang te weten dat bij sluipwespen, evenals bij een aantal andere groepen insekten, de bevruchte eieren vrouwtjes en de onbevruchte eieren mannetjes produceren. De vrouwtjes van vele sluipwespesoorten blijken het vermogen te bezitten de bevruchting van eieren te regelen nadat ze éénmaal door een mannetje zijn geinsemineerd. Hierdoor blijkt ook dat het produceren van veel dochters, mits hun kans op inseminatie ook groot is, de voorkeur verdient. Zij produceren immers het nageslacht.

De experimenten vonden plaats in een klimaatkamer bij 21°C, en voor een deel in een laagstam-boomgaard bij Lienden in de Betuwe.

Door de gemiddelde legselgrootte in het veld te vergelijken met het totaal aantal eieren dat een vrouwtje tijdens haar leven kan produceren werd aangetoond dat er twee à drie gastheren door een vrouwtje kunnnen worden geparasiteerd (Hoofdstuk 3). In het laboratorium werden alleen rupsen van het eerste, kleinste, stadium van de gastheer niet geaccepteerd. Rupsen van het tweede stadium werden in een kwart en die van het derde stadium werden in de helft van de gevallen geaccepteerd, duidelijk minder dan rupsen van de twee grootste stadia (elk voor ongeveer 85%). De legselgrootte, de overleving van ei tot volwassen wesp, en het percentage vrouwelijke nakomelingen bleken toe te nemen met toenemende gastheergrootte. Het gevolg is dat rupsen van de twee grootste stadia veel meer vrouwelijke nakomelingen opleveren dan die van kleine stadia, zodat de vraag gesteld kan worden of het voor de wesp niet voordeliger is rupsen van het tweede en derde stadium van de gastheer altijd te verwerpen. Het verwerpen van ongeschikte gastheren levert een tijdsbesparing op, want de beslissing accepteren dan wel verwerpen bleek door de wesp binnen anderhalf uur na aankomst bij de rups te worden genomen. Toen bovendien bleek dat in het veld bijna uitsluitend rupsen van het vierde en vijfde stadia worden geparasiteerd was de verwachting dat andere factoren naast de acceptatie van gastheren het uiteindelijke resultaat beïnvloeden. Dit werd in het veld onderzocht (Hoofdstuk 4).

Eerst werd aangetoond dat de accepteringspercentages van rupsen in de verschillende stadia in het veld niet verschillen van die in het laboratorium. Vervolgens werd door een gedetailleerde bemonstering van een aantal appelbomen aangetoond dat de grootste rupsen, in tegenstelling tot de andere, zich hoofdzakelijk bevinden op het jonge blad aan de periferie van de boomkroon. Hier bleken ook, van elk stadium, relatief de meeste geparasiteerde rupsen voor te komen. In een volgend experiment werd onderzocht of dit misschien betekent dat *C. florus* bij het zoeken naar gastheren een

voorkeur heeft voor die plaatsen in de boom waar normaliter de grootste gastheren zitten, onafhankelijk van de aanwezigheid van die gastheren. Grote en kleine rupsen werden aangebracht in een boomkroon, op zowel het jonge blad als op het oude blad. Daarna werden wespen losgelaten. Rupsen, ongeacht het stadium,bleken op het jonge blad een grotere kans op parasitering te lopen dan op het oude. De wespen zoeken dus meer op jong blad. Bovendien bleken grote rupsen, ongeacht de plaats in de boom, een grotere parasiteringskans te bezitten dan kleine rupsen. Blijkbaar vallen ze de wesp meer op. Het gecombineerde effect van de rupsverdeling over de boomkroon, de grotere opvallendheid van grote rupsen en het zoekgedrag van de wesp bleek precies het verschil te kunnen verklaren tussen de accepteringspercentages in het laboratorium en de in het veld in werkelijkheid geparasiteerde aantallen rupsen.

In Hoofdstuk 5 wordt ingegaan op de vraag of de legselgrootte door de wesp zo wordt gekozen dat er sprake is van maximalisatie van het aantal (vrouwelijke) nakomelingen. Het is opvallend, dat er een rechtlijnig positief verband bestaat tussen de legselgrootte en de lengte van de rups. Wanneer echter kunstmatig ingekorte rupsen werden aangeboden week de legselgrootte niet af van die in een controlegroep. De wesp bleek wel beïnvloedbaar in het aantal eieren dat ze legde, wannneer de breedte van de rups werd gevarieerd. Nu is de breedte van een rups niet een erg nauwkeurige manier om te meten hoeveel voedsel een gastheer het nakomelingschap van de wesp kan bieden. Door deze tekortkoming worden bij de kleinste rupsen te veel en bij de grootste rupsen te weinig eieren gelegd, wat misschien mede de extra sterfte in deze legsels verklaart. De meeste geparasiteerde gastheren behoren echter tot een middengroep, waar de sterfte, hoewel aanzienlijk (50-60%), het laagst is.

Doordat *C. florus* in tegenstelling tot de meeste andere sluipwespen de eieren los naast de gastheer deponeert,

bestaat de mogelijkheid voor de onderzoeker om eieren weg te halen bij, of toe te voegen aan het legsel. In een aantal laboratoriumexperimenten werd op deze wijze de verhouding legselgrootte/gastheergewicht, en daarmee de beschikbare hoeveelheid voedsel voor elke nakomeling, gevarieerd bij gastheren van het vierde stadium. Het bleek dat een legsel twee keer zo klein of twee keer zo groot kon worden gemaakt zonder dat dit de overlevingskansen noemenswaardig beïnvloedde. Wel werd hierdoor het lichaamsgewicht van de nakomelingen sterk beïnvloed. Werden er eieren aan het legsel toegevoegd, dan werden de nakomelingen kleiner, en werden er eieren weggehaald dan werden de nakomelingen groter. Uit waarnemingen aan wespen met verschillend lichaamsgewicht bleek dat bij wespevrouwtjes zowel de levensduur als het aantal eieren dat gelegd kan worden toeneemt met het lichaamsgewicht. Als we naar de afzonderlijke nakomelingen kijken geldt dus "hoe zwaarder hoe beter". Voor ouders telt echter niet het voortplantingssucces van de afzonderlijke nakomelingen maar dat van het totale nakomelingschap. Door berekeningen kon worden aangetoond dat relatief kleine legsels per gastheergewicht gunstig zijn in een situatie waarin het voortplantingssucces wordt bepaald door het beschikbare aantal eieren, bijvoorbeeld wanneer er een overvloed aan gastheren is. Grote legsels per gastheergewicht zijn gunstig wanneer het voorplantingssucces wordt bepaald door de beschikbare tijd om gastheren te zoeken en te parasiteren, bijvoorbeeld wanneer gastheren schaars zijn. In het laatste geval heeft elke nakomeling welliswaar een korte levensduur, maar dit wordt door de ouders gecompenseerd met een groter aantal nakomelingen.

Als de in werkelijkheid geproduceerde legselgrootte een aanpassing is aan een bepaalde gastheerdichtheid, is deze dichtheid gelegen tussen de twee zojuist genoemde uitersten. Van deze gastheerdichtheid werd tenslotte een schatting gemaakt, door aan te nemen dat in deze situatie een vrouwtje juist genoeg tijd heeft om haar eieren kwijt te raken.

In Hoofdstuk 6 werd allereerst de vraag gesteld welke getalsverhouding tussen de geslachten in het nakomelingschap als optimaal moet worden beschouwd. Wanneer, zoals bij *C. florus* het geval is, groepjes nakomelingen gezamenlijk opgroeien en er vrijwel uitsluitend paringen plaats vinden tussen broers en zusters, dan is het onvoordelig om broers met elkaar te laten concurreren tijdens deze paringen. Daarom werd geprobeerd om de geslachtsverhouding te begrijpen als resultaat van een mechanisme dat, met zo weinig mogelijk mannetjes, het aantal geïnsemineerde dochters maximaliseert.

Eerst werd de tijd gemeten die beschikbaar is voor paringen tussen broers en zusters. Deze tijd, gelegen tussen het uitkomen van de volwassen wespen en het verlaten van het gastheerspinsel, werd vervolgens toegepast in een experiment. Mannetjes van verschillend gewicht werden elk gedurende een dergelijke tijd samengebracht met een groot aantal maagdelijke vrouwtjes. Naderhand werd door sectie bepaald hoeveel vrouwtjes waren geïnsemineerd. De inseminatiecapaciteit van de mannetjes bleek afhankelijk te zijn van het lichaamsgewicht. Mannetjes van gemiddeld gewicht bleken juist het aantal vrouwtjes dat voorkomt in een groot legsel te kunnen insemineren. Dit resultaat toont aan dat elk legsel niet minder dan één mannetje van gemiddeld gewicht moet voortbrengen; een groter aantal of een hoger gewicht is overbodig en zal, in verband met de beperkte voedselhoeveelheid, het voortplantingssucces van de vrouwtjes schaden.

De hoge sterfte die optreedt tussen eileg en het uitkomen van de volwassen wespen maakt het noodzakelijk dat niet volstaan kan worden met het onbevrucht laten van slechts één ei. Enkele onbevruchte eieren zijn nodig om het verlies van een mannetje door sterfte te compenseren. Ook wordt het noodzakelijk aantal onbevruchte eieren hoger, wanneer elk ei dezelfde kans heeft op bevruchting. Dit laatste bleek echter bij *C. florus* niet het geval te zijn. Door van eieren met

behulp van een kleuringstechniek op de chromosomen het geslacht te bepalen, kon van een groot aantal legsels de volgorde van bevruchte en onbevruchte eieren worden bepaald. De onbevruchte eieren bleken aan het eind van een legcyclus geproduceerd te worden. Blijkbaar vindt in het vrouwtje tijdens het leggen een omschakeling plaats van bevruchte naar onbevruchte eieren. Dit maakt het waarschijnlijk dat het aantal onbevruchte eieren minder aan het toeval onderhevig is, dan wanneer op elk moment in de volgorde een onbevrucht ei kan worden geproduceerd.

Van elke legselgrootte werd vervolgens berekend welk aantal onbevruchte eieren in een legsel een maximaal aantal geïnsemineerde vrouwtjes tot gevolg zou hebben. Hierbij werd rekening gehouden met de sterfte, behorend bij elke legselgrootte. Na vergelijking met de werkelijke geslachtsverhoudingen die optreden in de verschillende legselgroottes bleek, dat welliswaar de geslachtsverhoudingen door deze berekening niet exact werden voorspeld, maar het uiteindelijk resultaat uitgedrukt in aantal geïnsemineerde vrouwtjes lag in alle gevallen binnen 8% van de maximaal mogelijke waarde.

In Hoofdstuk 7 worden de belangrijkste conclusies gegeven en besproken. Het onderzoek geeft inzicht in de manier waarop een sluipwesp de verschillende moeilijkheden bij het bereiken van een optimaal voortplantingsgedrag kan oplossen. Bij elke stap van het parasiteringsproces zijn beperkingen van het dier aan te wijzen. Toch is het gedrag door ons als een maximalisatie van het voortplantingssucces te begrijpen. Hierbij waren de gegevens uit het verrichte veldonderzoek onontbeerlijk.

Curriculum vitae

Lieuwe Jelte Dijkstra werd geboren op 27 oktober 1953 te Harlingen. Vanaf 1966 bezocht hij de Rijksscholengemeenschap te Zwolle, waar hij in 1971 het examen HBS-B aflegde. Vanaf 1971 studeerde hij biologie aan de Rijksuniversiteit te Groningen. Na het kandidaatsexamen in 1975 werden de volgende doctoraalvakken bewerkt:

- dieroecologie, voedseloecologie van de rotgans
- plantensystematiek, een systematisch onderzoek aan een mossengeslacht
- vakdidaktiek.

Het doctoraalexamen werd afgelegd in 1978 (cum laude).

Van 1979 tot en met 1982 was hij als wetenschappelijk assistent verbonden aan de vakgroep Dieroecologie van de Landbouwhogeschool te Wageningen, waar het onderzoek werd verricht dat resulteerde in dit proefschrift. Sinds 1984 werkt hij als vegetatiekundige bij de Stichting IJsselakademie te Kampen.