CLUTCH SIZE IN INSECTS

by

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Abstract

The aim of this thesis is to draw together the work on the reproductive ecology of insect clutch size which began in the mid-1980's and particularly to contribute to the body of experimental tests of theoretical models on clutch size using a variety of techniques and approaches.

In Chapter 1, I review the development of insect clutch size theory and the concept of gregarious egg-laying as an adaptive strategy. There is enormous diversity in behaviour among insects although studies have concentrated mainly on the Lepidoptera and Hymenoptera, despite the widespread occurrence of brood-laying throughout the insect class.

In Chapter 2, I study clutch size in an ectoparasitoid wasp. By establishing the relationship between clutch size and offspring size (using artificial manipulation of broods) and offspring size and fecundity I calculated the optimal clutch size which should maximise the total fitness returns from the brood. This estimated optimum proves to be far in excess of the observed mean and reasons for this are discussed.

Chapter 3 involves field work on a leaf-mining fly, *Pegomya nigritarsis*, and its parasitoids. Evidence is presented that females tend to lay the clutch size that maximises brood fitness (i.e. live unparasitised larvae). However the variation in clutch sizes and in parasitism was high. I also looked at the frequency distributions of parasitoids in host larvae. Superparasitism occurs more frequently than expected by random host encounters and appears to result from an adaptive strategy of maximising rate of encounter with hosts rather than time-consuming discrimination.

Finally in Chapter 4, I made a comparative study of reproductive behaviour in the hymenopteran sub-family the Bethylidae. In particular I looked at how the clutch size decision itself affects sex ratio. A general trend was found across the Bethylidae such that as mean clutch size increases, the proportion of males decreases.

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CHAPTER 1

CLUTCH SIZE IN INSECTS - A REVIEW OF THE LITERATURE

Introduction

The production of offspring in discrete batches is a widespread component of animal life histories. Since the number of individuals in a brood will often have direct bearings on a parent's fitness returns there is obvious potential for selection on decisions involving progeny allocation. Interest in brood allocation dates back to questions concerning clutch size in birds (Lack 1947), and litter size in mammalian broods (Lack 1948, Mountford 1968). However it is only recently that serious attention has focussed on invertebrates, and insects in particular, starting with the work of Charnov and Skinner (1984, 1985) and Parker and Courtney (1984). Subsequently there has been a substantial growth of work in this area and the potential for the optimality approach of demonstrating selection on insect oviposition decisions is now well recognised (Endler 1986).

The nature of brood production varies enormously among the insects from the long tendril-like strings of some geometrid moth eggs (Chew and Robbins 1984) to gregarious endoparasitic egg-laying in lepidopteran pupae by a pteromalid wasp (Takagi 1985). (In this review I shall mainly be concerned with clutches produced on or near some host resource such as butterflies on host plants, flies on fruit and leaf-miners on leaves.) Likewise a range of possible benefits which might favour brood-laying behaviour has been proposed, from practical observations on how it might increase fitness, to dynamic programming models which incorporate egg-laying as one component of an individual's survival strategy.

Clutch size theory is one of the oldest areas of ecology and is immensely suitable for applying behavioural ecological techniques. In recent years insect clutch size has become an exciting area of research where four areas of evolutionary

ecological theory have been combined and applied to study behavioural decisions; game theory, foraging theory, sex allocation theory and life-history theory (Charnov and Skinner 1985).

Godfray (1987a) has identified two main categories of theoretical tests of clutch size theory; mathematical optimality models of clutch size in different circumstances and comparative studies of behavioural patterns within and between related species.

Practical studies of clutch size must centre on quantifying the relationship between clutch size and individual offspring fitness in specific insect sytems (Godfray 1987a). Hence practical tests provide less general predictions about oviposition strategies than the theoretical approach. Practical tests are generally observational or manipulative. Observational tests utilise natural variation in clutch sizes on standard resource patches and the effect of this on individual fitness. However the observer can never guarantee to assess a resource as the insect would so the standardisation of a resource patch is not wholly reliable. Experimental tests manipulate the number of eggs on a given resource to see the effect of exceeding or falling short of the normal clutch size being laid. This may not only be technically difficult but it is unlikely to inform us about the selection pressures leading to the evolution of the clutch size, but only the selection pressures maintaining it (Chew and Robbins 1984). Also Reznick (1985) argues that such experiments are tests of phenotypic plasticity in adapted individuals rather than of costs of being genetically maladaptive. He argues that empirical studies of cost of reproduction must deal with genetically based trade-offs. However manipulative studies do serve to indicate the penalties of making a bad decision and can quantify the optimal utilisation of a patch by allocation of the correct number of individuals.

The evolution of clutch size in invertebrates has been reviewed by Godfray (1987a) who proceeds from a simple clutch size situation through progressively more complex situations as specific simplifying assumptions about insect behaviour are relaxed. While reference may be made to Godfray (1987a), I shall endeavour to approach this review from a slightly different perspective. In this review I shall introduce insect clutch size theory and show how it has emerged from avian models and subsequently been developed in accordance with the variety of reproductive behaviour of insects. The main advantages of batch laying as opposed to solitary egg-laying shall also be presented before proceeding with more specific work on insect orders. Much of the quantitative analysis of insect oviposition has been focussed on the parasitic Hymenoptera here for reasons which shall be discussed. The work on clutch size in the Lepidoptera and the Bruchidae shall also be described. Finally clutch size shall be considered as a determining factor in itself and its effect on egg-size, offspring size, sex ratio and other components of fitness shall be reviewed.

Clutch Size in Birds

In 1947 in a general comparative review, "The Significance of Clutch Size", David Lack briefly considered what the constraint on the upper limit of brood size might be, and how clutch size could be a heritable adaptive trait. He argued that natural selection should act on birds to produce the clutch that maximises the number of surviving young which the parents can successfully raise. Thus he envisaged that birds should aim to produce as many offspring in a brood as possible but parents exceeding the optimum would have lower reproductive success and would be selected against in the course of time.

Although continuing to look at reproductive effort in birds (Lack 1966), and mammals (1948), Lack never developed a full model to justify his hypothesis. Possibly by virtue of its intuitive appeal it was not fully tested until Charnov and Krebs (1974) recognised its suitability for quantitative behavioural ecological analysis. They showed that an incremental decline in individual offspring fitness as a function of increasing clutch size would result in an optimum which yielded maximum brood fitness for the parent (see Figure 1.1.ii). However, recognising the cost of reproduction (Williams 1966, Smith and Fretwell 1974, Pianka and Parker 1975), they went on to show that if there is an increase in parental mortality associated with maximising the brood fitness then the optimum will shift to the left and clutches smaller than those predicted by Lack are predicted. At the same time Perrins and Moss (1975) were finding experimental confirmation of this in Great Tits, by manipulating brood sizes and studying the resulting individual and brood fitness. They found that clutches were consistently lower than those that would be expected to maximise the fitness of the brood.

There has been much subsequent work on birds relating clutch size to aspects of bird reproductive behaviour including manipulative studies testing degree of parental care and lifetime fitness trade-offs (e.g. Askenmo 1979, Nur 1984, Lessells 1986). However the ground base of insect clutch size theory rests on Lack's hypothesis of the maximisation of brood fitness and the recognition that trade-offs with other life history components will lead to smaller broods than predicted by Lack.

Insect Clutch Size Theory

A range of benefits associated with the laying of eggs in discrete batches has been postulated (see Table 1.1). Nonetheless insect broods are generally characterised by having an upper limit on clutch size whereby some aspect of the ovipositing parent's fitness is constrained (see Table 1.2).

The Relationship Between *Per Capita* Fitness and Brood Fitness.

Brood fitness is taken to be the sum of the individual fitness of members of the brood. Consequently the relationship between clutch size and *per capita* fitness is crucial to the brood fitness profile when one is considering how fitness returns per clutch could be maximised (see Figure 1.1). If individual fitness remains constant as clutch size increases (Figure 1.1.i) then the parent's fitness does not diminish with increasing clutch size and theoretically there is no reason why the parent should not lay all its eggs in one clutch (Godfray 1987a). More commonly we'd expect *per capita* fitness to change in some way with increasing clutch size since developing individuals in a brood may well compete within the discrete resource patch with which they are associated. Probably the easiest *per capita* effect to measure is probability of juvenile survival as in birds (Perrins and Moss 1975). However the situation is usually complicated in insects by the relationship between adult size and fecundity. Hence the *per capita* effect will include change in offspring fecundity associated with the intensity of larval competition for resources. The change in fitness with increasing clutch size may be a monotonic decline (Weiss *et al.* 1983) or domed (Godfray 1986a) with a *per capita* maximum greater than one (see Figures 1.1.ii, 1.1.ii).

Regardless of the degree of parental investment at any single instance it should always hold that a female is seeking to maximise her lifetime fitness. In the simplest

possible situation where a female lays a single clutch in her lifetime, or where there are no trade-offs because fitness is limited wholly by the opportunity to lay clutches we can envisage that a female will maximise her lifetime progeny production by maximising brood fitness (Godfray 1987a). Charnov and Skinner (1984, 1985) applied Lack's hypothesis to this situation for insects, particularly parasitoids, so that as *per capita* fitness declines, a "Lack solution" clutch size is predicted (Figure 1.1). Natural selection will favour those females which can most accurately assess each host and lay the appropriate Lack solution for that resource.

Why Doesn't the Female Maximise Brood Fitness?

Charnov and Skinner (1984, 1985) tested their model for maximising brood fitness against experimental evidence with an egg parasitoid and a pupal parasitoid. However they observed that clutch sizes were slightly lower than their predicted Lack solution and proposed three possible reasons for this;

i.) A trade-off between female reproductive effort and survival leading to underinvestment in the interest of surviving for further oviposition (similar to Charnov and Krebs (1974)).

ii.) There is only partial recognition of the fitness benefit gained by offspring from smaller broods.

iii.) It may be misleading to regard maximising brood fitness as maximising a female's fitness. For example maximising brood fitness may not be the most productive strategy if this takes time and chances to oviposit elsewhere are plentiful.

Maximising Rate of Gain of Fitness.

Parker and Courtney (1984) describe the time considerations of an ovipositing female as having a clutch size independent component (including site location and preparation time), and a component that is a function of clutch size (e.g. time taken to lay eggs.) Charnov and Skinner (1984) and Parker and Courtney (1984) applied Charnov's (1976) marginal value theorem for optimal foraging to show that optimum brood size can be related to the independent component; travel time between sites (see also Skinner 1985). As a female exploits a patch her rate of gain of fitness will decline. She should remain on that patch until her rate of gain reduces to the environmental average. Thus under certain conditions it may pay to lay less than the Lack clutch size in order to maximise rate of gain of fitness (see Figure 1.2).

When time is the limiting factor, an animal should maximise its rate of gain of fitness by optimising the balance between time spent foraging and time spent ovipositing on a patch. When it is limited by opportunities to oviposit (when search time is large) the animal should remain with the patch and maximise brood fitness (Charnov and Krebs 1974, Parker and Courtney 1984). Experimental support is found in *Apantales glomeratus* (an endoparasitoid wasp) where clutch size decreases with increasing host encounter rate (Ikawa and Suzuki 1982) and similar effects are recorded in tephritid flies (Roitberg and Prokopy 1982). Pilson and Rausher (1988) showed that *Battus philenor* (a swallowtail butterfly) lays larger clutches as time since last oviposition increases.

Since the initial work by Charnov and Skinner (1984) and Parker and Courtney (1984), Gain Rate models have been applied to other aspects of clutch size such as egg production rate (Charnov and Skinner 1988, Charnov and Stephens 1988), and superparasitism (Charnov and Skinner 1984).

A Compromise Between Brood Fitness and Parental Mortality.

Charnov and Krebs (1974) showed that there may be a trade-off between current reproductive effort in the interests of survival and future reproduction and this trade-off is equally valid for some insects. Such a trade-off may be a physiological or energetic effect or may reflect risks of mortality associated with the act of oviposition (Charnov and Skinner 1985).

The physiological trade-off could occur if egg production rate is negatively correlated to female survival rate and Charnov and Skinner (1985) model this trade-off. Certainly in birds risk of mortality is argued to be the prime constraint on clutch size (Nur 1984) but in insects where parental investment is less common and not proportional to brood size this is less likely (Godfray 1987a).

The brood fitness may not be maximised if the mortality risk associated with oviposition is greater than that of searching (Weiss *et al.* 1983, Iwasa *et al.* 1984).

Maximising Egg Fitness - When Eggs Are Limiting.

In the trade-offs described so far, females have been limited primarily by their opportunities to oviposit on a patch. However under certain conditions the main constraint may be the availability of eggs.

In provigenic species, circumstances may arise where the limitation on egg supply becomes more important than the limitation on opportunities to produce clutches. Alternatively, even in synovigenic species, a female's egg production rate may limit egg availability and hence limit her fitness more than the opportunity to lay clutches.

Whenever a female's eggs are severely limited she should produce clutches that maximise egg fitness rather than brood fitness. i.e. the clutch size at the peak of the *per capita* profile. When the relationship is a monotonic decline individual fitness is maximised at a clutch size of one. In domed relationships the optimum will be the clutch size at the peak of the curve (Godfray 1987a). In reality there is more likely to be a compromise between maximising egg fitness and maximising brood fitness.

The situation for provigenic species with a constant inter-clutch mortality rate has been analysed by Weiss *et al.* (1983), Parker and Courtney (1984), Iwasa *et al.* (1984) and Waage and Godfray (1985). When the egg complement is large or when the risk of mortality between clutches is great then Lack's solution should be laid. However as the risk of running out of eggs before dying increases then the clutch size should decrease until, ultimately, individual egg fitness is maximised. It should be noted that this is not a linear decline and only when eggs are severely limiting should clutches be significantly lower than the Lack solution.

These models have been refined using constrained optimisation techniques (Parker and Courtney 1984, Begon and Parker 1986) and dynamic optimisation (Iwasa *et al.* 1984) to show that optimal clutch size decreases during the individual's lifetime as the risk of exhausting egg supply increases.

Is the Parent Capable of Maximising Brood Fitness?

It has been assumed up until now that females are capable of producing the Lack solution clutch size and their decision not to do so is a deliberate life history strategy. However there are several ways in which females may be incapable of achieving this.

Limited Fecundity.

Under certain conditions, we have seen that limited egg supply may favour smaller clutches than the Lack solution. However in situations where the Lack solution is large compared with the egg load carried by the parent (Godfray 1987a) the parent should dump the eggs she has or wait until her full complement is ready. Parker and Courtney (1984) also predict that females should lay their entire load on one spot (if the risk of predation is high and the egg load is less than the brood size that would maximise brood fitness.)

In some provigenic species there are records of females depositing their entire load in a single brood (e.g. Ito and Miyashita 1968, Morris 1972). Synovigenic species may be selected to deposit all available eggs and resume host searching especially if hosts are rare (Skinner 1985, Charnov and Skinner 1988). Some species are born with a batch of eggs which they dump and then mature more eggs through their life, leading to a large first clutch but subsequent small clutches (Labine 1968).

Physical Constraints on Clutch Size.

The female may be limited in the clutch size she can produce simply by physical oviposition factors such as in those insects who provide a protective egg case or jelly mass for their young (e.g. cockroaches, mantids and orthopterans (Hinton 1981)). Thus the number of eggs laid is constrained by the physical size of the protective resource.

Similarly certain oviposition sites have been recorded as placing a limit on the number of eggs which can be laid. For example cryptic concealment of eggs in cracks may physically limit the number of eggs which can be laid (Courtney 1987, Stamp 1980).

Stochasticity.

Clutch size models have generally been deterministic. That is they generally assume that individuals are selected to lay exactly the right clutch size on a resource and ignore individual variation and environmental stochasticity.

For example it is often assumed that individuals lay a precisely accurate clutch size. In fact it may be that while individuals lay the right clutch size on average there is variation around this mean (Godfray and Ives 1988). Individual variation may also occur as different individuals in a population pursue alternative strategies (Parker 1974) or as their priorities for maximising their lifetime fitness at a given time change (Iwasa *et al.* 1984, Mangel 1989) or as lifetime trade-offs are incorporated.

Environmental variation in patch quality will also occur. And while females can sometimes incorporate this in their clutch size decision (Klomp and Teerink 1962) some variation in clutch size may result.

Godfray and Ives (1988) examined the effect of incorporating stochasticity into simple invertebrate clutch size models and showed that inaccurate, simplistic models may underestimate or overestimate the clutch size an insect should lay. If stochastic variation above the optimum clutch size is heavily penalised then selection should favour a clutch size smaller than that originally predicted. Likewise, where the penalties for exceeding the original optimum are small, then models incorporating stochastic variation will predict clutches larger than originally expected. (On the brood fitness profile; a negative second derivative (i.e. deceleration) at the previously determined optimum indicates smaller clutch sizes than expected, while a positive second derivative indicates larger clutches.) This is supported by other work on behaviour affecting fitness (Real 1980, Rubenstein 1982).

Parent-Offspring Conflict.

Parent-offspring conflict occurs where the optimum solution for parental fitness differs from the optimum for larval fitness (Trivers 1974, Dawkins 1976). When individual fitness declines with increasing clutch size then this conflict of interests will occur. Generally larvae have no influence over their predicament but in some species larvae can kill some or all of their siblings and the parent's control over clutch size is impaired (Godfray 1987b).

Parker and Mock (1987) developed a model (for sexual diploids) to show how the optimum for the mother may differ to that for individual offspring. The degree of conflict (disparity of the two optima) increases with multipaternity and as the rate of initial decline in offspring fitness increases. The ESS depends on the direct costs and benefits of siblicide and on the nature of the indirect fitness penalties of loss of siblings.

Macnair and Parker (1979) have modelled a situation where asymmetric competition between larvae can evolve so that resources are not evenly distributed between offspring. They show that the occurrence of such a gene can be evolutionarily stable when the costs are evenly distributed among the brood. They also show that this type of parent-offspring conflict will be increased by multipaternity of broods. Hence in this situation a mother may not be able to maximise her brood fitness if the fitness gain of the soliciting larva is less than the loss in fitness incurred by the rest of the brood.

Optimal Oviposition When There is More Than One Female.

The majority of optimal oviposition models consider the optimal strategy of females in isolation rather than as members in a population. In fact it is probably more likely

that their strategy will depend on the presence and strategies of other females around them. We can expect their behaviour should subsequently be modified to incorporate the effect of other females ovipositing on the same patch either before or after them. Several different approaches have been adopted to understand this problem.

Fitness Gain Rate - Whether or Not to Superoviposit?

The perspective of a female encountering a previously oviposited patch - whether to superoviposit or not - has been treated as a fitness gain rate decision (Charnov and Skinner 1984, 1985, Skinner 1985, Smith and Lessells 1985). Generally a previously oviposited patch is inferior in quality to an unoviposited patch and superoviposition will not be selected if it lowers the fitness gain rate below the environmental average. However as the value of oviposition sites increases (e.g. if fresh patches are rare, or search time costs are high) then it may pay a female to superoviposit (Strand and Godfray 1989, van Alphen and Visser 1990).

Ikawa and Suzuki (1982) record innate reluctance to superparasitise in a wasp. Superparasitism has been shown to increase as unparasitised hosts become rare (Salt 1961, Jackson 1966, Glas et al. 1981, Ikawa and Suzuki 1982, Waage and Ng 1984). An interesting case of discrimination occurs where newly-hatched individuals will superoviposit on the first host presented to them. However if they subsequently encounter unattacked patches they become more selective over superoviposition in the future (Lenteren and Bakker 1975, Lenteren 1976, Klomp et al. 1980).

Fitness Gain Rate - How Many Eggs Should a Superovipositor Lay?

Several models have been devised to consider the quantitative clutch size decision of a female deciding to superoviposit. Parker and Courtney (1984) predicted that the clutch size of the first female on a patch should always be larger than that of the

second. (Skinner's (1985) model showed that theoretically it is possible for the second brood to be larger than the first if the environmental fitness gain rate changes. However for normal conditions he agreed that the second clutch should be smaller than the first.)

As the environmental rate of fitness gain declines (through increasing inter-patch search time, or shortage of free patches (i.e. high probability of superoviposition)) the difference between first and second clutches should decrease (Parker and Courtney 1984).

Skinner (1985) predicts that as time between the two oviposition events increases, the optimal second clutch size should decrease. The predicted Lack clutch for the second clutch will always be smaller than that of the first (although it is possible for the cumulative number of eggs to exceed the Lack solution for the first female.)

There is experimental support for second clutches being smaller than the first in parasitic wasps (Wylie 1966, 1973, Holmes 1972, Werren 1980, Ikawa and Suzuki 1982, Takagi 1985, Waage 1986). And discrimination against previously oviposited patches is reported for three lepidopteran species (Rothschild and Schoonhoven 1977).

The ESS For Many Females on a Patch.

Maynard Smith's (1982) game theory work on selection for the evolutionarily stable strategy (ESS) considers optimal strategies assuming that all females are behaving in their own best interests. When an ESS is adopted by all individuals in a population it cannot be bettered by an individual adopting an alternative strategy (Maynard Smith and Price 1973).

Whereas the superoviposition models of Charnov and Skinner (1984, 1985) and Skinner (1985) have assumed that the strategy of the first female is fixed, Parker and Courtney (1984) used game theory models to predict what all ovipositing females should do. They predicted that as the probability of superoviposition increases so the optimum clutch size of the first female should decrease (although the effect is weak.)

More generally it has been predicted that as the average number of hosts visiting a patch increases so the ESS clutch size should decrease (Parker and Courtney 1984, Skinner 1985). Under such conditions Parker and Begon (1986) predict smaller clutches of larger eggs.

However, Ives (1989), looking more closely at the relationship between superoviposition and larval competition has shown that the evolutionarily stable clutch size depends not only on the number of other females that oviposit but on the form of larval competition within the patch. When larval competition causes rapid mortality at high densities the evolutionarily stable clutch size should decrease. But when larval competition is more "benign" the ESS could increase.

The Relationship Between Clutch Size and Egg Size.

When egg size is variable and offspring fitness is a function of egg size as well as clutch size the parent must find a balance between laying many small eggs or a few large eggs. While offspring fitness increases with egg size, the rate of increase in gain will decrease until it asymptotes such that we can predict selection for the egg size that maximises brood productivity (Smith and Fretwell 1974). Where individual progeny benefit greatly from increased parental investment in egg size, smaller clutches of larger eggs are predicted. Likewise when the rate of gain in individual fitness from increasing egg size is small then brood fitness may be maximised by

laying larger clutches of smaller eggs.

More realistic models have subsequently been produced in the light of insect clutch size theory (Parker and Begon 1986, Begon and Parker 1986). These predict that clutch size and egg size may be optimised independently where reproductive effort is not a constant and sibling competition is not a major factor. However where sibling competition occurs (and it is usually more commonly a function of clutch size than egg size is), a particular optimal clutch size should be expected and the relationship with egg size will be less important. Parents can increase the brood fitness relative to other parents by laying larger eggs, particularly if they possess supplementary resources (e.g. through adult size) to those needed for producing the clutch.

A lifetime fitness model (Begon and Parker 1986) predicts that as it grows older and reproductive resources diminish, a parent should produce smaller eggs.

Dynamic Programming Models.

In recent years dynamic optimisation processes have been developed to look at complex questions of optimisation with several simultaneous variables. An example of these are the Markovian dynamic programming models which have been applied to insect reproductive strategies. Whereas clutch size models have considered the trade-off between brood fitness and other single parameters in gain rate models this dynamic approach may incorporate several factors such as time limitation, mortality, egg limitation and host availability simultaneously. e.g. Iwasa *et al.* (1984), Mangel (1987, 1989), Charnov and Stephens (1988).

An insect is described at any given time by one or more state variables. The state variable may simply be number of mature eggs available. Alternatively the insect may

be described by several state variables such as energy reserves, number of mature, number of immature eggs, which vary according to the insect foraging success of the individual. The optimal strategy of the individual is made to depend on these changing state variables by stochastic dynamic programming techniques. The optimal strategy depends solely on the present state and not on the route in which that state is reached (hence Markovian processes are adopted.)

This technique can be extremely powerful and its use in solving complex oviposition decisions is likely to increase in the future.

Clutch Size in Parasitic Wasps

The Suitability of Parasitic Hymenoptera.

It is obvious that the bulk of insect clutch size theory thus far has been applied or tested within the parasitic hymenoptera. This is due to the immense suitability of the order arising from the typical life-history of the insects. Eggs are laid on or near a host food resource of limited carrying capacity. Therefore in gregarious broods there will be larval competition leading to a *per capita* decline in individual fitness, hence a Lack solution optimum can be conceived. Broods are laid in succession during a short adult life (relative to birds and mammals). Thus the number of hosts which a female parasitises is a direct measure of her reproductive success (Takagi 1985). Solitary laying females should seek primarily to maximise their encounter rate with suitable hosts. In gregarious species the situation is more complex, but a female will seek to maximise her lifetime offspring production by balancing the benefits of high brood fitness with possible costs to future reproduction.

The fact that separate components of a female's lifetime strategy can be identified and controlled make these Hymenoptera ideal candidates for theoretical and practical tests of clutch size theory.

The Evolution of Clutch Size in Parasitic Hymenoptera.

We have been considering clutch size as a continuum of adaptive progeny allocation from single eggs to large clusters where larvae share the host resource. However Godfray (1987b) considers the solitary and gregarious habits as a discontinuum arising through a parent-offspring conflict of interests (Alexander 1974, Trivers 1974). This is certainly quite credible and there is evidence of this distinction in the Bethylidae (Evans 1964) and in the genus *Apantales* sensu lato (Le Masurier 1987).

Godfray (1987b) observes that in many solitary species the larvae are equipped with fighting mandibles, and though several eggs are laid in a host (either siblings or conspecifics) only a single individual emerges (Salt 1961, Fisher 1961). A simple genetic model shows that though a gene for larval fighting can spread and lead to the solitary habit, it is quite difficult for the reverse to occur. There has to be a considerable advantage to the larva associated with sharing the host for gregariousness to evolve. His model predicts that clutches of two and three eggs should be rare where larval killing can evolve and has support from observations by Le Masurier (1987) in *Apantales*.

Ridley (unpub.) has related gregariousness to polyandry and solitariness to monandry. He argues that polyandry is a response to gregariousness and will improve a mother's fitness by increasing the genetic variability within the brood. (He likens it to entering a lottery where having different tickets is preferable to having several copies of the same ticket.) His model receives some general support in the

Hymenoptera but does not work in other insect orders where monandry is exceptional.

Clutch Size in the Lepidoptera

In the Lepidoptera, a range of different factors may lead to the oviposition of eggs in batches. Larval competition is not always a primary factor in shaping the clutch size decision of females. For example gregarious larvae may feed and develop more efficiently than solitary ones (Long 1953, 1955). And they may be less prone to parasitism than solitary individuals (Stamp 1981). Unfortunately there are as yet few empirical tests of clutch size theory in the literature and typically one is given general behavioural observations on gregariousness rather than controlled ecological tests.

In Support of Clutch Size Models.

Nonetheless Pilson and Rausher (1988) have shown empirically that a swallowtail butterfly, *Battus philenor* adjusts its clutch size decision in response to variation in host quality (plant age) and time since last oviposition thereby supporting Parker and Courtney (1984) and Skinner's (1985) predictions in the Lepidoptera. Also Rothschild and Schoonhoven (1977) have shown that *Pieris brassicae*, *P. rapae* and *Danaeus plexippus* can assess egg load and discriminate against hosts with eggs on, even for inferior host plant species.

Predation avoidance strategies can be important in determining whether a species is solitary or gregarious. Single egg laying females average from a few seconds to a minute between ovipositions, while gregarious females may spend up to 1 hour depositing over 200 eggs on one patch (Labine 1968, Stamp 1980). Thus one would expect that the strategy of the female will depend on the nature of the predation risk she faces. If a female's principle predators occur on the foodplant she should

minimise her time on each patch and lay single eggs. If flying between oviposition sites incurs the greater risk, she should maximise the potential of each oviposition site (Stamp 1980). This supports Waage and Godfray's (1985) contention that clutch size should increase as the probability of surviving for another oviposition decreases.

Dispersal of Host Plants and Oviposition Sites on Host Plants.

Heliconiines show great variety in their preferences for morphological and ecological features of the host plant. The *sara-sapho* group of the genus *Heliconius* deposit egg clusters on the meristems of *Passiflora astrophea*, a woody shrub or vine in forests . This is probably a response to the spatial distribution of meristems (Stamp 1980). Also leaf buds afford better protection from predation than flat leaves (Alexander 1961) and younger leaves differ in quality to older leaves (Levin 1976). Thus owing to the scarcity of younger leaves as oviposition sites, some species may lay eggs in broods. Two heliconiine species are recorded laying egg clusters on mature leaves of *Passiflora distephana* but this may be a function of the search time necessary to find the host plant - a woody vine at the forest's edgs (Benson *et al.* 1975). *Dryas* and *Dryadula* spp. lay eggs singly on *Passiflora plectostemma* a much smaller vine in forest glades. Vines can only support one larva and do not have room for multiple oviposition and their leaves are too close to the ground for oviposition on the lower surface. Females discriminate against plants with eggs or larvae present, and such larvae are aggressive and cannibalistic (Stamp 1980).

Laying eggs in clutches may also be favoured if the adult food plant is patchily distributed or some distance from the larval host plant (Singer 1971).

Aposematism and Gregariousness.

The most widely accepted evolutionary mechanism for the evolution of gregarious brood laying in the Lepidoptera is aposematic effects (Stamp 1980, Chew and Robbins 1984). Considering the evolution of distastefulness Fisher (1930), argued that cluster-laying species should lay brightly coloured eggs in clusters. Thus the discovery of one distasteful individual would discourage a predator from further intrusion and the remainder of the brood would benefit. (This contributed to the concept of kin selection developed by Hamilton (1964).) Aposematic warning coloration together with distasteful or toxic effects would enhance the gregarious habit. Hence we can predict that aposematic species should tend to be brood layers.

As a general trend this has widespread support in the Lepidoptera. Eggs laid in clusters frequently develop bright colours while single eggs are usually cryptic (white, yellow or green) (Owen 1971). Aposematism with gregariousness is reported in the Heliconiinae, Acrainae, Ithomiinae, Nymphalidae, Pieridae and selected genera in other families (see Stamp 1980, and Chew and Robbins 1984, for full references.)

Exceptions to this pattern give more insight into factors leading to gregariousness. For example brightly coloured eggs are laid solitarily in some heliconiines (Benson 1971), but then Benson showed (1978) that this may be a response to larval competition for limited resources.

Conversely cryptic lycaenid and riodinid species may produce gregarious broods but this is explained by obligate myrmecophilous relationships such as honeydew production in exchange for protection by ants (Ross 1966, Owen 1971, Pierce and Mead 1981). Kitching (1981) has noted that all Australian lycaenids which lay more than 10 eggs are obligate myrmecophiles while only 30% of solitary egg-layers have

any relationship with ants. However Klots (1951) has noted some lycaenids which are attended by ants, yet exhibit strong tendencies toward larval cannibalism and are generally solitary layers.

Clutch Size in Larval Granivores

Many coleopterous and lepidopterous genera are internally feeding larval granivores. That is eggs are laid on or in seeds and immature stages feed and develop on that resource. Frequently the larvae remain solely with the seed with which their parent supplied them. If the fitness of such larvae decreases with the degree of larval competition within the seed and if all feeding occurs during the larval stages then clearly the oviposition decision of the parent is crucial.

Smith and Lessells (1985) related the larval competition curve (total brood fitness as a function of larval density) to the cost of searching. They used a marginal value approach (Charnov 1976) to predict that as search costs increase then the number of eggs per seed should increase. But for low search costs, eggs should be uniformly (as opposed to randomly or aggregatedly) distributed, in order to minimise larval competition by dispersing eggs evenly, hence maximising larval fitness.

Is Dispersal of Eggs on Seeds Uniform?

Uniform egg distribution is recorded in several *Callosobruchus* sp. including *C. maculatus* (Mitchell 1975, Giga and Smith 1984), *C. analis* (Umeya *et al.* 1975), *C. chinensis* and *C. rhodesianus* (Giga and Smith 1984). Wilson (1988) has shown that this mechanism probably works through an 'absolute' rule mechanism where females assess the egg load on the current seed and respond accordingly.

Does Clutch Size Increase With Search Costs?

There is less experimental evidence supporting the prediction that as search costs increase so clutch size increases. However the observation that *Prostephanus truncatus* (Curculionidae?) which lays its eggs in tunnelled galleries in seeds, lays larger clutches in harder maize varieties might be taken as such (Smith 1986). i.e. as the investment in preparing a site for oviposition increases (time spent tunnelling), so there is investment in more offspring. As yet though, many more specifically designed experiments are needed to test this prediction.

In the curculionid weevils of the genus *Sitophilus*, egg distributions tend to be clumped for reasons as yet unknown. (e.g. Arbogast and Miller 1978, Holloway 1984). Smith (1986) suggests that this could possibly arise since females are incapable of identifying other females' eggs (due to anti-ovicidal egg-concealment behaviour (Szentesi 1982)) but bear consistent preferences for certain types of intrinsically suitable seeds.

Effects of Clutch Size on Other Life History Traits

So far I have shown how clutch size is the result of determining factors which act to maximise an insect's lifetime fitness. The precise nature of these factors will depend on the reproductive ecology and life-history of the species in question. However the clutch size decision an individual makes can in itself act on other life history traits to affect other aspects of the insect's fitness.

Clutch Size and Egg and Larval Size.

The way in which clutch size and egg size interact has already been discussed in an earlier section. When clutch size influences the level of competition for a limited

resource then this will often influence the size of the larvae and ultimately the adult offspring emerging from the brood. However in insects, size usually confers advantages associated with fecundity (Charnov and Skinner 1984). Offspring size as a component of the clutch size decision to maximise brood fitness will therefore be optimised by the mother. Hence there is not likely to be much adaptive intra-specific variation in offspring size.

Clutch Size and Sex Ratio.

When a species has the ability to facultatively control the sex of the offspring at oviposition (as in many of the haplodiploid Hymenoptera species (Luck et al. 1982, Green et al. 1982, Putters and Van Den Assem 1985)) one of the most important decisions is not just how many eggs to lay but also what sex those eggs should be. These twin considerations have been described as the reproductive strategy of an individual (Waage and Lane 1984).

Fisher's sex ratio theory (1930) predicts that under normal conditions there should be an equal level of investment in both sexes. However Hamilton (1967) showed that in situations of in-breeding Fisher's theory does not apply. He predicted that as the level of inbreeding experienced by emerging siblings increases females should bias their investment in the sex that competes less. He predicted that where a female parasitoid's offspring are under the highest degree of Local Mate Competition she should lay only enough sons to fertilise all her daughters in order to minimise competition between her sons and maximise the reproductive potential of her brood. Thus where there is exclusive sibling mating the ideal number of males is effectively one per brood. This was subsequently developed for the arrhenotokous haplodiploid situation by Taylor and Bulmer (1980) to show that the best proportion of males in a brood is; $(n-1)(2n-1) / n(4n-1)$*. Since for a given situation, brood sex ratio should

* Where n = the number of females ovipositing on the patch.

remain constant then as clutch size increases, the number of males should increase. Precise sex ratio control has been demonstrated by Green *et al.* (1982), Luck et al. (1982), Putters and Van Den Assem (1985) and Mertins (1985).

Hamilton's prediction (1967) that as number of foundresses increases on a patch (hence the level of inbreeding decreases) the proportion of males increases, has been experimentally confirmed (Werren 1980, 1983, Waage and Lane 1984, Waage and Ng 1984, Takagi 1985). And his sex ratio theory has been used to explain patterns in taxonomically related species (Waage 1982, Griffiths and Godfray 1988) and in understanding field sex ratios in particular species (Godfray and Shaw 1987, Hamilton 1979). A range of models have followed including predictions of optimal sex and progeny allocation (Taylor 1981, Charnov 1982, Waage and Godfray 1985, Godfray 1986b.)

Other Deterministic Effects of Clutch Size.

Another particular instance of clutch size determining other aspects of larval fitness is in species where there is group cooperation and larval feeding rate is dependent on the size of the group (Long 1953, Ghent 1960, Kalin and Knerer 1977). Thus the time taken for larval development will be inversely proportional to the number of eggs which the female decides to lay. This could be very important in some species where larval development involves exposure to parasitism and it is advantageous to minimise development time.

Conclusion

The most striking conclusion at the end of such a review as this is of the enormous range and diversity of trade-offs which shape clutch size decisions in the insects and the resulting variety of strategies. By contrast while bird and mammal species vary in their costs of reproduction and parental care, their brood sizes are generally determined by the way in which energetic resources are channelled to the offspring and the resulting strategies are relatively consistent.

Charnov and Skinner (1984), note the great difference between the life-history strategies of the closely-related Pacific salmon (genus *Oncorynchus*) and the Trout (genus *Salmo*), which resolve present versus future reproduction trade-offs in extremely different ways. Clearly insects offer no less extreme examples of diverse behaviour as individuals maximise their lifetime fitness in their own environments. What is required now is more empirical testing of clutch size theory and more in-depth studies of insect oviposition systems.

A broadening in the scope of studies of oviposition in other insect orders would also be welcome. e.g. experimental tests on oviposition decisions in Orthoptera, Coleoptera and Diptera to name a few. Thus far the bulk of the empirical testing of clutch size theory has occurred in the parasitic Hymenoptera, and more sporadically in the Lepidoptera and selected Coleoptera.

Tables

Table 1.1 : The Advantages of Gregariousness Versus Laying Single Eggs.

1.) Maximising the parent's rate of gain of fitness, particularly if time or opportunities to oviposit are limiting (and eggs are not limiting.) Theoretical tests include Charnov and Skinner 1984, 1985, Parker and Courtney 1984, Iwasa 1984, Skinner 1985. Experimental evidence in wasps; Jackson 1966, Hirose *et al.* 1976, Podoler *et al.* 1978, and in butterflies; Courtney 1986, 1987, Pilson and Rausher 1988.)
2.) Co-operation between siblings - a.) In certain herbivores larval establishment and feeding rate are proportional to group size (Long 1953, 1955, Ghent 1960). b.) In certain parasitoids a minimum number of larvae may be required to overcome host defences (Askew 1968, Deloach and Rabb 1972, Puttler 1974, Kitano 1986). c.) In certain parasitoids pupation can not ensue until whole host is consumed (Taylor 1937, Godfray 1987a.). d.) In some sawflies there is communal exiting of the host pod and excavation of a pupation site which is aided by group size (Carne 1966, Seymour 1974). e.) Silkmoth larvae disperse silk trails more efficiently in groups (Stamp 1981).
3.) Avoidance of predation or parasitism - a.) Safety in numbers and active defence (Howard and Fiske 1911, Ford 1945, Prop 1960, Dowden 1961, Ito and Miyashita 1968, Van den Berg 1971, Emlen 1973, Knerer and Atwood 1973, Stamp 1980, Stamp 1981). b.) Aposematism - (Fisher 1930, and comprehensively reviewed by Stamp 1980 and Chew and Robbins 1984.) c.) Satiation of hosts before all eggs consumed (Godfray 1987a).

Table 1.1 Contd. : Advantages of Gregariousness.

4.) Protection against environmental extremes - a.) Decreased surface area to volume ratio with increased group size (Godfray 1987a). b.) Larval groups may build and share communal shelters (Ito and Miyashita 1968, Seymour 1974, Stamp 1980, Tsubaki 1981).
5.) Attraction of mutualist species may be increased by group size (Downey 1962, Kitching 1981, Chew and Robbins 1984).
6.) Increased aggregation of offspring leading to refuges and stabilisation of population (Myers 1976, Tsubaki and Shiotsu 1982, Davies and Gilbert 1985).
7.) Increased probability of matedness of offspring (Stamp 1980), including benefits of Local Mate Competition (Hamilton 1967, Taylor 1981, Waser *et al.* 1986).
8.) Reduced exposure to predation while flying between oviposition sites (Stamp 1980).

Table 1.2 : Limits on Parental Fitness Associated With Increasing Brood Size.

1.) Parental effort - resulting in reduced parental care or increased parental mortality (e.g. Williams 1966, Charnov and Krebs 1974, Charnov and Skinner 1985, Godfray 1987a).
2.) Increased Sibling Competition - resulting in; a.) Reduced fecundity of offspring (e.g. Charnov and Skinner 1984, 1985, Parker and Courtney 1984, Waage and Ng 1984, Takagi 1985). b.) Increased offspring mortality (e.g. either directly Godfray 1986, 1987a, or indirectly through necessity for more foraging Dethier 1959.) c.) Expiry of food resource (e.g. Dethier 1959, Stamp 1980).
3.) Increased apparency of aggregations to predators - Lyons 1962
4.) Instability/vulnerability of large clusters to physical damage e.g. in bad weather (Chew and Robbins 1964).
5.) Increased exposure to predation risks with increased time at oviposition site (Shapiro 1975, Stamp 1980).
6.) Reduced egg size resulting in reduced offspring fitness (e.g. Parker and Begon 1986).

Table 1.2 Contd. : Brood Size and Limits on Fitness.

7.) If there is maternal guarding of an egg cluster, peripheral eggs may risk greater predation as cluster size increases (Eberhard 1975, Rothschild 1979).
8.) Physical limits on mother. e.g. Carrying excessive numbers of eggs in flight is energetically expensive (Labine 1968).

Figures

Figure 1.1 : The Relationship Between *Per capita* Fitness and Brood Fitness and the Calculation of Lack's Solution.

Shaded blocks indicate individual fitness in a given sized clutch. The lefthand column shows the incremental fitness gain to the parent of adding another egg. In 1.1.i, *per capita* fitness stays constant with increasing clutch size and theoretically a parent maximises her fitness by laying all her eggs at once. In 1.1.ii, *per capita* fitness decreases with clutch size and there is a clear brood fitness optimum. In 1.1.iii, the relationship between *per capita* fitness and clutch size is domed, again resulting in a Lack solution. (N.B. This is not necessarily the same as the optimum for individual fitness.)

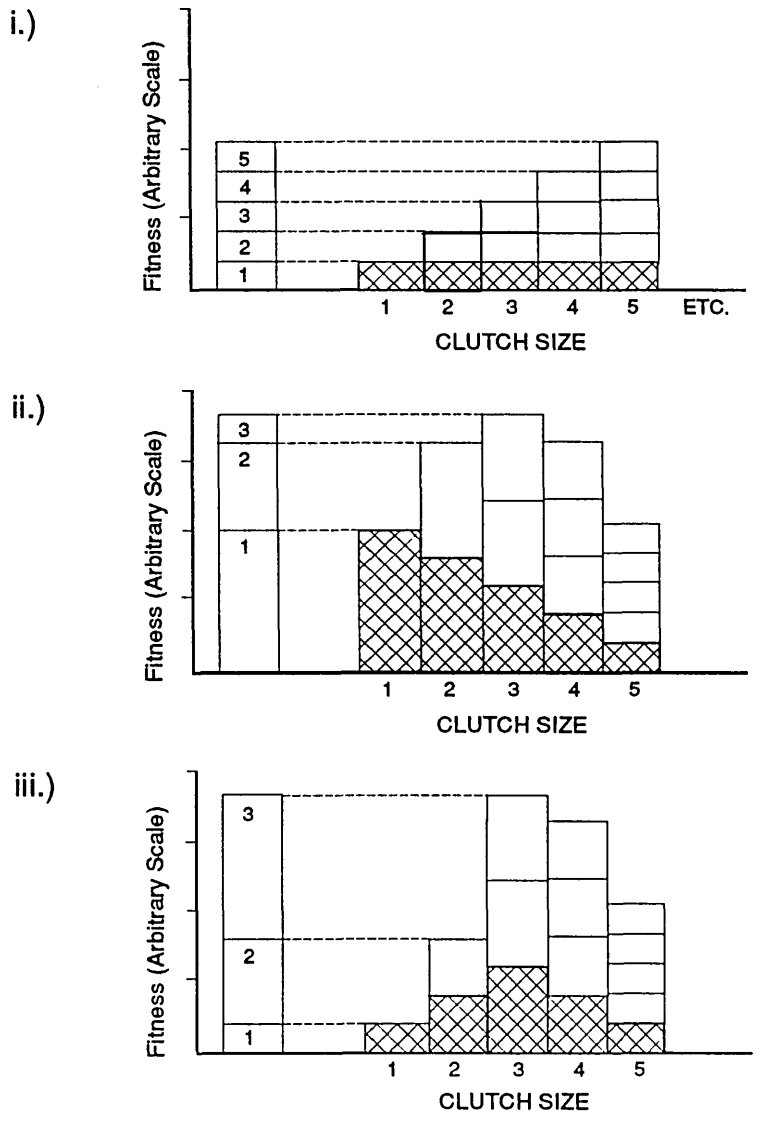
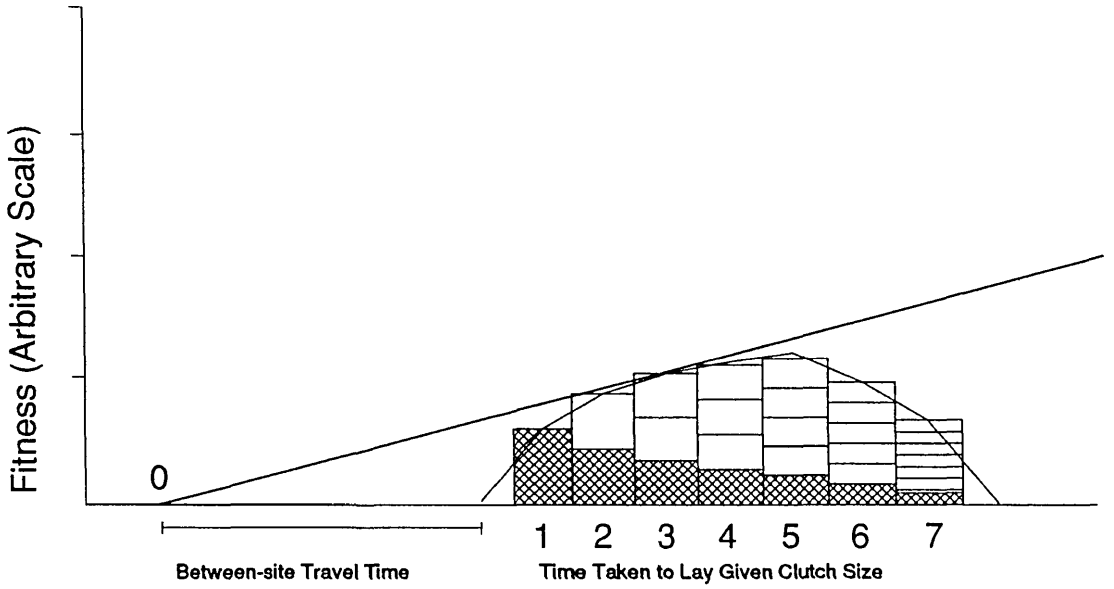


Figure 1.2 : Clutch Size, Patch Time and the Marginal Value Theorem.

The histogram of blocks making up the fitness curve is similar to those in Figure 1.1 except that the abscissa shows time taken to lay a given clutch size. The optimal clutch size maximises the rate of gain of fitness i.e. Fitness returns from a clutch divided by travel time plus oviposition time. Thus it occurs as shown at the tangential point from O to the fitness curve. As travel time between patches increases the optimum approaches Lack's solution, whereas as time between patches decreases then the optimal clutch size becomes progressively smaller than the Lack solution.



CHAPTER 2

OPTIMAL CLUTCH SIZE IN THE PARASITOID WASP, *Goniozus nephantidis*.

Summary

1.) *Goniozus nephantidis* lays gregarious ectoparasitic broods on lepidopterous hosts, adjusting its clutch size facultatively according to the size of the host larva. Females place a large investment of time into guarding their developing broods to pupation and it is argued that they should therefore maximise the fitness of each brood they produce.

2.) A manipulation experiment was used to show that as clutch size increases on a given host size, so offspring size decreases. (However offspring mortality was independent of clutch size.)

3.) It was experimentally shown that female size is positively associated with longevity and lifetime production of progeny. Larger females can also produce a higher total number of broods in a lifetime although there is no relationship between female size and number of fertile broods produced.

4.) From the regressions for the relationships between clutch size and female size and female size and lifetime production of progeny, it was calculated that the clutch size which should maximise the fitness of the brood was 22.9.

5.) The mean clutch size found on this size of host was 8.94. This huge discrepancy is most likely to be accounted for by the fact that laboratory measurements of fitness will probably underestimate the full range of benefits which adult female size may confer in the field (e.g. searching efficiency, longevity, temperature resistance). Hence smaller clutches offering larger offspring are selected in the field.

Introduction and Biology

There is now a significant body of theoretical work addressing specific aspects of insect progeny allocation (e.g. Parker and Courtney 1984, Charnov and Skinner 1984, 1985, Skinner 1985, Waage and Godfray 1985, Waage 1986, Godfray 1986b, 1987a). However practical tests of Lack's hypothesis are much scarcer. Indeed save perhaps for Dijkstra's (1986) attempt there have been no successful manipulative studies of insect progeny allocation analogous to the manipulations of Perrins and Moss (1975) on birds.

The scarcity of practical work partially reflects a range of problems associated with the empirical assessment of insect reproductive behaviour. Measuring *per capita* fitness changes in different-sized clutches is a major problem. While one may compare the fitness of broods in similar environmental conditions where the variation in clutch size is purely natural, it is difficult to guarantee that all factors relevant to the insect have been acknowledged in the experiment. Thus the best way of studying the effect of brood size on *per capita* fitness is, like Perrins and Moss (1975) to artificially manipulate real brood sizes on a controlled resource and study the resulting fitness of those broods. One obvious difficulty compared with the technique for birds is in the physical transplantation of insect eggs. Insects will often adopt a range of techniques for adhering their eggs onto a substrate or resource and consequently it is difficult to artificially mimic this behaviour in a realistic way and to avoid damage of the eggs.

Lack's hypothesis (1947) can be restated as saying that a bird should produce the number of eggs that maximises the number of offspring reaching reproductive maturity. (This recognises that clutch size may affect survival not just to the fledgling

stage as Lack suggested but through to adulthood.) In entomological studies it has become customary to refer to the clutch size which maximises brood fitness as the Lack solution (Charnov and Skinner, 1984).

The major problem in testing optimality in insect clutch sizes is in guaranteeing that the insect should maximise the fitness of every single brood. Generally as clutch size increases towards the optimum, the marginal gain in fitness for the parent decreases. Williams (1966), Klomp (1970) and Charnov and Krebs (1974) suggest that in birds, lifetime reproductive success may be maximised by producing broods below the Lack solution in order to save resources for future reproduction. Recent models of insect clutch size illustrate a number of potential trade-offs which are more directly applicable to insects. For example if there is some risk of mortality associated with remaining on a site to lay a Lack solution clutch it may pay to lay fewer eggs in favour of increasing one's chance of survival to lay more broods. Also if an individual's reproductive success is limited by time, it may pay a parent to lay a smaller clutch size than expected in favour of searching for a fresh host rather than waiting for the optimum number of eggs to be laid (Parker and Courtney 1984, Charnov and Skinner 1984). Alternatively if a parent has a limited number of eggs it may pay her to search for new sites and begin a new brood rather than completing a Lack solution clutch (Parker and Courtney 1984, Waage and Godfray 1985). Further trade-offs are discussed by Godfray (1986b, 1987a).

Reznick (1985) has been critical of the manipulative approach to studies of the costs of reproduction on the grounds that it is more a test of phenotypic plasticity in adapted individuals rather than of the true costs of being genetically maladaptive. True empirical studies of the costs of reproduction should, he says, measure genetically-based trade-offs. However I believe that there is still a good case for

manipulations of parental fitness when they serve to highlight the penalties of exceeding and/or falling short of an optimal solution. In the case of this experiment one cannot hope to emulate the evolutionary forces which led to the clutch sizes of current adaptive individuals, however one can still gain insight into the manner in which failure to lay the optimal number of eggs is manifested in the fitness of the parent's brood. Certainly the manipulative work on clutch size in birds by Perrins and Moss (1975), gives grounds for hope that the penalties of non-optimal clutch size-laying can be pin-pointed and quantified.

Goniozus nephantidis is an aculeate parasitic wasp from the family Bethylidae. Females search for lepidopterous larval hosts, subdue and paralyse them and lay roughly 5-15 eggs on the body surface (Jayaratnam 1941). The eggs hatch and the larvae develop on the outside of the host, feeding on the host tissue fluids. When larval development is complete (usually when all host tissues are exhausted), the larvae drop off the host carcass and pupate in the vicinity. Adult males emerge first and chew their way into their sisters' cocoons where they mate. (Thus there is a high level of inbreeding and local mate competition, leading to highly female-biassed sex ratios.) The mother remains with her brood until her offspring reach pupation (and sometimes adulthood). As brood maturation may take up to 10 days and adults only live for around 40 days this obviously imposes an enormous limit on the potential fecundity of the female. This cost is probably offset by the ability of a guarding female to exclude predators and parasites from her brood (Hardy and Blackburn, pers. comm.).

The reproductive behavior of *G. nephantidis* makes it a suitable model species for empirical tests of insect clutch size theory. Since females invest so much time in each brood a strong *a priori* argument can be made that a female should maximise the fitness of each brood as she lays it. Thus a Lack solution clutch size is expected. Additionally, since eggs are laid gregariously on the surface of a limited resource, one can expect density-dependent larval competition for resources. (In gregarious parasitoids this is usually scramble competition with resources being equally shared among the competitors but in solitary species it may be a contest competition with a single survivor (Godfray 1987b.)) Further, it should be possible to manipulate brood size by transferring eggs between hosts. In this way the *per capita* changes in fitness associated with brood size can be quantified and hence the Lack solution identified by comparing the total fitness from each brood size.

The measurement of brood fitness is also facilitated by the local mating behaviour of the wasp. Not only are sex ratios of clutches highly female-biased (normally there is only one male per brood), but one can argue that in the absence of competition for matings, male reproductive success will not be significantly affected by competition within the brood. Thus *per capita* changes in offspring fitness arising from clutch size decisions will be reflected chiefly in the fitness of daughters rather than sons.

The work described in this chapter was undertaken in collaboration with my fellow student Mr. Ian Hardy with statistical assistance from Dr. Charles Godfray.

Materials and Methods

Insect Cultures

Goniozus nephantidis is a gregarious ectoparasitoid of the Black-headed Coconut Caterpillar, *Opisina arenosella* Walker (Lepidoptera, Oecophoridae) (Cock and Perera 1987) in coconut growing areas of the Indian Subcontinent. Our experimental stock was obtained from the Coconut Research Institute in Sri Lanka where it has been cultured for over 10 years on the Rice Moth, *Corcyra cephalonica* Stainton (Lepidoptera, Pyralidae). *C. cephalonica* was reared in the lab. on a diet of bran, yeast and glycerol. Female wasps were introduced to individual larval hosts in glass test-tubes (a streak of fresh honey solution was also supplied for the adult to feed on.) To maintain genetic diversity in the *Goniozus* stock culture parasitised hosts would be placed together in the same tube so that emerging wasps could inter-breed with non-siblings. All stock cultures were maintained at 30° C and 80% relative humidity.

Experiment 1: The Relationship Between Clutch Size and Host Size.

In order to control the degree of larval competition for the host resource, gregarious parasitoids should be able to facultatively control the number of offspring according to the size of the host (Green *et al.* 1982, Griffiths and Godfray 1988). Therefore it is important to establish the nature of the relationship between clutch size and host size; what index of host size should be measured? Does host size affect the size of emerging adult offspring or their mortality at any stage? Finally in following experiments the amount of resource available to female wasps should be fixed, to study their strategy in the limited host size range. What will be a suitable host size (or size range) for future experiments and what is the corresponding mean clutch size that

is laid?

Individual mated female wasps with one previous breeding experience were presented with a single host larva selected from a range of sizes (between approximately 10-60 mg.) Mothers were allowed to remain with their broods until pupation. For each brood a record was made of host size (weight, length, headwidth), clutch size, maximum and minimum number of larvae, number of pupae and number of adult offspring (from which mortality at the different stages could be calculated). Sex ratio and the adult size of progeny were also recorded.

Experiment 2: Manipulation of Clutch Size.

To study the effect of varying the degree of larval competition for resources the amount of resource has to be standardised or fixed. Then it becomes possible to examine the result of increasing brood size (and the resulting competition) on the fitness of individual offspring. The purpose of this experiment was to identify the aspects of individual fitness which change with clutch size and quantify the relationship.

From the results of Experiment 1 the most representative and convenient measure of host size was taken to be host weight. The range was standardised as between 30-40 mg (because hosts in this range are abundant and can be easily picked out.) Hosts within this size range were supplied for individual wasps to attack. A store of eggs was built up by manually removing all the eggs from parasitised hosts using a fine mounted hair. Then artificial clutches* were created by replacing eggs onto paralysed hosts using the mounted hair. A range of clutch sizes was generated in this way. Clutches were then left to develop through to adult emergence (with no parental care. Initial clutch size, pre-adult mortality (as above) and sex ratio and size of adult

* Ranging from 1 to 54 eggs.

progeny (thorax length) were recorded.

Experiment 3: The Relationship Between Female Size and Fitness.

Since competition within broods leads to decreased adult size, an experiment was devised to indicate which aspects of a female wasp's fitness would be affected by its size.

Newly-hatched females were taken and introduced to a succession of hosts, transferring the female to a fresh host as the current brood reached pupation. This continued until the female died whereupon the size of the female was measured (from its thoracic length). Hence for each female there was a record of female size and longevity, plus the number and size of broods and their mortalities at different stages, and the size and sex of her progeny.

Experiment 4: The Effect of Maternal Brood Guarding.

Since, in the manipulation experiment (Experiment 2), broods developed in the absence of a maternal guard, an experiment was devised to test whether this affects survivorship of the brood at any stage under laboratory conditions. Using hosts between 30-40 mg, pre-adult mortality and progeny size was measured for 40 broods in which mothers were allowed to guard their broods until pupation. The data was then compared with results from the broods in the manipulation experiments where no maternal care was available.

Statistical Analysis Using Generalised Linear Interactive Modelling.

Data was analysed using the Generalised Linear Interactive Modelling (GLIM) package, (McCullough and Nelder 1983). This fits models using a maximum

likelihood procedure. It can be used for fitting regressions and analyses of variance and covariance and can assume normal, poisson or binomial error variance in the dependent variable without transformations. Owing to the large sample sizes, changes in deviance (chi-squared tests) and differences in sample means (t-tests) may often be statistically significant although the reduction in deviance due to the fit of the model may not have been very marked. Thus the strength of the relationship arising from the fit of a model is also described here in terms of the percentage change in deviance in the response variable when the model (independent variable) is fitted.

In analyses with non-normal error variances, there is no exact theory available for the distribution of the deviances and a chi-squared approximation is used. For analyses of this sort, conservative significance levels are used. The standard errors of parameter estimates are frequently asymmetric and are displayed here in the form: mean = 10, S.E. = 9.5/11.0, where 9.5 and 11.0 are the lower and upper standard errors respectively, around the mean of 10. When poisson error variances are assumed, the logarithm of the response variable is assumed to be a linear function of the explanatory variable(s): in the case of binomial errors, the 'log odds' of the response variable (i.e. the 'logit') is assumed to be the linear function. All logarithms are of base e.

In Experiment 1, a series of regressions are fitted in order to explain the variance in the response variable, clutch size. Poisson errors are assumed because of the low and discontinuous nature of clutch size values. First of all clutch size was fitted against three different parameters of host size to determine which index would be most appropriate for future experiments. The mortality (proportion surviving) at each stage was recorded. Also to see the effect of increasing host size on other factors a series of y-variables were fitted; mortality at any stage (binomial errors), offspring

size (normal errors), sex ratio (binomial errors).

Experiment 2 measures the proportions surviving as a function of increasing clutch size so binomial error variance is assumed on the y-axis. (Owing to the way in which GLIM operates with binomial errors it is the change in deviance in the response variable (rather than in variance) which is explained by clutch size.) Mortality at the different stages was compared with the unmanipulated hosts in Experiment 1. Finally offspring size is fitted against clutch size (assuming normal errors) treating the sexes separately.

In Experiment 3, adult female size is an independent variable against which a series of indices of fecundity are fitted all of which are assumed to have normally distributed errors. These include, lifetime progeny, number of females produced and longevity. When total number of broods laid was treated as a function of clutch size Poisson errors were assumed.

In Experiment 4 the presence/absence of a guarding mother was fitted as a factor to see if it explained any of the deviance in mortality (binomial errors) or variance in adult offspring size (normal errors).

Results

Experiment 1: The Relationship Between Clutch Size and Host Size.

Clutch size was strongly correlated to host size. Host weight explained 47.1% of the deviance in clutch size, host length explained 46.7% of the deviance, and host headwidth explained 43.2% of the deviance. For reasons of convenience host weight was taken as the index for future experiments (see Figure 2.1) and the selected size

range for future experiments was 30-40 mgs. Mean clutch size in this range was found to be 9.26 (S.E. = 8.51/10.10). Mean number of hatched eggs in this range was 8.94 (S.E. = 8.28/9.66).

The appearance of a number of all male broods suggested that some mothers were unmated, but there was no statistical difference between mated and unmated broods in the relationship between clutch size and host size ($X^2 = 0.39$ df=1.)

When female wasps encounter smaller hosts they compensate by laying smaller broods in order to reduce the larval competition for the limited resource. Hence it is conceivable that host size itself exerts little effect on survivorship or size of individual offspring. What may be more relevant is the ratio of host resource to eggs which changes as host size increases. (Figure 2.2). Thus offspring mortality was regressed against host size and also against resource availability (host size divided by number of hatched larvae gives a crude measure.)

Mortality was not related to host size or competition for host resource at any life-history stage. The probability of survivorship of the different life-history stages can be taken from the proportion of individuals surviving at each stage. The probability of eggs hatching into larvae is 0.907 (S.E. = 0.896/0.917). The probability of surviving death during the larval stage is 0.938 (S.E. = 0.930/0.946). Survivorship of larvae to the pupal stage is 0.950 (S.E. = 0.942/0.956). The probability of pupae surviving through to adulthood is 0.923 (S.E. = 0.914/0.931). The proportion of individuals surviving through from eggs to adulthood is 0.729 (S.E. = 0.712/0.745).

Female offspring size was strongly related to host size ($r^2 = 34.2\%$) so that as the size of the host increases so the size of adult females reared on it increases (see Figure

2.3). However there was no relationship between male offspring size and host weight (r^2 = 7.3%). In neither case did the measure of the level of competition (units of host per egg) affect offspring size.

There was no evidence to suggest that sex ratio changes with host size (mean proportion of males = 0.068 (S.E. = 0.055/0.085)) (see Figure 2.4 A). As broods increase in size so the number of males increases although the relationship is weak (percentage deviance explained = 13.7%), the maximum number of males in a brood was two (see Figure 2.4 B).

Experiment 2: Manipulation of Clutch Size.

Exceeding the optimal clutch size will increase larval competition for the limited resource of the host. Thus a reduction in brood fitness is expected and might be detected through finding direct density dependent mortality in developing broods. Density dependent competition could also affect brood fitness indirectly by leading to reduced adult size of offspring, which in turn affects their fecundity.

The survivorship at the different life history stages can be compared for Experiment 1 and Experiment 2. Mean survivorship from egg to larval stage in the manipulation experiment was 0.428 (S.E. = 0.419/0.436). This is substantially lower than the survivorship of unmanipulated broods at the corresponding stage (mean survivorship = 0.907 (S.E. = 0.896/0.917)) which implies that the process of manually transferring eggs is responsible for the high mortality. Table 2.1 shows that mortality at every stage but the larval is significantly higher in the manipulation experiment although the only severe difference is at the initial egg-larval stage. It seems that once larvae have been successfully introduced onto the host then survivorship is still fairly high. The survivorship of larvae is not significantly related to increasing clutch size

(X^2 (1 d.f.) = negligible). Thus although the survivorship from the larval stage is reduced slightly by the manipulation process, the effect is density independent. The measure of competition which was adopted in the optimal clutch size calculation was number of hatched eggs, not initial number of eggs.

Mean larva/pupa survivorship was 0.885 (S.E. = 0.876/0.893) and was weakly related to number of hatched eggs (percentage deviance explained = 12.0%). The probability of survival from pupation to adulthood was 0.843 (S.E. = 0.854/0.831) and was not related to number of hatched eggs.

Thus it seems that apart from a slight effect of clutch size on survival of larvae to pupation, the survivorship of offspring is not affected by the degree of competition which may be imposed by clutch size (at least not in the range of clutch sizes which was created in this experiment.)

When brood fitness is considered in terms of the size of adult offspring, male and female wasps are considered separately due to the marked sexual dimorphism in size. Moreover the process of manipulation results in random allocation of sexes to broods. Therefore we can look at asymmetries between sexes in competition for resources by seeing if brood sex ratio explains any of the deviance in either male or female size.

Female offspring size was strongly influenced by clutch size (r^2 = 45.9%) and was described by the regression;

$$FemaleSize = -0.5210.ClutchSize + 55.58 \quad (1)$$

(see Figure 2.5.) However neither size of host nor sex ratio had any effect on female size.

Male offspring size was influenced less strongly by clutch size (r^2 = 17.8%), and was unrelated to either host size or sex ratio (see Figure 2.6).

Experiment 3: The Relationship Between Female Size and Fitness.

These data came from 76 females (ranging from 0.5mm - 1.3mm thoracic length.)

Lifetime production of adult offspring was strongly correlated to female size (r^2 = 29.7%) and was explained by the regression;

$$\text{Log}\,No.ofAdultProgeny = 0.0876.FemaleSize - 0.7383 \quad (2)$$

(see Figure 2.7.) A Log function was selected because although Number of Progeny in itself explains slightly more deviance (31.7%), it shall be seen that using the Log function facilitates the calculation of the optimal clutch size.

There was no significant difference between mated and unmated females in the relationship between their size and fecundity. (Fitting matedness as an additional factor after female size only explained another 1.2% of the deviance in lifetime fecundity).

The relationship of female size to the number of females produced was weaker (r^2 = 15.7%) and described by the regression;

$$\text{Log}\,No.ofFemales = 0.06345.ParentSize + 0.1887 \quad (3)$$

(Figure 2.8.)

Female size was also related to the total number of broods produced (percentage deviance explained = 25.0%) (see Figure 2.9), yet interestingly, not to the number of fertile broods (percentage deviance explained = 6.6%), to longevity (r^2 = 31.3%) (see

Figure 2.10) and total number of eggs laid (r^2 = 34.9%). There was no difference between mated and unmated females in each case (with the obvious exception of number of fertile broods.)

The size of a female parent had no relation to the probability of an egg surviving through to adulthood, 0.770 (S.E. = 0.764/0.776), furthermore neither male nor female progeny size was related to the size of the parent. Female size had no effect on the sex ratio of the brood.

In summary, a female's size has important consequences in terms of her fecundity and longevity but the fitness of individual progeny is unaffected by female size.

Experiment 4: The Effect of Maternal Brood Guarding.

There was no difference between broods with guarding mothers and broods without, in the survivorship of eggs through to adulthood (percentage deviance in survivorship explained by fitting maternal care = 3.1%). (See Table 2.2 for full t-test details.)

Moreover the presence or absence of a maternal brood guard did not affect the adult size of either the male progeny (percentage deviance explained = 3%) or the female progeny (percentage deviance explained = 12.5%).

This suggests that the function of maternal guarding has more to do with defensive behaviour such as the exclusion of predators and parasites than with the nurturing and tending of developing broods. In fact this has been experimentally confirmed by Hardy and Blackburn (pers.comm.) who showed that females actively defend their broods against other conspecifics and against multiparasites.

Discussion

Calculation of the Optimal Clutch Size.

Since the brood guarding comprises such a large investment of time for the female wasp, I argue that the wasp should maximise the fitness of each brood that she lays (W) (see the chapter on Clutch Size Theory for the full argument). As the number of individuals in the brood (c) increases, individual fitness will be a function (f(c)) of clutch size and decrease as the degree of larval competition increases. The fitness of a brood can therefore be described by;

$$W = (c - 1)gf(c) \qquad (4)$$

where g is the probability of surviving to adulthood (independent of clutch size), and (c - 1) describes number of females per brood if it is assumed that on average one male is laid per brood as is the case in this host size range, and this male fertilises all his sisters. Since the single male does not have to compete for mates, I assume that male reproductive success is not influenced by body size and the individual fitness (f(c)) will only be important for the female offspring. Hence a female wasp should seek to maximise the number of daughters in her brood but these daughters must be fertilised thus for total fitness f(c) is multiplied by (c - 1).

Using standard optimisation techniques (e.g. Parker and Courtney 1984) one can calculate an expression for the optimal clutch size (c^*). This is taken from the peak of the fitness against clutch size profile which is found by taking the first derivative (i.e. where $dW/dc = 0$). Thus;

$$dW/dc = 0 = (gf(c)) + ((c^* - 1)gf'(c))$$

where f'(c) is the first derivative of the fitness function f(c). So alternatively;

$$(c^* - 1)gf'(c) = -gf(c)$$

Dividing both sides by (g f'(c)) we get;

$$(c^* - 1) = -gf(c)/gf'(c)$$

The density independent larval mortality, g can be cancelled out. Therefore c^* does not depend on the larval mortality and we get;

$$c^* = -(f(c)/f'(c)) + 1 \qquad (5)$$

(Apart from the +1 which arises from the adjustment for one male per brood this is the same as Parker and Courtney's expression (1984).)

Female size (S) is related to clutch size (c) in the form S = mc + n (see equation 1), where m represents the slope of the regression and n is the intercept. Furthermore, our measure of offspring fitness f(c) is related to female size (S) in the form log f(c) = bS + a (see equation 2), where b is the slope of the regression and a is the intercept. Hence substituting our expression for female size into the regression for female fitness we get;

$$\log f(c) = b(mc + n) + a \qquad (6)$$

(This is plotted in Figure 2.11 A, using the values found from regressions 1 and 2.) Alternatively;

$$f(c) = \exp(bmc + bn + a) \qquad (7)$$

(See Figure 2.11 B.) Thus from equation 7 we can substitute an expression for (f(c)) and its first derivative (f'(c)) into equation 5, and c^* can be solved;

$$c^* = -(\exp(bmc + bn + a))/(bm \exp(bmc + bn + a)) + 1 \qquad (8)$$

This cancels to give the simple expression;

$$\bar{c}^* = -1/bm + 1$$

Thus the optimum clutch size can be calculated from the slopes of the two regressions (equations 1 and 2), where bm = -0.0456.

An approximate confidence limit can be taken from the product of the standard errors of b (S.E. = 0.01697) and m (S.E = 0.05623). Thus from the regressions (1 and 2) we can predict the optimum clutch size (i.e. the optimum number of hatched eggs) that will maximise the second generation (i.e. grandchildren);

$$c^* = -1/-0.0456(+/-0.001) + 1$$

This gives c^* to be 22.9 (S.E. = 22.5/23.4). This would produce females of mean size 43.7 (equation 1), who could each produce 21.9 offspring (equation 2) i.e. a second generation of 501.5 (product of individual fecundity and clutch size). (Figure 2.11 C shows brood fitness as a function of clutch size with the Lack solution at the peak of the curve.)

The clutch size that maximises number of grand-daughters (equations 1 and 3; such that bm = -0.0331 and S.E.of m = 0.0307), is 31.2 (S.E. = 30.7/31.7) and would produce females of mean size 40.3, who could each produce 15.6 daughters. (See Figures 2.12 A, B, C, where equations 1 and 3 are used to project a regression of *per capita* fitness versus clutch size which is then used to find the clutch size which maximises brood fitness.)

In fact from experiment 1, the mean number of eggs hatching on hosts in this size range was 8.94. This would give female offspring of mean size 50.9, who would on average produce 41.4 offspring, including 30.6 females (the total second generation would be 370.1).

Interpretation of Results.

Although it is common for insects to lay clutches that are smaller than the Lack solution (Godfray 1987a), it is clear that my prediction of what should constitute the optimal clutch is far in excess of the actual brood sizes exhibited by *G. nephantidis* females.

In the derivation of the predicted clutch size one crucial assumption is that brood fitness (W in equation 4) is proportional to lifetime fitness (equation 6). It may be that the assumption that females maximise their fitness from each brood is wrong and that there are other future trade-offs that have not been considered.

In the introduction to this chapter I outlined three main penalties associated with guarding which may lead to a trade-off between current brood fitness and future reproductive success. Firstly, during the time spent guarding the host, the female may be exposed to a high risk of mortality. However, in *G. nephantidis*, maturation time is independent of clutch size so guarding time is unlikely to be a function of clutch size. Secondly, for some species, egg-laying may in itself represent a time cost which may outweigh the benefit of laying a full clutch and favour the female to search for a new host. Again, however, it is hard to envisage how such a cost could act as a function of clutch size, particularly in *G. nephantidis* where egg-laying comprises only a fraction of the total time spent with the brood. Thirdly, it may be that a female is egg-limited and rather than staying on one host to mature a whole Lack solution brood, it pays her

to lay what she has, guard it, and then search for a new host. However when a guarding female is removed and offered a new host she can immediately lay more eggs. Thus such a female is obviously not egg-limited.

Therefore there is still a strong argument to suggest that a female should maximise the fitness of each brood as she lays it. One obvious short-coming in this method is that while clutch size decisions are likely to be the same in the laboratory as in the field, estimates of lifetime fitness can only be based on reproductive behaviour under laboratory conditions. However it is quite unlikely that any laboratory experiment could allow the full benefit of female size to be expressed. Hence if female size in the field is associated with advantages such as searching efficiency, longevity or resistance to temperature changes then selection would favour larger females and therefore larger clutches than are predicted in the laboratory (effectively causing a leftwards shift towards smaller broods in the optimality curve, see Figs. 2.11 C and 2.12 C).

The most likely benefit of size is that it facilitates host searching. Not only is this well documented for other species but it implies some scarcity of the hosts and hence is consistent with the host guarding behaviour of females.

It is worthwhile reconsidering what would constitute an ideal insect species for studying *per capita* decline in fitness with increasing brood size. *G. nephantidis* lays manipulable gregarious broods (although the manipulations were not without problems) within which the larvae undergo density-dependent competition for a limited resource and also there is a good argument for maximising brood fitness. But it would appear that the full consequences of clutch size (i.e. in this case adult size) are not easily quantified. In addition an ideal model species should suffer direct density-dependent effects such that increasing clutch size will act directly on

individual fitness. In Great Tits increasing clutch size leads to density-dependent pre-fledging mortality (Perrins 1979) and clutch size has little effect on individual fitness after fledging. Furthermore, the consequence of brood size should be fully expressed in the developing brood and have no further effect in the field after the adults have hatched. However the relationship between larval competition and adult size, and adult size and fecundity is a common one. Given that this effect is so common in insects one wants to work with a species in which the full benefit of adult size is directly expressed in terms of the capacity to produce adult progeny or where lifetime reproductive success in the field is easily quantified. Thus, ideally, the consequences of the clutch size decision should have no additional expression in the field other than directly determining offspring fecundity.

The work on female size in experiment 3 produced the apparently contradictory results that female size is correlated to longevity, number of offspring, number of daughters and total number of broods laid, but not to number of fertile broods laid. Looking at this sceptically one might argue that individuals were sustained for artificially long lifespans in the laboratory (up to 80 days) when in fact longevity in the field is much lower, e.g. 40 days (Jayaratnam 1941). Hence while there is a definite correlation between size and lifespan in the laboratory this may have little biological relevance in the field where the primary concern is location of hosts over a mean lifespan of 40 days. Moreover it appears that although large females attack more hosts than small females, they do not necessarily produce more fertile broods. The benefit of greater fecundity would be questionable if it merely entailed producing large numbers of males at the end of a protracted lifespan. (N.B. Nonetheless the correlation between adult size and production of daughters does suggest that size has realistic benefits for lifetime fitness.) Overall, in studies of this sort where optimal reproductive strategies are calculated from manipulation of adapted individuals,

caution should be exercised when quantifying the costs of failure to be optimal. It may be that it is wrong to extrapolate fitness estimates from what is basically a test of phenotypic plasticity in adapted individuals (Reznick 1985).

Conclusion

Even in species where there is a strong *a priori* argument that an individual should seek to maximise brood fitness with each host it can be difficult to demonstrate optimality in insect oviposition behaviour. Quite apart from the mechanical difficulties of simulating oviposition, one cannot necessarily determine how individual offspring fitness is expressed. In this particular experiment it appeared that the full benefits of offspring size could not be demonstrated in the laboratory and that mothers produced fewer but larger larvae than predicted from laboratory estimates.

Furthermore assuming a species could be found in which offspring fitness could be directly measured under laboratory conditions one must still be sure that the insect has the ability to lay an accurate clutch size. One can see in Figure 2.1 that even within the 30 to 40 mg host size range there is enormous variation between individuals in the clutch sizes which are laid. One possible assumption from this could be that individuals are not able to produce the optimal clutch size accurately and there is stochastic variability around the optimum (Godfray and Ives 1988). Alternatively it may be that the insects have a more accurate index of host quality than larval body weight and adjust their clutch sizes accordingly.

In conclusion, there are many new complexities involved in predicting optimal clutch sizes in insects which do not exist for workers on mammalian litter sizes and bird clutch sizes. There are increased technical problems with manipulation, problems in measuring offspring fitness and problems with working in a realistic natural environment. Future work should perhaps involve careful selection of species to minimise these difficulties. Furthermore there might be some benefit in paying closer attention to variability in clutch sizes - in identifying both its source and its adaptive significance.

Tables

Table 2.1 : Survivorship in Manipulated and Unmanipulated Broods.

Stage	Normal Survivorship	Survivorship Manipulated Broods	Student's t-value
Egg-Larva	0.907 (+/-0.010)	0.428 (+/-0.009)	39.2 (df=191)
Larval	0.938 (+/-0.008)	0.939 (+/-0.007)	0.09 (df=207)
Larva-Pupa	0.950 (+/-0.007)	0.885 (+/-0.008)	5.74 (df=214)
Pupa-Adult	0.923 (+/-0.008)	0.843 (+/-0.012)	5.10 (df=213)

Table 2.2 : The Effect of Maternal Care on Survivorship.

Stage	Survivorship (Mother Present)	Survivorship (Mother Removed)	Student's t-value	Percent Deviance of Model
Larval	0.955 (+/-0.012)	0.935 (+/-0.016)	1.0 (df=154)	0.5%
Larva-Pupa	0.971 (+/-0.008)	0.960 (+/-0.009)	0.914 (df=150)	0.5%
Pupa-Adult	0.917 (+/-0.020)	0.811 (+/-0.032)	2.809 (df=138)	4.6%
Larva-Adult	0.865 (+/-0.021)	0.778 (+/-0.026)	2.603 (df=140)	3.1%

Figures

Figure 2.1 : Clutch Size (Hatched Larvae) Versus Host Weight.

Showing the regression fitted by GLIM of y = EXP (x. 0.01356 + 1.656), which explains 46% of the deviance. N.B. The curved regression is due to assumption of Poisson error variance by GLIM's fitting procedure. Dotted lines highlight the host size range of 30-40 mg which was selected for subsequent experiments.

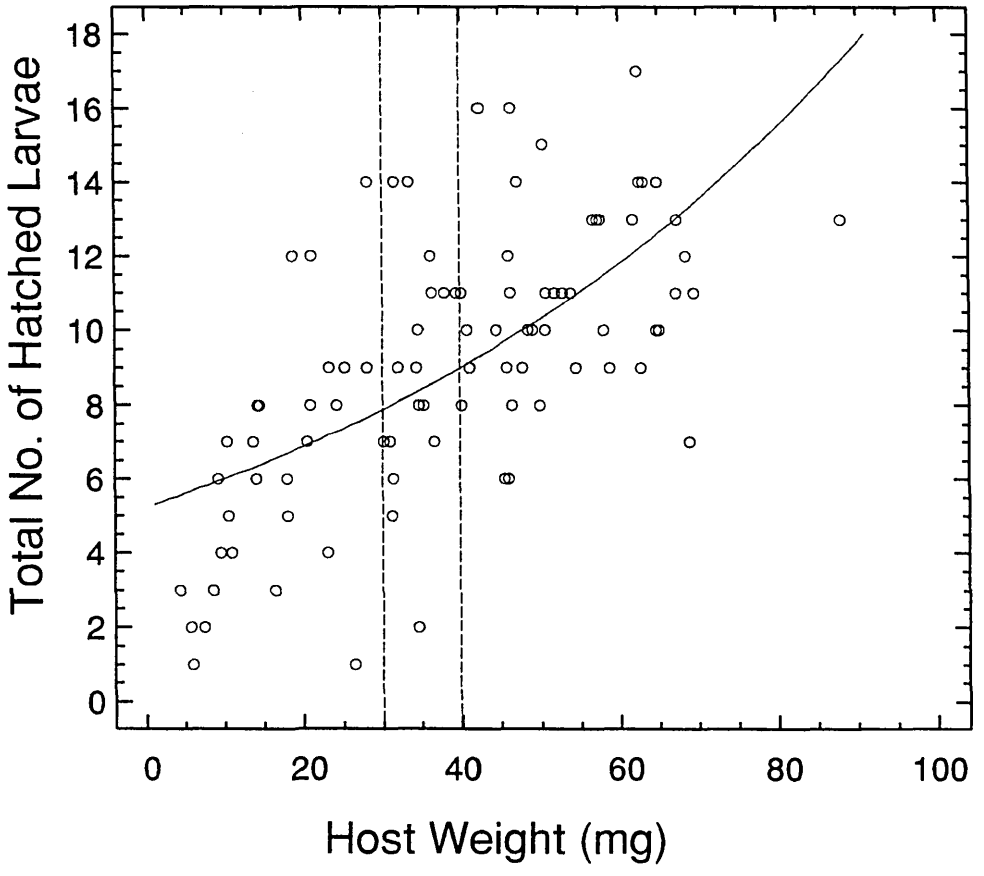


Figure 2.2 : The Amount of Host Resource Per Egg Versus Host Weight.

Showing the regression line of best fit of y = 0.048.x + 2.73 (deviance explained = 28.8%).

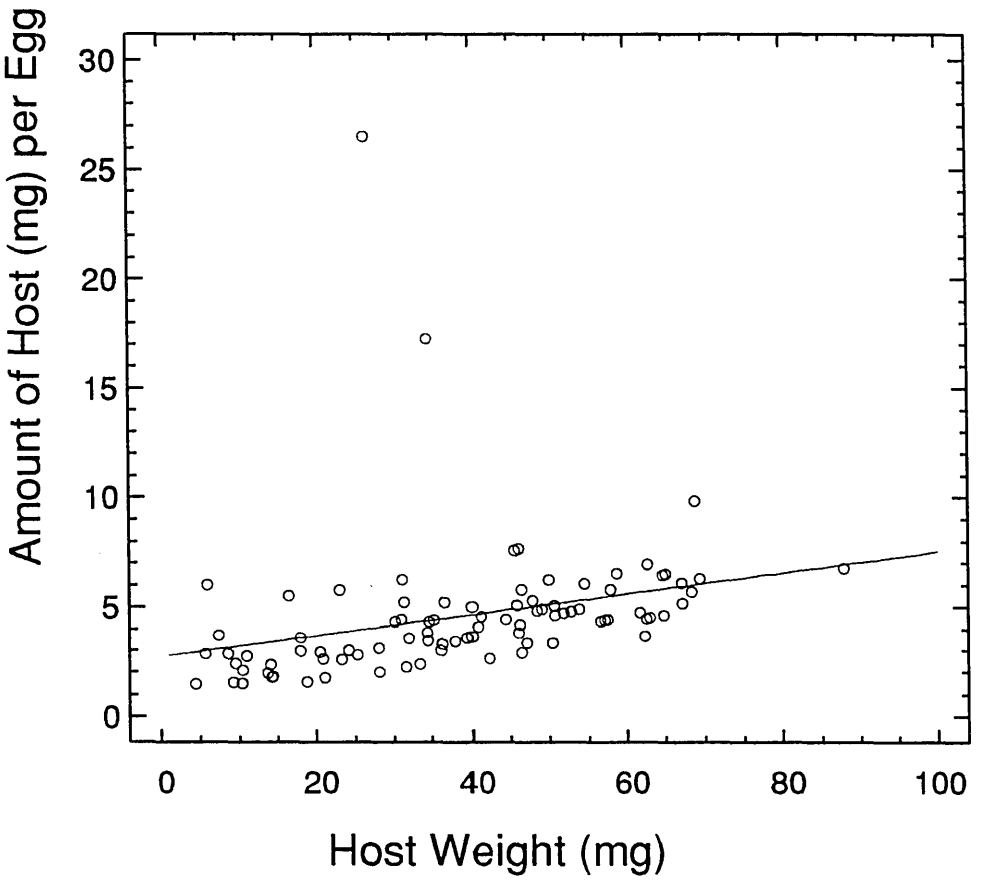


Figure 2.3 : Female Offspring Size Versus Host Weight.

Showing the regression line of best fit of $y = 0.171.x + 47.14$ ($r^2 = 34.2\%$). NB. Female size measured in graticule units. 50 units = 10^{-3} m.

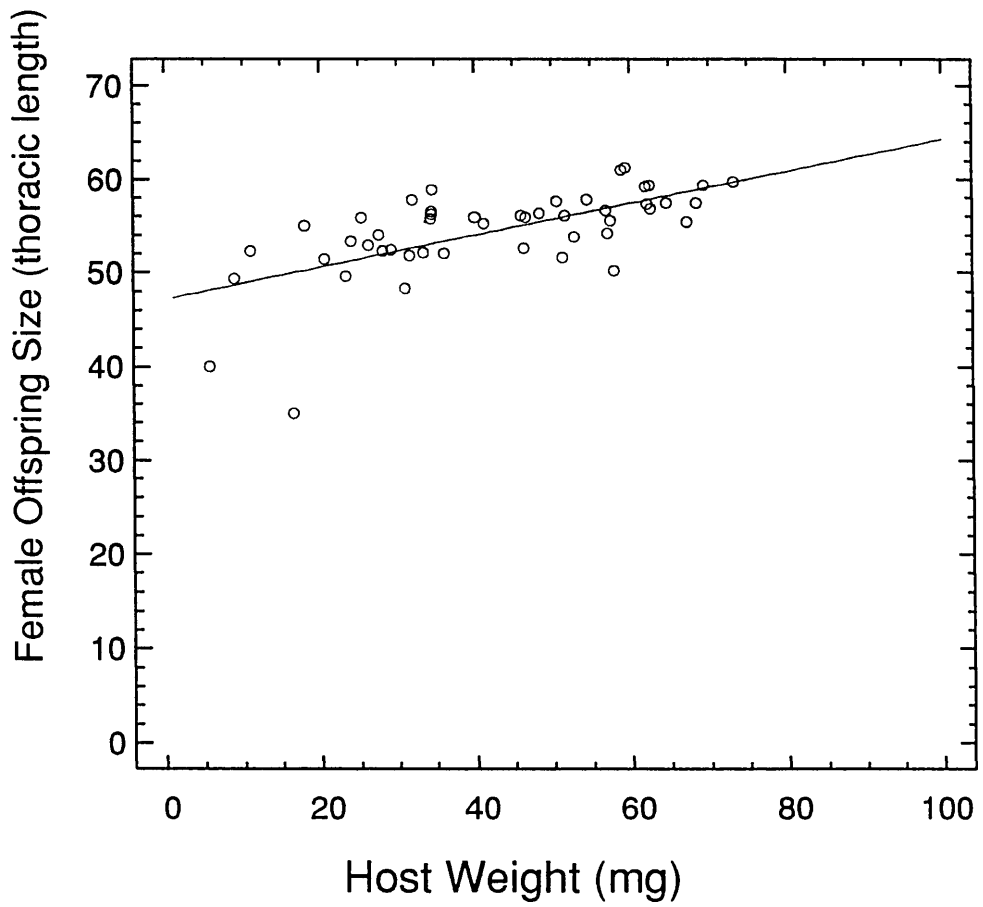


Figure 2.4 : Sex Ratio and Host Weight.

The relationship between sex ratio and host weight is illustrated in Fig 2.4 A, with proportion of males versus host weight (N.B. includes all male broods), and Fig 2.4 B, with number of males per brood versus host weight. In Fig 2.4 B, all datapoints are taken from fertilised broods only i.e. broods which include at least one female offspring. (Regression line of best fit is given by y = 0.0063.x + 0.71 which explains 13.7% of the deviance.)

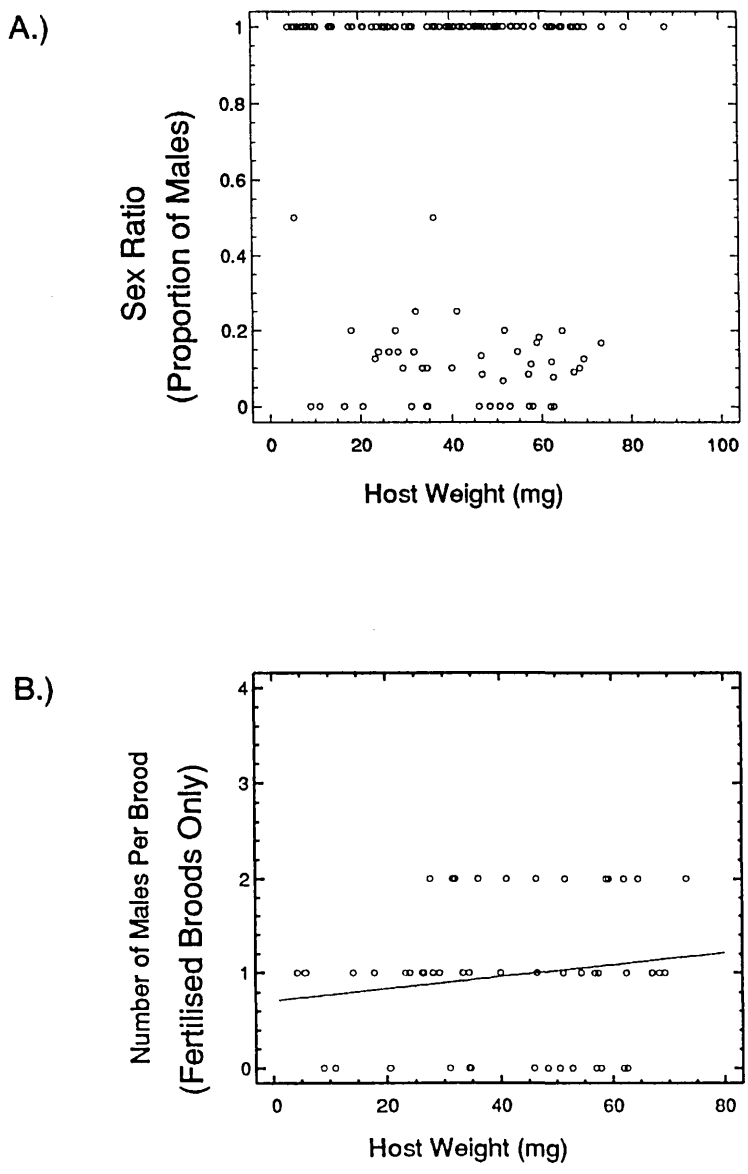


Figure 2.5 : Female Offspring Size Versus Clutch Size (Hatched Larvae).

NB. Female size measured in graticule units. 50 units = 10^{-3} m.
Fitted regression is given by y = -0.521.x + 55.6 (r^2 = 45.9%).

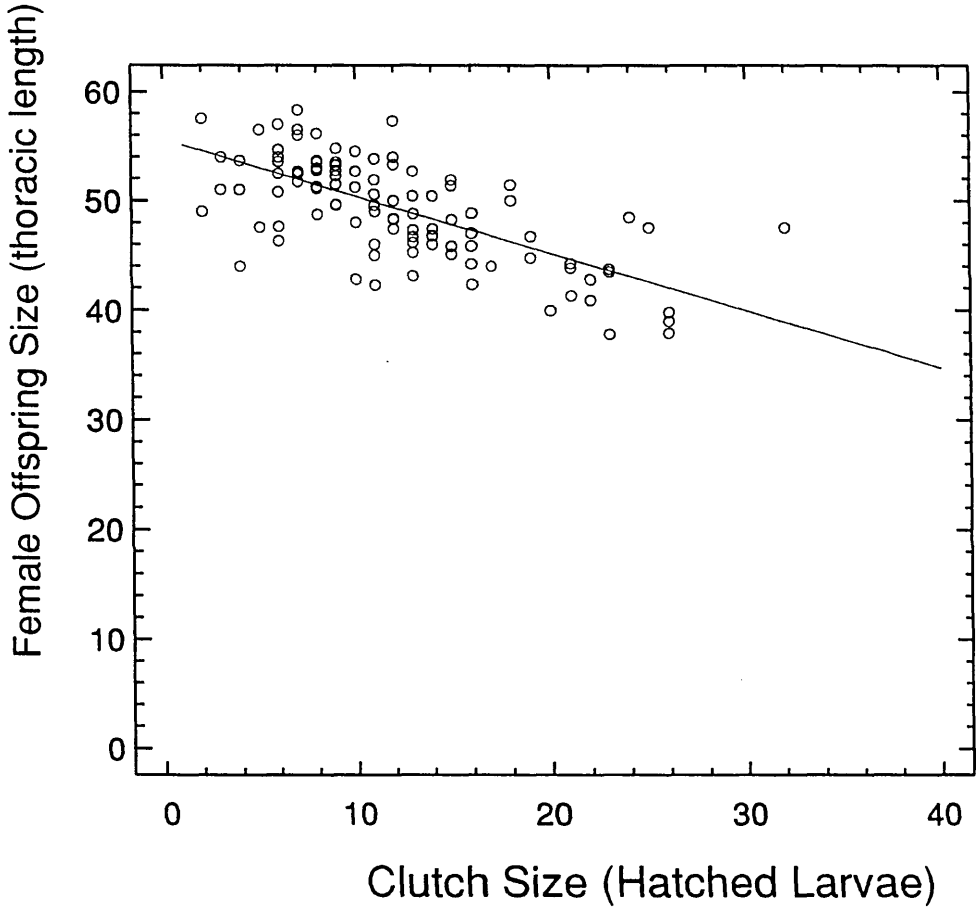


Figure 2.6 : Male Offspring Size Versus Clutch Size (Hatched Larvae).

NB. Male size measured in graticule units. 50 units = 10^{-3} m.
Regression is given by y = -0.32.x + 45.5 (r^2 = 17.8%).

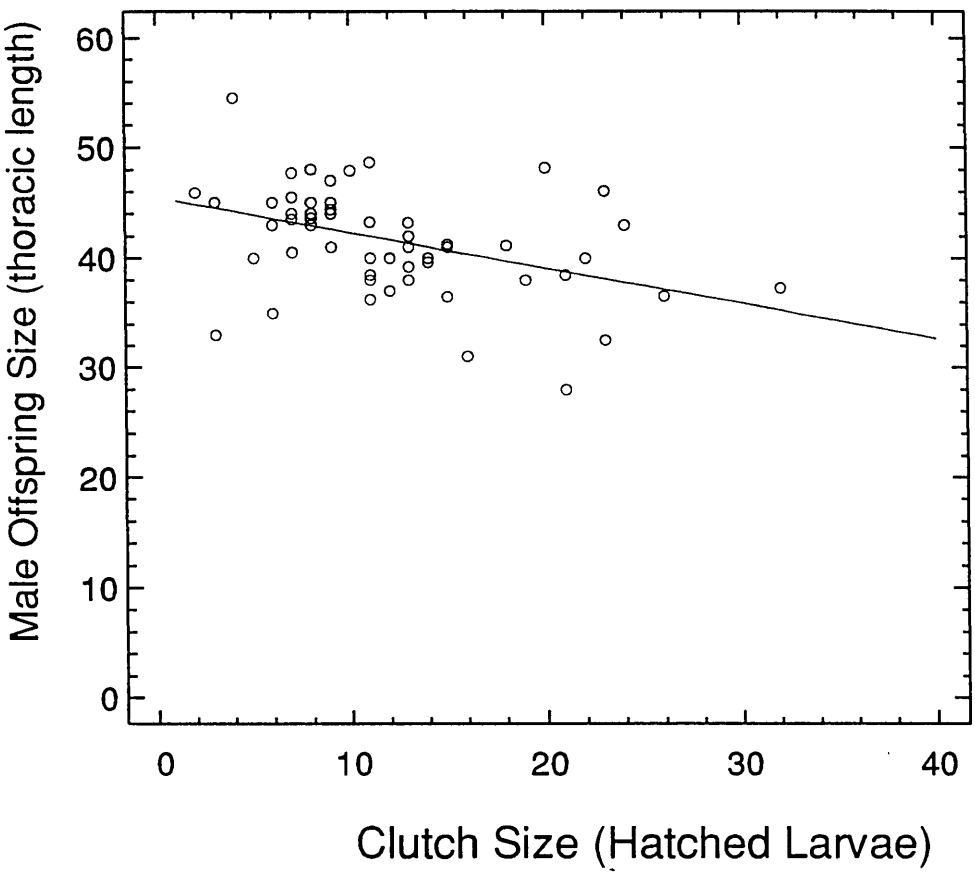


Figure 2.7 : Log. Lifetime Fecundity Versus Female (Parent) Size.

NB. Female size measured in graticule units. 50 units = 10^{-3} m.
Fitted regression is given by y = 0.0876.x - 0.7383 (r^2 = 29.7%).

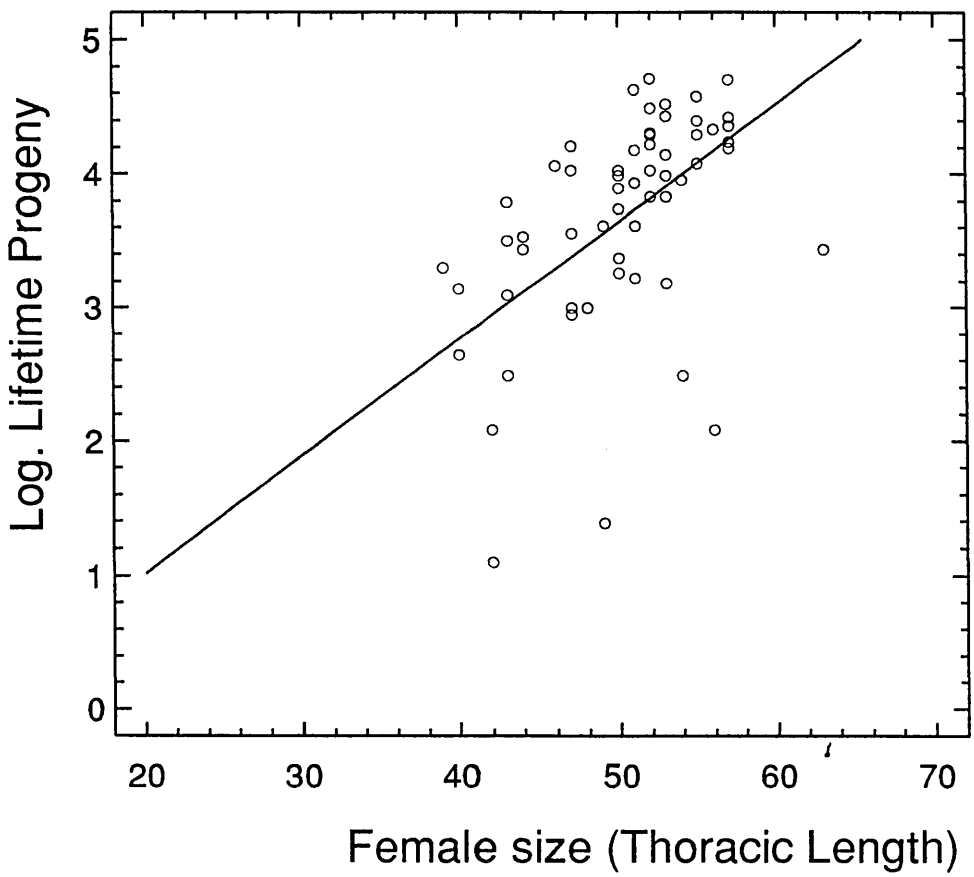


Figure 2.8 : Log. Lifetime Production of Daughters Versus Female (Parent) Size.

NB. Female size measured in graticule units. 50 units = 10^{-3} m.
Fitted regression is given by y = 0.06345.x + 0.1887 (r^2 = 15.7%).

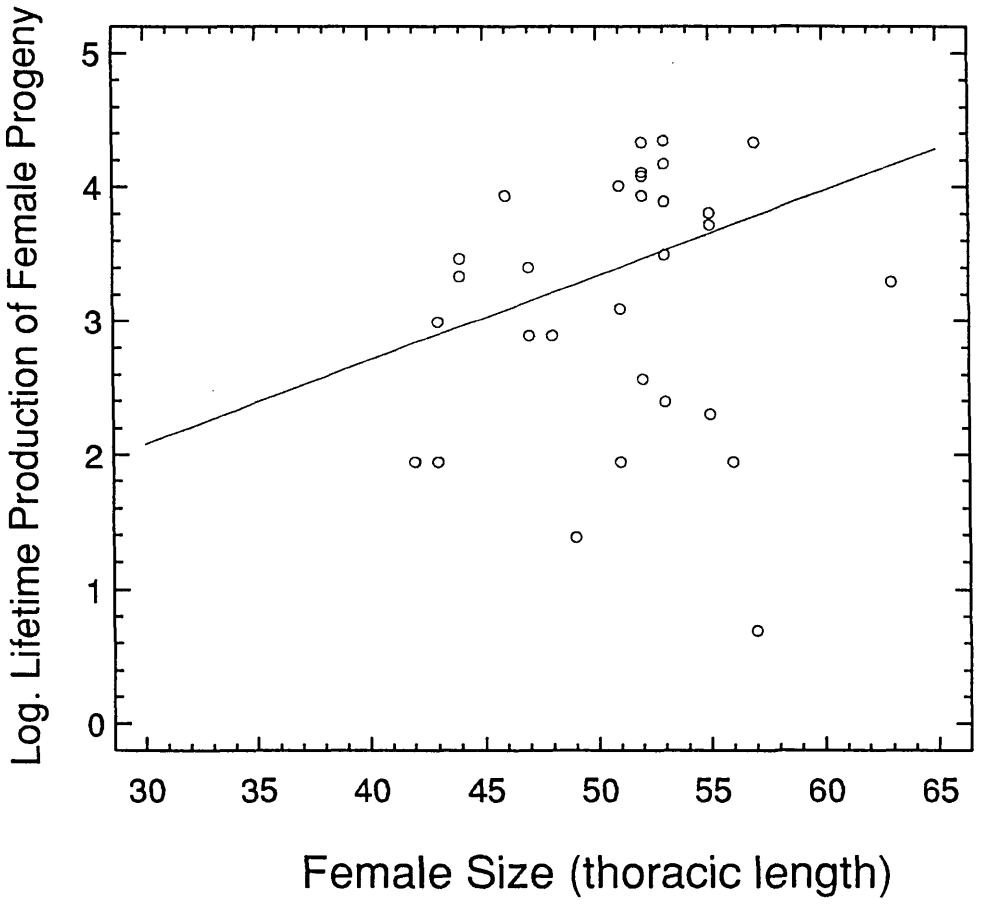


Figure 2.9 : Total Number of Broods Laid Versus Female (Parent) Size.

NB. Female size measured in graticule units. 50 units = 10^{-3} m.
Regression is given by y = 0.288.x - 8.117 (which explains 29% of the deviance).

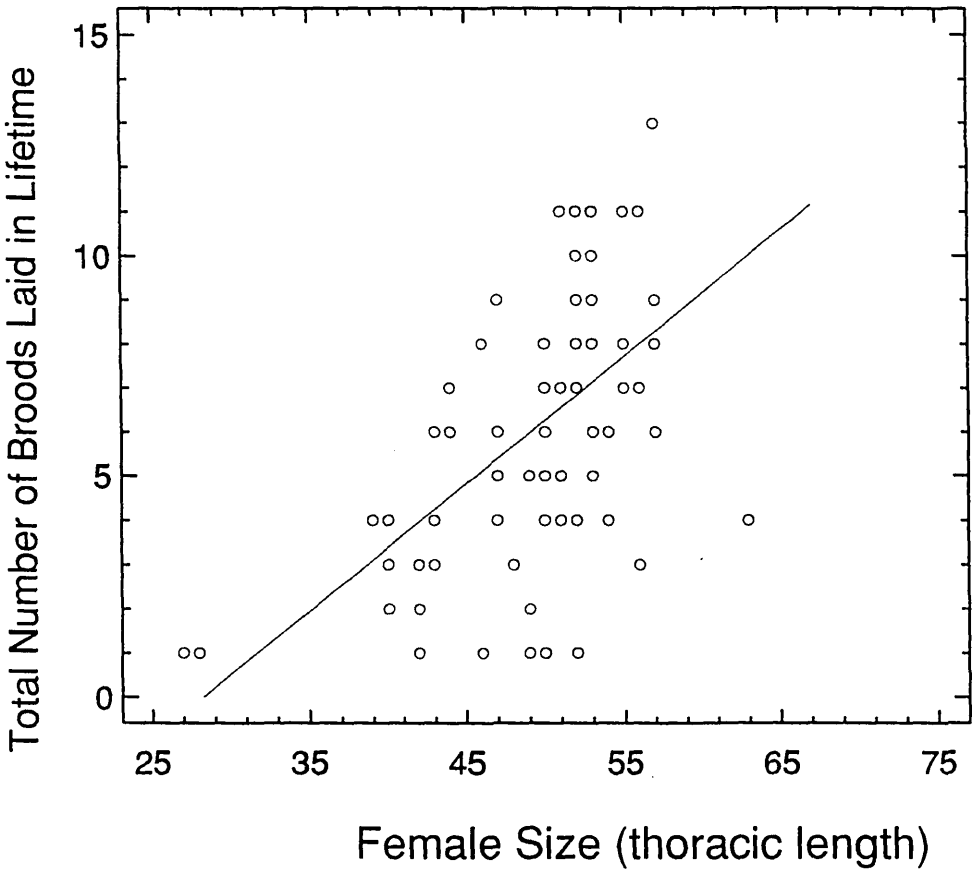


Figure 2.10 : Longevity Versus Female (Parent) Size.

NB. Female size measured in graticule units. 50 units = 10^{-3} m.
Regression is given by y = 1.76.x - 52.75 (r^2 = 31.3%).

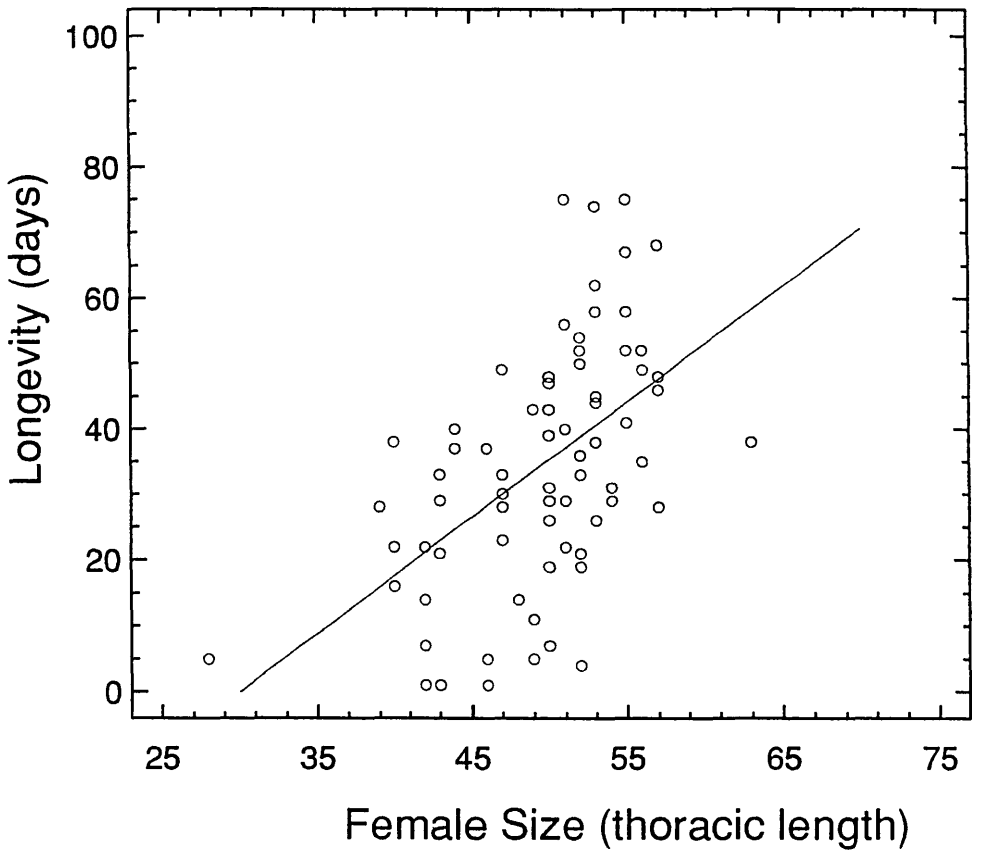


Figure 2.11 : Calculating the Lack Solution Clutch Size.

The projected fecundity of individuals in a given clutch size is calculated by inserting the regression for Female Size (Equation 1) into the regression for Log Total Progeny (Equation 2.) and shown in Figure 2.11 A. This can be simply converted to predict total progeny from females from a given clutch size in Figure 2.11 B. Finally, by multiplying total progeny by clutch size, one can determine where brood fitness is maximised in terms of total F2 generation (Figure 2.11 C). The dotted line indicates that brood fitness is maximised in clutch sizes of 22.9.

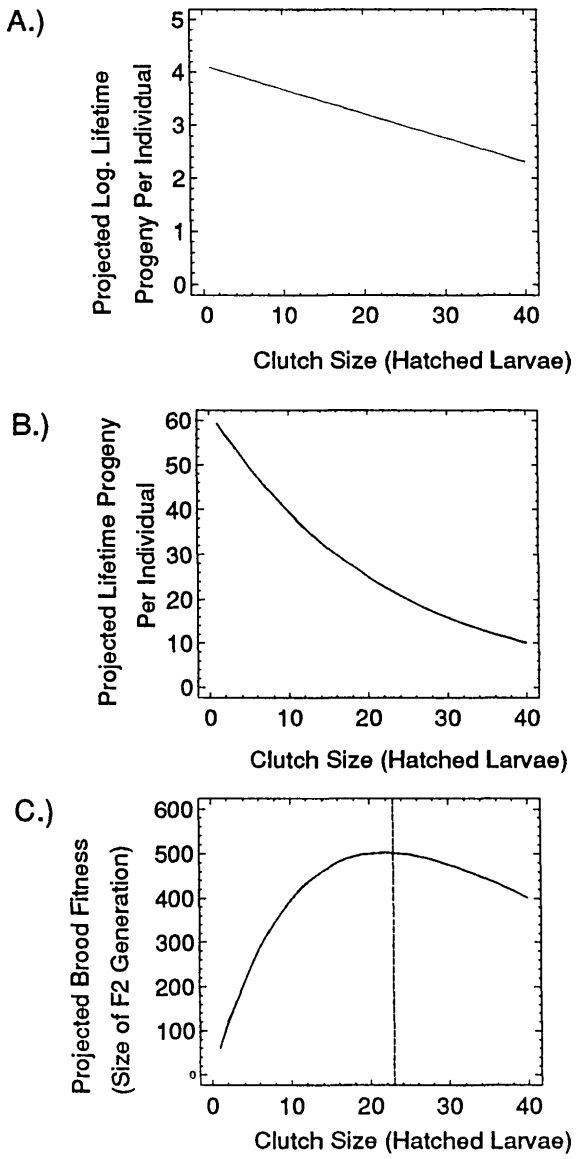
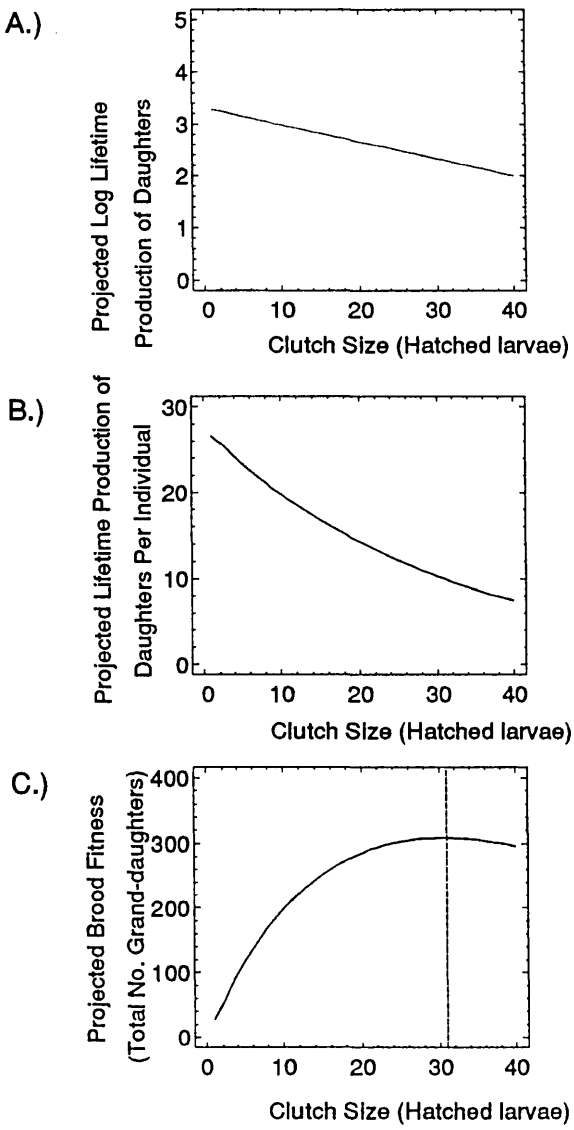


Figure 2.12 : Calculating the Clutch Size That Maximises Grand-daughters.

The projected Log Total No. of Females resulting from individuals in a given clutch size is calculated by inserting the regression for Female Size (Equation 1) into the regression for Log Total Number of Females (Equation 3.) and is shown in Figure 2.12 A. This is converted to predict lifetime production of daughters from females in a given clutch size in Figure 2.12 B. Finally, by multiplying the predicted total number of daughters by the associated clutch size, one can determine where brood fitness returns are maximised in terms of total number of grand-daughters from a given brood (Figure 2.12 C). The dotted line in Fig 2.12 C indicates the optimum at 31.2.



CHAPTER 3

OVIPOSITION BEHAVIOUR IN *Pegomya nigritarsis* (Anthomyiidae), A LEAF-MINING FLY

Chapter 3: Section 1

An Introduction to Oviposition in *Pegomya nigritarsis*

Background to the Study

The leaf-miner *Pegomya nigritarsis*, lays its eggs in discrete batches on the underside of leaves of its various *Rumex* spp hosts. The fitness of larvae varies according to clutch size and it is possible to predict an optimal clutch size, or "Lack solution" which maximises brood fitness returns for the parent. However unlike Lack's Great Tits or the parasitoid *Goniozus nephantidis* (see Chapter 2) where brood size is limited by fitness constraints associated with food availability, the optimal clutch size appears to be associated more with survivorship from parasitism. Godfray (1986a) showed that *P. nigritarsis* has a domed fitness function resulting from high levels of parasitism in small and large clutches and higher larval mortality in larger clutches. Hence intermediate-sized *P. nigritarsis* broods have, on average, a higher chance of surviving to maturity.

Godfray's study was made from a single collection site over a relatively short time period and therefore does not give any impression of seasonal and spatial differences in the behaviour of either the leaf-miners or the parasitoids which attack them. Also, while Godfray's work suggests that the most productive brood sizes are also those laid most frequently in the field, an alternative study (Cook, 1987 unpublished) indicates that the commonest clutch size is the one which maximises individual egg fitness. In both cases their data were drawn from single samples in a restricted time period leading to conclusions based on limited information. Furthermore while providing interesting results this kind of study does not satisfactorily explain the large amount of variation in leaf-miner clutch sizes which is found.

This project set out to continue and expand on Godfray's initial work by studying seasonal and spatial differences in the overall survivorship profile and by

attempting to explain the variance in clutch size by different factors such as date, leaf size and site. An attempt is made to study the oviposition patterns seen on individual plants through their life and dissections are used to predict the probability of larval mortality in different-sized clutches in order to predict a Lack solution. The change in the Lack solution (and in the modal clutch size observed) is monitored in four different sites through four sampling periods through a summer field season. The behaviour of individual parasitoid species is considered in terms of the probability of host attack according to clutch size and the distribution of attacks by the different parasitoids in individual host larvae.

The Biology of *Pegomya nigritarsis*.

P. nigritarsis is a leaf-mining anthomyiid fly, 5-6mm long, with a grey thorax and light brown abdomen. (See Hennig (1973), for a full taxonomic description and extensive bibliography.) It is a member of a monophyletic species group of leaf-miners within the genus *Pegomya*, which contains thirteen species in the British Isles.

Adult *P. nigritarsis* are active from May to November. Females lay their eggs singly or side-by-side in batches on the undersides of leaves of various *Rumex* spp. Eggs are approximately 3 x 0.5 mm in size, white with an areolated surface. Once the egg has hatched the larva mines directly into the leaf from the egg and feeds inside the leaf usually just beneath the upper epidermis (but occasionally utilising the full depth of the leaf). As larvae consume the leaf tissue the extent of the mine can be easily seen as a discoloured patch on the leaf. Occasionally, if the leaf tissues are exhausted, the larva may evacuate the leaf and initiate a new mine on another leaf.

This behaviour tends to be limited to final instar larvae. When ready for pupation, final instars leave the mine and enter the soil or leaf litter. Individuals either overwinter as pupae or emerge as adults later in the same season.

As it progresses through to adulthood *Pegomya nigritarsis* is exposed to several different mortality factors. Egg parasitoids have been recorded (Fulmek 1962), although in numerical terms they are relatively unimportant. Godfray (1986a) found a strong density dependent "larval death" mortality with increasing clutch size, comprising mortalities due to larval competition and starvation, invertebrate predation and parasitoid host-feeding. There are ectoparasitoids, although as with the egg-parasites their numbers do not lead to a significant independent mortality effect.

The greatest and most important mortality is due to endoparasitoid attack of the larval stage. Eggs are normally injected into first instar larvae. They hatch into their larval stage but do not pupate until the host itself has entered the soil and pupated whence they eventually emerge instead of the fly. It seems that oviposition is only successful when the leaf-miner host is in its first instar. As they get larger potential hosts become more adept at avoiding parasitism and eggs are rarely found in second and third instar larvae. Godfray (1986a) found that the cumulative effect of attack by different endoparasites led to a domed mortality function with maximum survivorship in intermediate clutch sizes.

A total of 9 different parasitoid species were found during the course of this study. For purposes of convenience, these can be divided into three categories (see Table 3.1.1 and Figures 3.1.1, 3.1.2). The most common parasitoid of *P. nigritarsis* on *R. obtusifolius* is the solitary braconid wasp species *Adelurola florimela*

(*Alysiinae*). The next major category of parasitoids is the solitary braconid wasps in the genus *Biosteres* (*Opiinae*). The remaining minor species including chalcid and braconid wasps are grouped together and considered *en masse*.

There is also parasitism of the larval and pupal stages in the soil although its effect on overall mortality levels is small. Furthermore parasitism in the soil is unlikely to be a selective force on the clutch size decisions of *P. nigritarsis* since it does not vary according to clutch size.

Table 3.1.1 The Endoparasitoids Found in *P. nigritarsis* Larvae, 1988

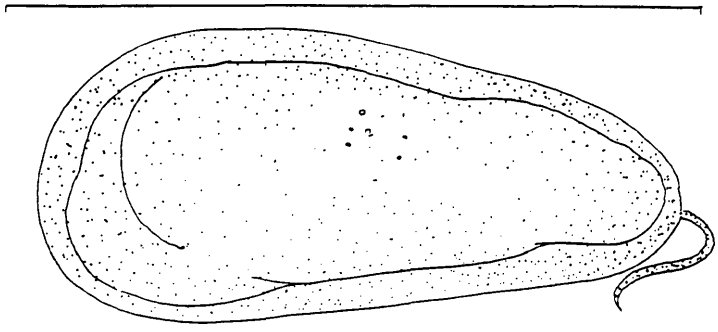
Estimates of proportion of hosts parasitised by each species obtained from rearing of pupae. (NB. These should not be confused with proportion of hosts attacked by the species since multiparasitism is common and there may be different parasitoid species in the same host larva, yet only one parasitoid can emerge.

Parasitoid Category	Species	Family	Proportion of Hosts Parasitised
A. florimela	*Adelurola florimela*	Braconidae	45.9%
Biosteres spp.	*Biosteres carbonarius*	Braconidae	14.7%
	Biosteres X	Braconidae	3.0%
	Biosteres waesmali	Braconidae	0.3%
	Biosteres rusticus	Braconidae	0.1%
Minor Parasitoids	*Lamprotatus splendens*	Pteromalidae	3.7%
	Trybliographa gracillipennis	Eucoilidae	1.8%
	Opius rufipes	Braconidae	1.0%
	Opius ruficeps	Braconidae	0.1%

Figure 3.1.1 Identification of the Egg and Larval Stages of *Adelurola florimela*

(N.B. Scale bars indicate 0.5mm length)

i.) Egg Stage - An immotile "tail" is clearly visible at one end. As hatching approaches the form of the developing embryo is clearly seen in the centre.



ii.) Larval Stage - N.B. hardened fighting mandibles and extended posterior spine (tail).

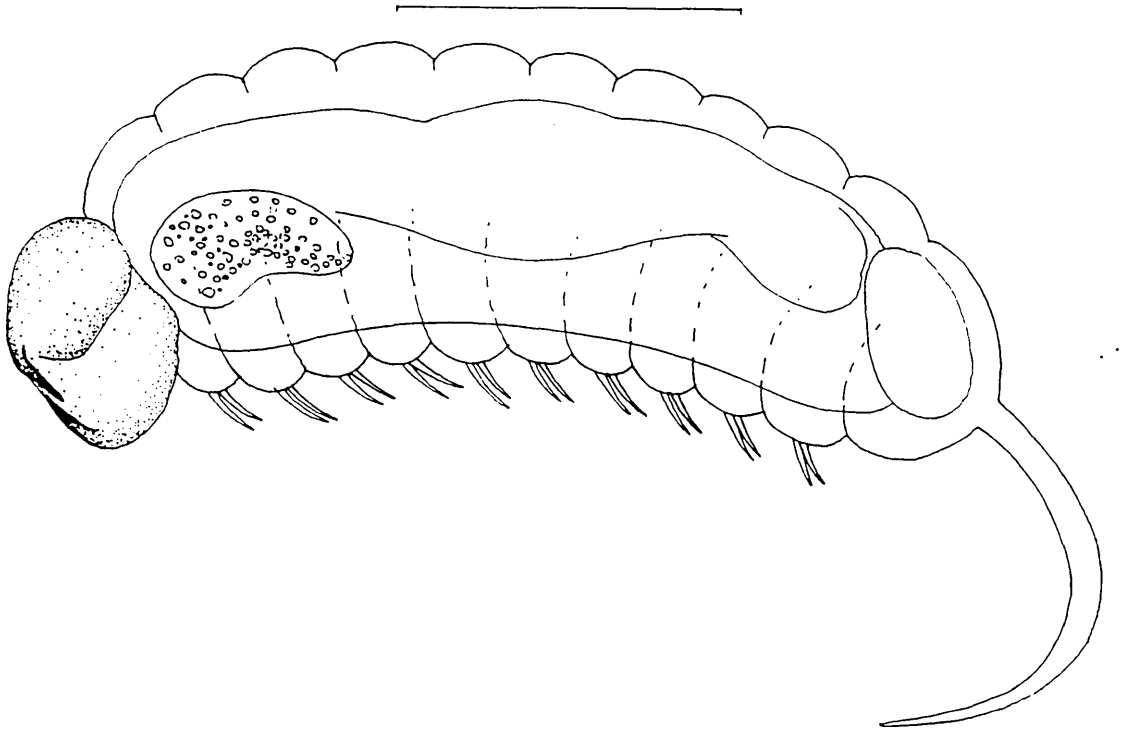
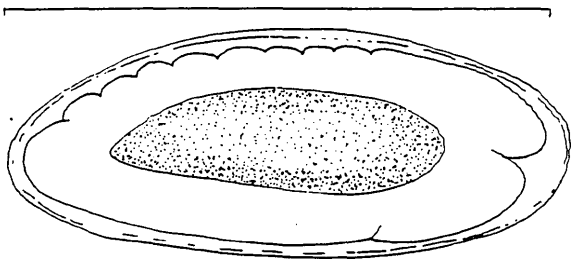
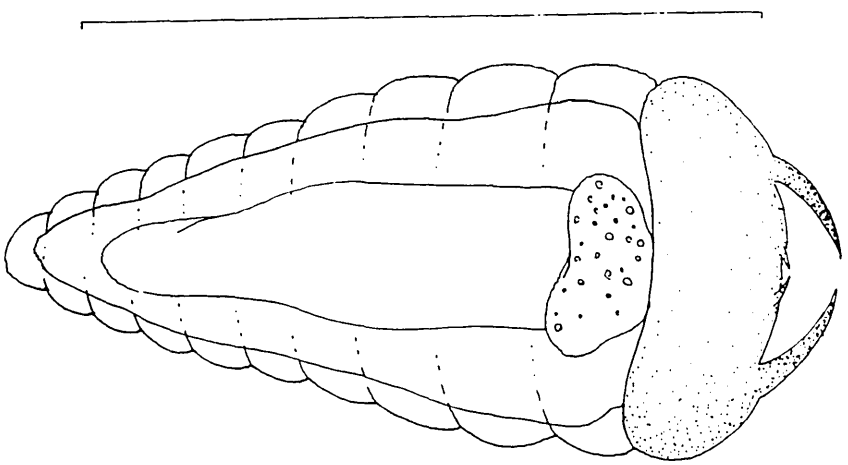


Figure 3.1.2 Identification of the Egg and Larval Stages of a *Biosteres spp.* Endoparasitoid (N.B. Scale bars indicate 0.5mm length)

i.) Egg stage - N.B. Tail-less and with less texture and pattern than *A. florimela.*



ii.) Larval stage - N. B. hardened fighting mandibles



Chapter 3 : Section 2

Selection of an Oviposition Site by *Pegomya nigritarsis*

Summary

1.) A tagging experiment following the progress of ten *Rumex obtusifolius* plants through the season was used to study oviposition decisions of *P. nigritarsis* through the season.

2.) The frequency distribution of new *P. nigritarsis* broods on leaves is highly aggregated implying that certain leaves in the field have a higher probability of being attacked than others.

3.) Ovipositing females tend to select leaves of a specific age range and size range. They also exhibit a preference for previously attacked leaves although it is not clear whether this is related directly to the presence of other eggs or whether this implies that certain leaves are universally more popular as oviposition sites.

4.) Once a suitable leaf has been selected for oviposition it is not clear what influences the clutch size decision of the female. A series of one-way analyses of clutch size deviance failed to find any effect of leaf size, leaf age, leaf position, stage of the season or pre-existing broods on the clutch size strategy of the leaf-miner.

5.) After all these different factors are fitted a large amount of variance in clutch size is still unaccounted for. There is also significant variation in clutch size between plants. It is suggested that this variation might be related to clutch size-specific parasitism anticipated at these different sites.

Introduction

This experiment was devised to study the discriminatory ability of ovipositing leaf-miners searching for suitable oviposition sites. Godfray (1986a) sampled leaves at random from stands of *Rumex obtusifolius* to see how clutch size might vary according to leaf size. Thus he could only compare oviposition from leaf to leaf and could not compare attacked leaves with unfavoured leaves. His study failed to show how insect behaviour might vary from plant to plant, or how insects might favour certain leaves on plants more than others.

In order to study effectively the leaf-miners' selection of oviposition sites, it was necessary to follow individual leaves throughout the season to see if progressive factors such as age or relative position of leaf on plant have a significant influence on their preferability for the leaf-miner.

When one is asking whether leaves are selected as oviposition sites at random or whether non-random selection preferences can be demonstrated one can compare the frequency distributions of leaf-miners' broods on leaves with a random frequency distribution such as the Poisson distribution. One can also test for aggregation of broods and compare the frequencies of leaf-miner broods on leaves with an aggregated frequency distribution such as the negative binomial distribution.

Another approach to studying the selection of oviposition sites is to look at the incidence of new clutches and relate this to aspects of the leaves on which they occur. Thus I also perform T-tests and Chi-squared tests on the differences between attacked and unattacked leaves in aspects such as age, size and position on the plant.

Finally I look at possible factors which influence the number of eggs which are laid, once a site has been selected. A series of factors and variables are fitted against the dependent variable, clutch size, to see if any of the variability in clutch size can be explained.

Methods 1 : Experimental Procedure

Ten separate *Rumex obtusifolius* plants were selected in May and their leaves individually labelled around the leaf stem. All plants were examined every week for new leaves (which were labelled) and new clutches. For each new clutch a record was made of date of first observation, size of brood, size of leaf, age of leaf (i.e. days since leaf was first recorded on plant), relative position of leaf on plant (a vertical index based on number of leaves above and below at the time of oviposition), the area of leaf surface already covered by previous mines and the number and size of any pre-existing clutches.

Methods 2 : The Analysis of Data

The Frequency Distributions of *P. nigritarsis* Broods on Leaves.

A helpful method when studying the selection of leaves as oviposition sites is to compare their frequency distributions with standard statistical distributions, which are known to describe particular patterns of dispersal. In order to test whether the distribution of individuals of a given category is non-random one tests to see whether the distribution differs significantly from a random model. Hence, the frequencies of the different numbers of clutches on leaves (including zero) were compared with a random (Poisson) distribution using a X^2 Goodness of Fit test to see if they differed

significantly from the distribution that would be expected if leaves were attacked at random. Under random conditions in a Poisson process, the variance of the number of broods per leaf will be equal to the mean.

In order to test whether the distribution of individuals conforms to an aggregated distribution, I compared the data to a negative binomial distribution, again using a X^2 Goodness of Fit test. The negative binomial distribution describes a form of aggregated distribution when the variance in scores is greater than the mean, resulting in a clumped distribution of individuals in the different frequency categories. The distribution is obtained from an expansion of;

$$(Q - P) - k$$

where P is the overall probability of an event (e.g. a leaf being laid on), Q is the probability of the event not occurring and $Q + P = 1$. k is related to the distribution of the event and can be used as an aggregation index with large values representing low aggregation and low values (e.g. less than 1) signifying high aggregation. This distribution has two parameters; the arithmetic mean μ (e.g. mean number of broods per leaf), which is equal to P x k, and the variance, σ^2, which is equal to the product of kPQ. When this series is given values for μ and σ^2 and is expanded for each frequency category (0, 1, 2, 3...etc.) it gives an aggregated frequency distribution where the variance is greater than the mean. This can be used to compare with real data using a X^2 Goodness of Fit test to see whether or not the data is significantly different to an aggregated distribution. Furthermore, the k-value for the data will indicate the extent of the aggregation (see above).

Preferences for Sites of Oviposition

Since all of the leaves on each plant were followed through the season it was possible to look at the distribution of new clutches according to three different aspects of the leaf; length, age and the presence of existing broods. Chi-squared tests were used to compare the observed frequency of new broods on leaves in different categories with the frequency expected if oviposition were entirely random. T-tests were also used to see whether the leaves selected for oviposition are significantly different to other leaves in terms of size and age.

One-Way Analyses of Deviance in Clutch Size

The results were statistically analysed on GLIM with the assumption of poisson-distributed errors in the response variable clutch size. Different factors such as leaf dimensions, leaf age, stage of season, previous oviposition on the leaf and position of the leaf on the plant were fitted against clutch size to see how much of the clutch size deviance they accounted for and determine their influence (if any) on the clutch size decision.

Results

The Frequency Distributions of *P. nigritarsis* Broods on Leaves.

The total number of individuals is found by multiplying each frequency by its corresponding frequency size class and taking the sum total. The total number of hosts (either leaves or host larvae) is simply the sum of the frequencies since each score represents the number of broods on a single host unit.

The frequency distribution and Goodness of Fit tests for *P. nigritarsis* broods on leaves are shown in Figures 3.2.1 i.) to iii.). As in the case of the parasitoids' frequency distributions, the total number of broods is the sum of frequencies multiplied by their respective size classes. The total number of leaves available is obtained from the sum of the frequencies since each score represents the number of broods per leaf.

Table 3.2.1 ii.) shows clearly that not only is the distribution of leaf-miner clutches on leaves significantly different to a Poisson distribution but it conforms closely to the negative binomial distribution and hence is highly aggregated (Table 3.2.1 iii.)). (In the frequency distribution in Table 3.2.1 i.) one can see that despite there being many more broods laid than available oviposition sites (i.e. leaves), over 50% of leaves had no clutches on at all.)

Preferences for Sites of Oviposition

The results of the analysis of selection of leaf size are shown in Table 3.2.2.1. A T-test showed that selected leaves are larger than average leaves ($P<0.001$) (Table 3.2.2.1.ii.). In all there were three sampling dates (a fortnight apart) when sufficient numbers of new broods were recorded for an analysis of their frequency on different leaf sizes. When leaves were divided into six size categories (each covering a range of 10 cm) a chi-squared test showed that the selection of leaves was non-random in two out of the three samples (Table 3.2.2.1.i).

Since new leaves were recorded it was possible (after the first sample on day 4) to use a rough estimate for age to see whether the incidence of new clutches on leaves was random or whether a particular age was preferred. Studies on oviposition preferences for certain ages of leaf are summarised in Table 3.2.2.2. A T-test showed

that new clutches are laid on younger than average leaves ($p<0.001$) (see Table 3.2.2.2.ii.). A chi-squared test showed that the distribution of new clutches was significantly non-random in both samples for which there was enough data on new clutches (Table 3.2.2.2.i.).

Additionally, females seem to favour leaves which have been previously attacked. Table 3.2.2.3 shows a study on the oviposition preference regarding leaves with or without eggs already present on them. A 2 x 1 Chi-squared test shows that the frequency of new clutches on leaves is non-random ($p<0.001$) (see Table 3.2.2.3.i.). However it is not clear whether they are attracted to other broods *per se* or whether some aspect of certain leaves makes the leaf universally popular with ovipositing females. A T-test showed that leaves with a single clutch present were significantly smaller than leaves with more than one clutch ($p<0.001$) (Table 3.2.2.3.ii.).

One-Way Analyses of Deviance in Clutch Size

The results of the one-way analyses of clutch size deviance are summarised in Table 3.2.3. The results are presented in terms of the change in clutch size deviance and loss of degrees of freedom resulting from fitting the factor or variable. This can be Chi-square tested, hence the statistical significance of the change is given. Also the change in deviance is given as a percentage of the total deviance in clutch size which is explained by that factor or variable. This indicates the biological significance of the change in deviance. When the total deviance is very high a change in the deviance may be shown to be statistically significant although the actual reduction in total deviance may be relatively small. When looking for factors and variables which might affect the clutch size decision we are mainly interested in the biological significance of their explanatory effect.

In this analysis it was found that neither leaf length nor any other leaf dimension could explain a significant amount of the deviance in clutch size. For each leaf size variable there was statistical evidence for a slight positive correlation but the effect on the total deviance in clutch size is marginal.

The time in the season did not appear to account for a significant proportion of the deviance in clutch size. Furthermore the number of eggs and the number of broods already on the leaf at the time of oviposition did not explain any of the deviance in clutch size. The area of leaf already covered by mines had virtually no effect on the clutch size decision. The position of the leaf on the plant made a negligible difference to clutch size as did the age of the leaf at the time of oviposition.

However when plants were considered as 10 separate factors in the analysis the deviance in clutch size was reduced by 24% which was significant. In other words the variation in clutch size between plants is much greater than the variation within plants.

When the variation between plants was controlled for by fitting plants as a factor before fitting either date or leaf length, the effect of stage of the season or leaf size on the clutch size decision was even less.

Discussion

The study of frequency distributions and the tests of selection of new oviposition sites showed that *P. nigritarsis* broods are not distributed randomly on leaves but are distinctly aggregated. i.e. Certain leaves have a higher probability of being attacked than others. This may occur either through some aspect of the distribution of leaves in the field (e.g. females maximise their encounter rate with leaves by concentrating on areas of high leaf density) or by virtue of some aspect of the leaves themselves. Unfortunately this study made no measure of leaf density in the vicinity of the tagged plants so it is not possible to test this further. However, the tests of oviposition site preferences clearly show that the occurrence of new broods on leaves is non random with respect to certain aspects the leaves.

Females prefer a specific leaf size range (in which, overall, leaves are larger than average) and a specific age range (where leaves are younger than average.) Females prefer to oviposit on leaves on which broods have already been laid although it is not clear whether other broods are in themselves attractive or whether their presence signifies suitability of that leaf as a site of oviposition. Most probably the latter is the case since preferences for particular age and size have been demonstrated. Leaves with more than one clutch were, on average, significantly larger than those on which just one clutch was laid.

Once the decision is taken to lay it is not clear what influences a female in the actual number of eggs she lays in that clutch. This experiment failed to find evidence of a correlation between her decision of how many eggs to lay and any aspect of the leaf or plant biology. There was no evidence to suggest that clutch size is related to leaf size or leaf age, position on plant or time of season. Neither did the number of

broods (or eggs) already on the leaf appear to affect the clutch size laid. It remains to ask what accounts for all the variation in clutch size. Clutch sizes range from 1 to 9, so what is the female *P. nigritarsis* responding to?

Godfray (1986a) showed that survivorship of parasitism varies according to the clutch size which is laid. In this case the chief fitness constraint is the mortality inflicted by parasitoids which depends on the clutch size decision. Thus we might expect variation in clutch size to be associated with parasitism rather than with physical attributes of the larval resource. Certainly there is little evidence here for correlation between clutch size and larval resource availability (i.e. leaf size). Moreover the fact that super-ovipositing females (i.e. on previously attacked leaves) did not alter their brood size in response to existing eggs or broods also suggests that larval competition for the food resource is not the main influence on the clutch size decision. It then remains to ask how ovipositing females might respond to parasitism to produce the variation in clutch size. If the female is capable of assessing the risk of parasitism at the oviposition site in some way then she might facultatively control each clutch size to maximise the fitness of the brood. More probably, variation in clutch size is stochastic, either as a result of random quirks, mistakes, interruptions etc., or as many females pursue separate strategies.

The fact that variation in clutch size between leaves and plants is so high might therefore be attributed to the different levels of parasitism associated with each individual leaf or plant. Obviously a study of the relationship between clutch size and parasitism is beyond the scope of this particular experiment.

Tables

Table 3.2.1 : The Frequency Distributions of *P. nigritarsis* broods on Leaves.

Using the data from the 1987 Leaf-tagging Experiment (Section 1 of this chapter) the total frequency distribution of *P. nigritarsis* broods on *R. obtusifolius* leaves was fitted against a random (Poisson) distribution and an aggregated (Negative Binomial) distribution. A significant X^2 value means the distributions are significantly different to each other.

i.) : Frequency Distribution of *P.nigritarsis* broods

No. of Broods	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total/ Leaves
Freq.	77	20	7	9	5	2	3	4	0	0	0	4	0	1	194/132

ii.) : Test of Goodness of Fit With POISSON Distribution

X^2 Value	D.F.	Significance of the Difference	Conclusion About Distribution
375.5	5	**$p < 0.001$ *****	Non-random

iii.) : Test of Goodness of Fit With NEGATIVE BINOMIAL Distribution

X^2 Value	D.F.	Significance of Difference	Value of k	Conclusion About Distribution
2.04	5	p = NS	0.313	Highly Aggregated

Table 3.2.2 : Selection Criteria For a Site

Table 3.2.2.1 : Leaf Size and The Occurrence of New Broods

i.) Chi-Squared Test; Leaf Size Selection

Leaves were divided into six size categories and at each sampling period, the number of new clutches found in each category was compared with that which would be expected if leaves were targeted randomly. (Sample size = 461.)

Date	Chi-squared Value	Significance
4	3.91 df=5	NS
18	34.90 df=5	$p < 0.001$
32	15.47 df=5	$p < 0.01$

ii.) T-Test; Leaf Size Selection

Mean size of leaves on which new clutches were laid was compared with mean size of unattacked leaves. (Sample size = 461.)

	Mean Length (cm)	Standard Error	t-value
Unattacked leaves	21.46	0.317	6.75 df=459
Oviposited leaves	26.99	0.756	$p < 0.001$

Table 3.2.2.2 : Leaf Age and the Occurrence of New Broods

i.) Chi-Squared Test; Leaf Age Selection

Since the date of appearance of new leaves was recorded, leaves in any sampling period could be approximately aged. The frequency of new clutches in the different age categories was compared with that which would be predicted if leaf selection were entirely random. Unfortunately the scope of this analysis was limited by the range of known leaf ages and the scarcity of new clutches later in the season on these plants. (Sample size = 297.)

Date	Chi-squared Value	Significance
18	11.36 df=1	$p < 0.001$
32	8.47 df=2	$p < 0.05$

ii.) T-Test; Leaf Age Selection

Mean age of leaves on which new clutches were laid was compared with mean age of unattacked leaves. (Sample size = 297)

	Mean Age (days)	Standard Error	t-value
Unattacked leaves	34.89	0.927	3.47 df=295
Oviposited leaves	25.24	2.622	$p < 0.001$

Table 3.2.2.3 : Presence of Existing Clutches and the Occurrence of New Broods

i.) Chi-Squared Test; Presence of Other Broods

A 2 by 1 contingency table was used to compare the frequency of new clutches on fresh leaves with the occurrence of new clutches on leaves with broods on already. (Sample size = 137.)

	Observed Frequency	Expected Frequency	Chi-Squared
Only Brood on Leaf	42	68.5	20.5 d.f.= 1
Other Broods on Leaf	95	68.5	$p < 0.001$

ii.) T-test; Size Difference Between Leaves With First Clutch and Leaves with More Than One Clutch

The mean size of leaves with one brood on was compared with the mean size of leaves with more than one brood (sample size = 137)

	Mean Length (cm)	Standard error	t-value
Only Brood on Leaf	21.90	0.849	6.59 d.f.= 135
Other Broods on Leaf	28.62	0.565	$p < 0.001$

Table 3.2.3 : Factorial Analysis of Experiment 2

Summary of the results of a series of one-way analyses of deviance in clutch size showing the maximum amount of the deviance attributable to each factor.

Factor	Change in deviance (Chi-square test)	Percent change (Biol.sig.)	Sample Size
Leaf Length	11.31 df=1 X^2<0.005	8.8%	137
Leaf Width	7.49 df=1 X^2<0.010	5.8%	137
Leaf Area	8.85 df=1 X^2<0.005	6.9%	137
Date	3.00 df=1 X^2=NS	2.3%	137
Prior broods	2.39 df=1 X^2=NS	1.9%	137
Prior eggs	4.05 df=1 X^2<0.05	3.2%	137
Mined area	1.24 df=1 X^2=NS	1.3%	108
Pos.on plant	0.07 df=1 X^2=NS	0.05%	137
Age of leaf	1.34 df=1 X^2=NS	2.4%	62
Ind. plants	30.73 df=9 X^2<0.005	24.0%	137
Fit After Ind. plants			
Leaf Length (after plant)	0.80 df=1 X^2=NS	0.8%	137
Date (after plant)	2.30 df=1 X^2=NS	2.3%	137

Chapter 3 : Section 3

Optimal Clutch Size in the Leaf-miner, *Pegomya nigritarsis*.

Summary

1.) An analysis of deviance shows that neither site nor month of oviposition explain any of the large amount of variation in clutch size. And while leaf size explains up to 11% of the deviance in clutch size the majority of the deviance still remains unaccounted for.

2.) Similarly an analysis of deviance in larval mortality found that neither site, month, clutch size nor leaf length could explain a significant portion of the large amount of deviance in either of the two main larval mortalities nor in the total larval mortality.

3.) Despite the high variation in this experiment, a study of survivorship versus clutch size through the season suggested that larval fitness (the probability of survival from egg to pupal stage) decreases with clutch size.

4.) The Lack solution, i.e. the clutch size which is predicted to maximise brood fitness is 4 in the first month and 3 for the rest of the season. Accordingly the modal clutch size observed every month was 3, suggesting that females attempt to lay the clutch size which maximises the fitness returns from each brood. Clutch size theory predicts this behaviour when a female's fitness is limited chiefly by her opportunities to produce broods.

5.) The monthly profiles of survivorship in different clutch sizes were broken down for each of the three parasitoid categories. Host survival from attack by *Adelurola florimela* shows considerable variation with both clutch size and the month from which the sample came. Since *A. florimela* is the most abundant of the parasitoid groups it has the strongest effect on the monthly profiles of survival from larval endoparasitism versus clutch size.

Introduction

The clutch size decision of a female *Pegomya nigritarsis* has strong implications for her fitness (Godfray 1986a). Females have the ability to control clutch sizes for different situations and broods range in size from 1 to 9 eggs per brood. Assuming that the variation in clutch sizes is adaptive and that the female is maximising her fitness at all times then we must understand what influences each decision she makes. Consequently, in this section there are three main areas of investigation of clutch size in *Pegomya nigritarsis*.

First of all I study the variability in clutch size and relate this to possible explanatory variables such as the size of the leaf, the site of the leaf and the stage of the season at which the clutch was laid.

Second, I study the variability in individual larval mortality according to factors such as clutch size, leaf size and site and month of oviposition. As a female seeks to maximise her lifetime fitness through her oviposition strategy she will experience an upper limit on her fitness gain from each clutch which will determine her clutch size decision. A study of the variability in larval mortality (a measure of offspring fitness) should help to indicate how a female can maximise her fitness gain rate through her ovipositing life.

Third, I look at the direct relationship between clutch size and larval fitness and the subsequent relationship between clutch size and brood fitness (found by multiplying individual fitness by the number of individuals in the brood). The major mortality imposed upon developing *Pegomya nigritarsis* larvae is inflicted by the hymenopterous endoparasitoids which attack the larvae in the mines. After leaving the leaf, larvae pupate in the soil and any mortality factors which arise thereafter are unlikely to be a function of clutch size (see Section 1 - Biology). Hence, the

probability of individual larval survival to pupation should be a good indicator of the individual fitness of *P. nigritarsis* eggs in relation to the clutch size in which they are laid.

Godfray's (1986a) study showed that individual host larval survivorship of endoparasitism varies with clutch size. He found that the female's fitness returns per brood (the sum of the individual offspring fitnesses) could be maximised in intermediate clutch sizes. Clutch size theory predicts that when opportunities to produce clutches are rare, females should maximise their fitness returns per brood and lay the Lack solution clutch (Charnov and Skinner 1984, 1985, Godfray 1986a). If eggs are limiting then females should maximise individual fitness. i.e. They should lay the clutch size at the peak of the individual survivorship profile. For a monotonically declining profile this will be at clutch sizes of one. (However Godfray (1986a) found a domed individual survivorship profile for *Pegomya* whereby individual fitness was maximised in intermediate clutch sizes.)

Hence in this Section, profiles of individual survivorship versus clutch size are used to repeat and expand on Godfray's (1986a) initial study. Individual fitness and brood fitness profiles are studied through a complete field season along with the corresponding mean and modal clutch sizes which are found in order to understand how females maximise their fitness.

The profile of larval survival of endoparasitism is the cumulative effect of a total of nine different parasitoid species which attacked *P. nigritarsis* larvae through the season. One can envisage that inter-specific variation in their host searching strategies may lead to differing profiles of survivorship with host clutch size and the effect of each parasitoid on the overall survivorship profile will be proportional to its relative frequency. Thus I look at the relationship between clutch size and the probability that

a host is attacked by a member of one of the three main groups of parasitoid (described in Section 1 of this chapter). In leaf mines collected through the season *Adelurola florimela* was reared from approximately 65% of parasitised hosts, *Biosteres* spp. from 25% and the remaining 10% were parasitised by minor parasitoids. The clutch size profiles were broken down into host survivorship from each of the three groups of parasitoids to see if differences in the parasitoids' response to clutch size could be identified and related to the overall profile of parasitism in that month.

Thus the aim of the work in this section is primarily to understand what influences variation in *Pegomya* clutch sizes; to study the frequency of different clutch sizes through the season and to identify influential factors and variables. An analysis of larval mortality of *Pegomya* is conducted to see whether any factors or variables may be identified as exerting a significant influence on larval fitness. Finally, profiles of probability of larval survival against clutch size are analysed to establish how individual larval fitness and overall brood fitness change according to the stage of the season at which the brood is laid. The overall profiles are also broken down into survival from each of three different parasitoid categories to look for interspecific differences in their distributions in different clutch sizes.

Methods 1 : Field Sampling

Mined *Rumex obtusifolius* leaves were sampled randomly from four separate sites on a monthly basis from May through to September. Individual samples consisted ideally of at least 200 clutches. (However this was not possible in the last month of the season and the final sample (month 4) contains data collected from August and September.)

For every *Pegomya* brood collected, a record was made of date and site of collection, size of host leaf, number of eggs, number of larvae, and the coexistence of any other broods on the same leaf.

Leaf-miner larvae were removed from their mines and dissected using mounted needles by removing the anterior segments and squeezing out the body contents under water. All endoparasitoid eggs and larvae visible under X7 magnification on a binocular dissecting microscope were identified and recorded. Parasitoids were divided into three general categories (see Section 1 of this chapter for figures of egg and larval stages). The first category is a braconid species called *Adelurola florimela.* The second is, more correctly, a group of species which is referred to as the *Biosteres* group comprising braconids - predominantly of the species *Biosteres carbonarius* although three other closely-related species were reared from pupae. Since these are not distinguishable at the egg or larval stages they are grouped together for this analysis. The remaining parasitoids are grouped together and considered as a third category of minor parasitoids.

The approximate width of the anterior segment of the host larva was measured to give an indication of host size. For this analysis broods were only used where the larvae were between the second and fourth instars (inclusive) i.e. an age range within which they would have been fully exposed to parasitism yet would not be old enough to evacuate the leaf (see Section 1 concerning biology of *Pegomya nigritarsis*).

Thus for every *Pegomya* brood there was a record of date and site of collection, size of host leaf, number of other co-existing broods on the leaf, clutch size, number of live larvae and number of unparasitised larvae. For every host larva there was a record of larval headwidth, and the number of parasitoids (eggs and larvae) from each category.

Methods 2: The Analysis of Data

The range of information recorded for each brood, allows us to study the egg-laying decisions of *P. nigritarsis* in several ways. As outlined in the introduction to this section, there are three main areas of study;

1.) An analysis of the variation in clutch size can be performed using other variables such as site and month of oviposition to identify the main effects that influence the egg-laying decision.

2.) A similar analysis can be performed on the variation in offspring mortality to identify factors which affect the probability of survival of larvae in different clutch sizes, at different stages.

3.) A study of how brood fitness varies with clutch size. As clutch size increases so the probability of offspring survivorship of mortality changes. The relationship between survivorship of different mortalities and clutch size was studied from samples collected through the summer.

The methods for these procedures are described below.

General Procedure For The Three-Way Analysis of Codeviance

The purpose of an analysis of deviance is to study how variation in a character such as clutch size can be explained by fitting different effects to see if they have a significant effect on its deviance. When there is normal error variance GLIM fits models by minimising the sum of the squares of the differences. Thus the total deviance in the dependent variable is the sum of the squares of the differences of data points from the Grand Mean for that variable. When the error variance is more complex e.g. with Poisson or Binomial error variance, the method GLIM uses is a more complex

maximum likelihood method analagous to a least squares procedure. If an explanatory effect (i.e. fitted model) is a continuous variable then the deviance is reduced as the differences of points from the fitted regression line is reduced. If the model is a discontinuous factor, deviance will be reduced if fitting each level of the factor reduces the sum of the differences of data points from the model.

A two-way analysis of codeviance involves applying a combined model of two factors and a variable to explain variation in the dependent variable. Table 3.3.1 outlines the general procedure for analysing the deviance in a variable Y (which has 1000 data points) according to two factors, F1 (with 3 levels) and F2 (with 5 levels) and a variable V. The values of deviance shown were made-up arbitrarily.

Interactions between explanatory effects can account for a significant proportion of the deviance if some effect of the combined terms leads to variation in the response variable. It is important to establish how the dependent variable responds to the three separate effects and therefore interaction terms must be included in the model.

Initially the total deviance in the variable Y is found by a general fit through the dependent variable (Stage 1). In this case the total deviance is 1000. Next, a "complete" model is fitted comprising both factors, the variable, the three-way interaction term and all two-way interactions (Stage 2). The remaining deviance (500.0) signifies the variation in clutch size alone after the main effects have been applied.

Next the 3-way interaction between F1, F2 and V is removed from the complete model. (Stage 3). The resulting increase in clutch size deviance signifies the amount of deviance explained just by that interaction term, in the case of the example it is 10.0. The 3-way interaction term is now excluded from the model and the three 2-way interactions are removed individually (and replaced) to see how much deviance each

accounts for (Stage 4). Note that the change in deviance is measured from the model after the 3-way interaction has been excluded but that the 2-way interactions are removed one at a time and replaced before the next interaction is removed.

In the next stage a model with the three characters without the interactions is fitted against clutch size (Stage 5). Single effects are then removed from the model individually (and then replaced) to see what is the minimum amount of deviance attributable to that factor (or variable) alone.

Finally in Stage 6, a grand mean is fitted to find the total deviance in clutch size (This is the same model that was fitted in Stage 1.) The single effects are fitted against this to see how the total deviance in clutch size decreases. This indicates the maximum deviance attributable to that factor (or variable). (N.B. Due to some orthogonality between variables it may occasionally occur that the maximum attributable deviance is actually less than the minimum. However since this effect usually occurs for small and insignificant changes in deviance it is unlikely to be critical.)

Since the change in deviance after fitting a model can be conceived as the sum of the differences between observed and expected values of deviance (differences squared), the statistical significance of changes in deviance can be tested using the Chi-squared statistic. The degrees of freedom lost by fitting the particular model in question are used to look up the level of significance on Chi-squared tables.

In addition the change in deviance was measured as a percentage of the deviance explained by the model to ascertain the biological significance of an effect. Particularly when sample sizes are large it is possible to show that a change in deviance is statistically significant when in real terms the effect on the total deviance is minimal.

The statistical analysis of the data was carried out using the generalised linear interactive modelling (GLIM) program (McCullagh and Nelder 1983). This is highly suited to analyses of this sort as it fits models by a sophisticated maximum likelihood procedure and has the particular benefit of being able to incorporate assumptions of abnormal error variance in its fits.

Analysis of Codeviance in Clutch Size Due to Leaf Length, Month and Site

An analysis of codeviance was carried out to see how the dependent variable clutch size might vary according to leaf length, site and month of collection. Site and Month were treated as factors (with 4 and 5 levels respectively) and leaf length was fitted as a variable.

Since the y-variable was clutch size, the error variance was assumed to be poisson-distributed and the assumption was built into the GLIM fitting program.

Analysis of Codeviance in Survivorship of Each Mortality

An analysis of codeviance in larval survivorship was conducted to see whether variation in larval survivorship could be explained by certain factors or variables.

As outlined in the introduction, the survivorship profile of *P. nigritarsis* larvae to pupation is the result of two main mortalities. There is a monotonic density dependent "larval death" mortality (including components such as starvation, predation, interference from other larvae), and a mortality due to endoparasitic attack. In separate analyses each mortality was considered as the dependent variable and finally the dependent variable was total probability of survivorship of larvae to pupation. A measure of survivorship of larval death was taken for each brood by

dividing number of live larvae by clutch size. Survivorship of parasitism was calculated by dividing number of unparasitised larvae in a brood by total number of live larvae. Overall probability of survivorship was the proportion of unparasitised larvae out of the original clutch size.

Two different sets of effects and variables were used to study variation in each of the three mortality analyses. In one set of analyses clutch size is treated as a variable with site and month of oviposition as factors. In the other set of analyses clutch size is treated as a discrete factor with month of oviposition, the continuous explanatory variable being leaf length.

The analyses were performed on GLIM. Since all records of survivorship are given as a proportion of survivors out of an initial total, binomial errors in the dependent variable were assumed.

Deviance Explained By Site, Month and Clutch Size

Site and Month were treated as factors (with 4 and 5 levels respectively) along with clutch size as a variable. These were used to study the deviance in both of the individual mortalities and in the total mortality (with both combined.)

Thus far clutch size has been fitted by linear regression. However Godfray's (1986a) analysis suggested that the profiles of survivorship of parasitism versus clutch size and total survivorship versus clutch size may be domed. Thus for these two analyses a curvilinear regression was simulated by fitting a quadratic expression (i.e. $C + C^2$ where C is clutch size) against mortality.

Deviance Explained By Leaf Length, Month and Clutch Size

One other possible variable which might influence larval mortality is Leaf Length. However since this type of analysis only allows one variable, clutch size was fitted as a factor with all clutch sizes of five and greater being grouped together. An alternative three-way analysis of codeviance in mortality was conducted, this time with Clutch Size and Month as the discrete factors (with 5 and 5 levels respectively) and Leaf Length as the variable. The analysis was performed separately for both mortalities and then for total survivorship from eggs through to pupation.

Larval Survivorship of Endoparasitism and Clutch Size

There are two main mortalities faced by *Pegomya* larvae (Godfray 1986a) which vary with clutch size and thereby influence brood fitness. The first is a "larval death" mortality comprising death due to starvation and interference from other larvae. The second is mortality due to endoparasitic attack. The relationship between clutch size and each of these mortalities was studied individually and in combination. Probability of survival of larval death in a brood is found by dividing the number of live larvae in a brood by the number of eggs. Probability of surviving parasitism is taken by dividing number of unparasitised larvae in a brood by the total number of live larvae in that brood. The probability of overall survivorship is found by dividing number of unparasitised larvae by the original clutch size.

An analysis of survivorship of *Pegomya* larvae against clutch size was conducted by plotting mean probability of survival of a given mortality against clutch size through the season. This is effectively a profile of individual larval fitness according to clutch size.

Brood fitness is calculated by multiplying probability of survival by the size of the brood in question. The Lack solution, where fitness returns to the female are maximised is found at the peak of the profile. For each month a profile of brood fitness versus clutch size was produced.

Survivorship Profiles For Each Parasitoid Category

Using the data from each host larva the presence or absence of the given parasitoid was scored as either zero (present) or one (absent) such that the mean score for each clutch size represents the probability of survival from that parasitoid category. Since the mean for each clutch size is calculated from live larvae from a given clutch size, individuals suffering larval death are excluded. Hence this value will represent the probability of host larval survival of parasitism by the given parasitoid in a given clutch size and is effectively an endoparasitoid mortality for each group.

(Note that this is not strictly a mortality in the sense of the probability of death due to the species in question but the probability that a host will be attacked by that species. Given the levels of multiparasitism which occur (see Section 4), there will be some overestimation of death due to each parasitoid category since some hosts contain more than one type of parasitoid.)

Probability of host larval survival of endoparasitism was plotted against clutch size for four months through the field season; May, June, July and August (plus September) for each of the three parasitoid categories.

Results

The frequency distributions of different clutch sizes through the season are shown in Table 3.3.2 and illustrated graphically in Figure 3.3.1. The modal clutch size is always three and the second most common clutch size is always two with the notable exception of the first month where it is four. This leads to the trend for mean clutch size to decrease through the season in each of the different sites (Table 3.3.6 and Figure 3.3.2).

In all a total of 2,494 mines were dissected over the five months from May to September. Due to interference in Site 4, sampling was only possible during the first two months. Similarly it was not possible to obtain a full sample in Site 1 during the second month. Thus from the four sites a total of 16 monthly samples was made.

Analysis of Codeviance in Clutch Size Due to Leaf Length, Month and Site

The results of this analysis are summarised in Table 3.3.3. The complete model accounted for 15.0% of the total deviance in clutch size.

None of the interactions between leaf length, site and month are either statistically or biologically significant.

Leaf length is positively correlated to clutch size and accounts for a statistically significant change in the total deviance. However in terms of the total deviance in clutch size this effect is only slight - between 10.2 and 11.2 %.

Similarly the change in deviance due to fitting month as a factor is statistically significant but biologically the change is relatively unimportant (between 2.7 and 4.4 %.) In other words there is a slight variation in clutch size according to the month of

oviposition but this is just a small proportion of the total deviance associated with clutch size. Mean clutch sizes in different sites through the season are given in Table 3.3.6 and are shown in Figure 3.3.2.

The maximum deviance attributable to site is statistically significant although the deviance due to site alone is minimal. This may well arise through some orthogonality with leaf length, if leaf lengths vary according to site.

Analysis of Codeviance in Survivorship of Mortality Due to Site, Month and Clutch Size

In this analysis virtually every change in deviance is statistically significant due to the size of the sample and the very high total variability in each mortality.

The complete model accounts for 38.5% of the total deviance in the larval death mortality (see Table 3.3.4.i). There is slight variation according to site of oviposition although this is just a small fraction of the total deviance. Clutch size is negatively correlated to survivorship of the larval death mortality although the strength of this relationship is weak. There is significant variation in the larval death mortality according to month which accounts for between 6.5 and 14.5% of the total deviance.

23.8% of the total deviance in mortality due to larval parasitism is accounted for by the whole model (Table 3.3.4.ii). Neither site, month, or clutch size appear to exert a significant individual influence on the probability of survivorship although the interaction between site and month accounts for 8.4% of the deviance in mortality due to parasitism. The non-linear fit of clutch size ($C + C^2$) accounts for less deviance than fitting clutch size alone. Thus there is no evidence to suggest that the relationship between endoparasite mortality and clutch size is non-linear.

Similarly in the analysis of deviance in total survivorship of larval mortality (Table 3.3.4.iii), site, month and clutch size all fail to account for much of the total variation. The entire model only accounts for 11.2% of the total deviance. The quadratic term for clutch size makes virtually no difference to clutch size alone in explaining the deviance.

Analysis of Codeviance in Survivorship of Mortality Due to Leaf Length, Month and Clutch Size

Similarly to the previous analysis the large sample sizes result in a lot of deviance in the dependent variable so that most changes in deviance prove to be statistically significant while not representing a biologically significant change in the overall deviance.

In the analysis of deviance in survival of the larval death mortality, the entire model of leaf length with clutch size and month as factors only explains 9.8% of the total deviance (Table 3.3.5.i). Neither the 3-way interaction nor any of the 2-way interactions account for much of the total deviance. The larval death mortality is positively correlated to leaf length ($p<0.005$) although the effect is very weak (maximum 5.9%). In this analysis it appears that the variation in mortality according to month of oviposition is low. Similarly clutch size (as a factor) only explains between 4.8 and 5.1% of the deviance.

Fitting the complete model accounts for 26.5% of the total deviance in the mortality from larval parasitism (see Table 3.3.5.ii). Again leaf length exerts little if any influence on this mortality and the maximum deviance explained by either clutch size or month is less than 10% of the total deviance in each case.

Looking at overall larval survivorship, the complete model accounts for 10.7% of the total deviance (see Table 3.3.5.iii). Neither leaf length, month, nor clutch size have any significant effect on the total deviance either alone or in their interactions.

Larval Survivorship of Endoparasitism and Clutch Size

Since it appears from the analysis of clutch size deviance that the effect of site is minimal, all sites were combined together to give large, monthly samples. Also since the samples collected in the last month were generally fairly small, mines collected in months four and five are grouped together.

It appears that overall survivorship in given clutch sizes changes from month to month (see Figure 3.3.3). This is due to the fluctuating degree of survivorship of parasitism from month to month (Figure 3.3.5) although the general trend appears to be for survivorship of parasitism to decrease with clutch size. Likewise survivorship of the larval death mortality decreases with increasing clutch size (Figure 3.3.4).

Brood fitness profiles are shown in Figure 3.3.6 and summarised in Table 3.3.7. Since individual fitness decreases with increasing clutch size, the profiles of total brood fitness (individual fitness multiplied by number of individuals in that brood) against clutch size are domed with clear optima at the clutch where brood fitness is maximised i.e. the Lack solution. This is 4 during the first month and decreases down to 3 for the rest of the season.

Comparing the brood fitness profiles predicting optimal clutch size with the frequency distributions actually found (see Figure 3.3.1) there is clearly a close association whereby the most commonly laid clutch size is that which maximises

fitness returns to the female. Interestingly, in the one month where brood fitness should be maximised in clutches of four eggs, the relative frequency of clutches of four is much higher (see Figures 3.3.1 and 3.3.6).

Survivorship Profiles For Each Parasitoid Category

The survivorship profiles for the three parasitoid categories are given in Figures 3.3.7 to 3.3.10. Figures 3.3.7 to 3.3.9 show the probability of hosts surviving attack by each parasitoid group in turn in successive months through the season. Figure 3.3.10 shows the same profiles on the same graphs, side-by-side for each month, along with their combined overall effect; the mortality profile. Thus the relative contributions of the three types of parasitoid to the total endoparasitoid mortality can be compared for each month.

There are two main ways in which parasitoid survivorship varies in this study. There is variation in individual host survival of endoparasitism according to the size of the brood in which the host occurs (the survivorship profile.) Also there is variation in this profile from month to month both in the shape and height (i.e. the relative level of parasitism). To understand the overall survivorship profiles (Figure 3.3.5) we need to identify these types of variation in the different parasitoids' behaviour.

Survival from attack by the minor parasitoid species shows little variation with clutch size (see Figure 3.3.9 i.) to iv.)) apart from a marked decrease in survivorship in clutch sizes of 6 and 7 in Month 4. There is little variation in the response from month to month apart from in the larger clutches (5 and above) where the level of survival becomes less predictable, owing to smaller sample sizes.

Similarly survival from parasitism by *Biosteres* spp. seems not to vary very much with host clutch size (Figures 3.3.8 i.) to iv.)). While the overall level of parasitism varies from month to month, the shape of the profile according to clutch size is remarkably consistent.

The response of *A. florimela* seems to show rather more variation both with clutch size and with the month of the season (Figures 3.3.7 i.) to iv.)). No clear trends emerge although in each profile there is a suggestion of non-linearity in the response to clutch size. Furthermore, with the exception of Month 4, there seems to be a greater probability of parasitism in larger clutches.

The profiles of host survival from endoparasitism are the cumulative effect of all three groups of parasitoids. However the relative contributions of the three parasitoid groups will be proportional to their relative abundance (i.e. the proportion of hosts parasitised). A comparison of the separate parasitoid mortalities in each month with that month's cumulative profile of parasitism (Figures 3.3.10 i.) to iv.)) does serve to illustrate that the resulting profile is most strongly influenced by the varying response of *A. florimela* to host clutch size. It appears that the group of minor parasitoids has little effect on the variation in the overall endoparasitism mortality. Similarly, while the *Biosteres* spp. may contribute to some variation in survival with clutch size I would expect them to have a lesser effect on the changing monthly profiles of endoparasitism versus clutch size.

Discussion

Unexplained Deviance in Clutch Size and Mortality

One striking aspect of the results of the analyses of deviance is the large amount of variation be it in clutch size or in mortality which is still unaccounted for after a model is fitted. For example in the analysis of clutch size, the entire model of leaf length, site and month only explains 15% of the total clutch size deviance.

If, as might be expected, a female's fitness varies with clutch size, why is there so much unexplained variation in clutch size? The most obvious explanation is simply that there is some other independent variable which affects the decision of the ovipositing female which has not been taken into consideration. But whatever the character to which ovipositing females might respond, the very idea of modifying clutch size would suggest that females were maximising their fitness directly through the egg-laying decision. In other words the fitness of individual larvae depends directly on the ability of their mother to accurately assess the effect and adjust clutch size accordingly. Thus even if such an effect explained deviance in clutch size it still does not explain why there is still such high deviance in larval mortality after clutch size is fitted.

A range of clutch sizes may arise through there being more than one strategy being employed in the *P. nigritarsis* population. Such alternative behaviour may be phenotypically fixed with different individuals adopting different strategies to maximise their success (e.g. male *Centris pallida* bees adopt different strategies for gaining matings depending on their size (Alcock *et al.* 1977)).

Alternatively a range of strategies may be adopted by the same individual. This may simply be age-related (e.g. Young females are limited by opportunity to lay clutches and maximise brood fitness while old females are egg-limited and maximise egg fitness.) Or the clutch size decision may be the result of a more complex strategy of maximising a female's total fitness by optimising several different fitness parameters simultaneously (e.g. Availability of eggs versus time during which weather conditions are favourable versus risk of predation versus stage of adult life etc.) Hence at any given time, different females will pursue different strategies according to their own needs and priorities.

Other sources of variation in clutch sizes may simply be mistakes, disruptions and random quirks and other types of non-adaptive noise which would accumulate during a field sampling experiment of this sort.

Clutch Size and Larval Survivorship of Parasitism

An alternative way of studying the relationships between clutch size and other effects is to accept the high variability but look for underlying trends in individual larval fitness which might lead certain strategies to have long term adaptive value through natural selection. Whatever the source of the great variation in both clutch size and mortality we should expect natural selection to work on even the slightest trends if these can be shown to have some genuine genetic basis. Thus even where there is wide scatter in a response variable it is valid to look for the general patterns affecting individual fitness in order to understand how individuals can maximise their success. Clear patterns of survivorship according to clutch size should still indicate the egg laying strategies which are most likely to be successful in terms of selection.

The profiles for total larval survivorship (Figure 3.3.3) show a decline in individual fitness with increasing clutch size. Since survival from density-dependent larval competition is relatively high (from approximately 95% in clutches of 1 to approximately 70% in clutches of 5) the more influential mortality factor influencing individual larval fitness in different clutch sizes will be larval endoparasitism. Thus the decline in individual fitness with clutch size is not simply the result of density-dependent larval competition for a limited food resource (i.e. the leaf) as in many insect systems, but is strongly influenced by increasing endoparasitism as clutch size increases.

One possible explanation for increasing parasitism may be simply that as clutch size increases, so the associated leaf mines become more conspicuous to parasitoids and therefore larger broods experience higher levels of predation. An alternative hypothesis could be that the time taken for an egg to develop through to pupation is proportional to brood size. Hence the larger a brood is, the longer it takes for individual larvae to develop and therefore the greater their risk of detection by parasitoids. Naturally these questions cannot be addressed by the available data and require further investigation.

Survivorship of the larval death mortality consistently decreases with clutch size in all months (Figure 3.3.4). However the mortality due to parasitism is much less consistent (Figure 3.3.5). Firstly, the shape of the profile varies from month to month and is at various times a straight line with equal survivorship in all clutch sizes (Month 1), a monotonic decline (Month 2) and a dome shape (Month 4). Secondly, the actual levels of survivorship vary according to month from around 20% in Month 1 to around 35% in Month 2. This variation can be explained by the fact that while the density dependent larval death mortality (due to starvation, interference between

larvae etc.) will be consistent through the season, the mortality due to parasitism will vary according to the relative levels of the parasitoid and host populations at the given time (See Results; Larval Survivorship Versus Clutch Size and Figure 3.3.5.)

Godfray (1986a) fitted orthogonal polynomials to his data to show that the individual survivorship profile of *P. nigritarsis* was domed. Individuals from intermediate clutch sizes had the highest probability of surviving to pupation. However, when larval endoparasitism is broken down for each of the parasitoid groups, the only evidence which may be suggestive of this is in the monthly profiles for *A. florimela* (Figure 3.3.7), where an individual's probability of survivorship in Months 1 and 3 is lower in clutch sizes of 1 than it is in clutch sizes of 2. But even so the standard errors overlap and the difference is not marked. Furthermore in Months 2 and 4 the trend is reversed with higher survivorship in clutch sizes of 1 than in clutch sizes of 2. The mortalities due to *Biosteres* spp. and the minor parasitoids do not appear to vary with clutch size although this may simply be because their numbers relative to the host numbers are too low to show a pattern.

In the Introduction I refer to rearing data which shows that of all parasitised hosts, 65% gave rise to *A. florimela* adults, 25% to *Biosteres* spp. and 10% to minor parasitoids. By contrast, the table of rearing data given by Godfray (1986a) gives 43.3% *A. florimela*, 33.5% *Biosteres* spp. and 23.2% Minor Parasitoids. Thus there are differences between the composition of the parasitoid community which he sampled at Silwood in 1986 and the community which I sampled in 1988. Furthermore he accumulated data over a more limited time period and grouped it all as one sample. It is conceivable that the dome-shaped survival function which Godfray found is an artefact which occurs at certain stages of the season with certain

relative levels of host and parasitoid species. If so then one need more data describing absolute numbers of hosts and parasitoids in the field through the season in order to understand it further.

The decline in individual fitness with increasing clutch size (Figure 3.3.3) leads to domed brood fitness profiles with clear optima where a Lack solution can be identified for each sampling period. The Lack solution clutch size was always 3 apart from in the first month when it was 4.

The frequency distributions of clutch sizes in different samples show that there is a clear association between the relative frequencies of different brood sizes laid by *P. nigritarsis* females and their fitness returns. Throughout the season the modal clutch size was 3 (Figure 3.3.1). In months 2, 3 and 4 the modal clutch size matches the optimal brood size predicted for that month (Figure 3.3.6. (ii),(iii),(iv)). In the first month when the predicted optimum is 4 eggs (Figure 3.3.6. (i)), the corresponding relative frequency profile shows a distinctly greater proportion of broods of that size compared to other months (Figure 3.3.1). The decline in mean clutch size through the season reflects the fact that while the mode is constant the relative frequency of larger clutches decreases through the season and clutch sizes of 1 and 2 become relatively more common.

In the Introduction to this study two extreme oviposition strategies were described depending on how the female's fitness is constrained. If she is limited by the number of eggs she has or the time available to lay them the female should maximise her fitness by maximising individual offspring fitness. If she is limited by brood laying opportunities she should maximise the total fitness of each brood. The fact that the modal clutch size laid by *Pegomya nigritarsis* females corresponds to the

most productive one for the female suggests that ovipositing females are constrained chiefly by their opportunities to lay broods. If they were limited either by number of eggs or by time we would expect them to produce the clutch size with the highest predicted individual fitness (Figure 3.3.3). In this analysis these profiles have been interpreted as indicating a decrease in fitness with increasing clutch size. Thus larval survivorship is maximised in clutch sizes of 1. However Table 3.3.2 shows that the frequency of solitary eggs is relatively low.

Apart from the profiles of clutch size and individual fitness there is some agreement with Godfray's study (1986a). He found a monotonic decline in survivorship of the larval death mortality. Also he predicted that the most productive clutch size should be 3 and found that this was the modal clutch in the field.

Tables

Table 3.3.1 : Demonstration Analysis of Codeviance in Variable, Y.

Y is the dependent variable and has 1000 data points. Initially a "complete model" is fitted comprising two factors F1 and F2 (with 3 and 5 levels respectively), a variable V and all interactions, such that all remaining deviance is due to Y. Individual terms are then removed from the model to find how much deviance they each account for.

Component	Deviance Explained	X^2 Test	% Change (Biol.Sig.)
STAGE 1 Total Deviance in Y	1000.0 df=999		
STAGE 2 Remaining Deviance in Y After Fit of Complete model	500.0 df=972		
STAGE 3 - 3-Way Interaction (F1.F2.V)	+10.0 df=7	X^2=NS	2.0%
STAGE 4	510.0 df=979		
- 2-Way Interaction (F1.V)	+2.0 df=2	X^2=NS	Negligible
- 2-Way Interaction (F2.V)	+3.0 df=4	X^2=NS	Negligible
- 2-Way Interaction (F1.F2)	+15.0 df=7	X<0.05	2.9%
STAGE 5 No Interactions (F1+F2+V)	530.0 df=992		
- V	+50.0 df=1	X^2<0.005	9.4%
- F1	+200.0 df=2	X^2<0.005	37.7%
- F2	+10.0 df=4	X^2<0.05	1.9%
STAGE 6 Total Deviance in Y	1000.0 df=999		
+ V	-100.0 df=1	X^2<0.005	10.0%
+ F1	-220.0 df=2	X^2<0.005	22.0%
+ F2	-12.0 df=4	X^2<0.025	1.2%

Table 3.3.2 : Relative Frequencies of Clutch Sizes in Each Month

Summary of the relative frequencies of the different-sized clutches recorded in each month.

	Clutch Size						
	1	2	3	4	5	6	7
Month 1	8.6%	16.2%	29.3%	26.4%	14.2%	3.9%	1.3%
Month 2	6.9%	24.9%	39.8%	17.5%	8.0%	2.6%	-
Month 3	3.5%	29.1%	38.8%	22.3%	5.0%	0.9%	0.4%
Month 4	11.7%	29.3%	35.7%	15.9%	5.3%	1.5%	0.6%

Table 3.3.3 : Comprehensive Analysis of Codeviance in Clutch Size (Month, Site, Leaf Length)

Beginning with the "complete model" comprising all factors and interactions such that all deviance is due to clutch size alone, interactions and factors are removed to find how much deviance they each account for.

Component	Deviance Explained	X^2 Test	% Change (Biol.Sig.)
Total Deviance in Clutch Size	1237.7 df=2493		
Complete model	1052.1 df=2462		
- 3-Way Interaction (Month.Site.LLength)	+4.9 df=8	X^2=NS	0.5%
- 2-Way Interaction (Month.L.Length)	+7.5 df=4	X^2=NS	0.7%
- 2-Way Interaction (Site.L.Length)	+1.0 df=3	X^2=NS	Negligible
- 2-Way Interaction (Month.Site)	+7.5 df=8	X^2=NS	0.7%
3-Factor Model, No Interactions	1072.6 df=2485		
- Month	+25.0 df=4	X^2<0.001	2.7%
- Site	+0.6 df=3	X^2=NS	Negligible
- Leaf Length	+109.0 df=1	X^2<0.001	10.2%
Total Deviance in Clutch Size	1237.7 df=2493		
+ Month	-54.8 df=4	X^2<0.001	4.4%
+ Site	-9.7 df=3	X^2<0.05	0.8%
+ Leaf Length	-138.3 df=1	X^2<0.001	11.2%

Table 3.3.4 : Comprehensive Analysis of Codeviance In Survivorship (Month, Site, Clutch Size)

An analysis of deviance beginning with the "complete model" comprising two factors (month and site) and a variable (clutch size) and their interactions. The different explanatory components are withdrawn until all deviance is due to the mortality alone. (Each mortality is analysed separately, and then combined for overall survivorship.)

i.) : Survivorship of Larval Death Mortality

Component	Deviance Explained	X^2 Test	% Change (Biol.Sig.)
Total Deviance in Mortality	3459.5 df=2493		
Complete Model	3190.6 df=2462		
- 3-Way Interaction (Month.Site. Clutch size)	+13.0 df=8	X^2=NS	Negligible
- 2-Way Interaction (Month.Clutch Size)	+11.7 df=4	X^2<0.05	Negligible
- 2-Way Interaction (Site.Clutch Size)	+0.6 df=3	X^2=NS	Negligible
- 2-Way Interaction (Month.Site)	+36.0 df=8	X^2<0.005	1.0%
3-Factor Model,No Interactions	3254.6 df=2485		
- Month	+76.3 df=4	X^2<0.005	2.3%
- Site	+14.2 df=3	X^2<0.005	Negligible
- Clutch Size	+140.9 df=1	X^2<0.005	4.3%
Total Deviance in Mortality	3459.5 df=2493		
+ Month	-48.4 df=4	X^2<0.005	1.4%
+ Site	-8.3 df=3	X^2<0.05	Negligible
+ Clutch Size	-121.2 df=1	X^2<0.005	3.5%

ii.) : Survivorship of Endoparasitism Mortality

Component	Deviance Explained	X^2 Test	% Change (Biol.Sig.)
Total Deviance in Mortality	12888.0 df=2493		
Complete model	9817.0 df=2462		
- 3-Way Interaction (Month.Site.Clutch size)	+425.0 df=8	X^2<0.005	4.3%
- 2-Way Interaction (Month.Clutch Size)	+167.0 df=4	X^2<0.005	1.6%
- 2-Way Interaction (Site.Clutch Size)	+348.0 df=3	X^2<0.005	3.4%
- 2-Way Interaction (Month.Site)	+862.0 df=8	X^2<0.005	8.4%
3-Factor Model No Interactions	11424.0 df=2485		
- Month	+804.0 df=4	X^2<0.005	7.0%
- Site	+502.0 df=3	X^2<0.005	4.4%
- Clutch Size	+417.0 df=1	X^2<0.005	3.7%
Total Deviance in Mortality	12888.0 df=2493		-
+ Month	-616.0 df=4	X^2<0.005	4.8%
+ Site	-333.0 df=3	X^2<0.005	2.6%
+ Clutch Size	-187.0 df=1	X^2<0.005	1.5%
+ Clutch Size + Clutch Size^2	-124.0 df=1	X^2<0.005	1.0%

iii.) : Survivorship of All Mortalities

Component	Deviance Explained	X^2 Test	% Change (Biol.Sig.)
Total Deviance in Mortality	5252.5 df=2493		
Complete model	4666.5 df=2462		
- 3-Way Interaction (Month.Site.Clutch size)	+23.1 df=8	X^2<0.005	0.1%
- 2-Way Interaction (Month.Clutch Size)	+39.2 df=4	X^2<0.005	1.0%
- 2-Way Interaction (Site.Clutch Size)	+12.7 df=3	X^2<0.001	Negligible
- 2-Way Interaction (Month.Site)	+67.2 df=8	X^2<0.005	1.4%
3-Factor Model, No Interactions	4818.8 df=2485		
- Month	+274.7 df=4	X^2<0.005	5.7%
- Site	+91.6 df=3	X^2<0.005	2.0%
- Clutch Size	+66.4 df=1	X^2<0.005	1.4%
Total Deviance in Mortality	5252.5 df=2493		
+ Month	-272.4 df=4	X^2<0.005	5.2%
+ Site	-51.5 df=3	X^2<0.005	1.0%
+ Clutch Size	-107.4 df=1	X^2<0.005	2.0%
+ Clutch Size + Clutch Size^2	-117.1 df=1	X^2<0.005	2.2%

Table 3.3.5 : Comprehensive Analysis of Codeviance In Survivorship (Month, Clutch Size, Leaf Length)

An analysis of deviance beginning with the "complete model" comprising two factors (site and clutch size) and a variable (leaf length) and their interactions. Explanatory components are withdrawn until all deviance is due to the mortality alone. Each mortality is analysed separately and then combined for overall survivorship.

i.) : Survivorship of Larval Death Mortality

Component	Deviance Explained	X^2 Test	% Change (Biol.Sig.)
Total Deviance in Mortality	3459.5 df=2493		
Complete model	3119.7 df=2444		
- 3-Way Interaction (Month.Clutch Size. Leaf Length)	+18.0 df=16	X^2=NS	Negligible
- 2-Way Interaction (Month.L.Length)	+37.0 df=4	X^2<0.005	1.2%
- 2-Way Interaction (Clutch Size. LLength)	+5.7 df=4	X^2=NS	Negligible
- 2-Way Interaction (Month.Clutch Size)	+37.9 df=16	X^2<0.005	1.2%
3-Factor Model, No Interactions	3212.2 df=2484		
- Month	+68.0 df=4	X^2<0.005	2.1%
- Clutch Size	+154.0 df=4	X^2<0.005	4.8%
- L.Length	+12.0 df=1	X^2<0.005	Negligible
Total Deviance in Mortality	3459.5 df=2493		
+ Month	-48.4 df=4	X^2<0.005	1.4%
+ Clutch Size	-178.1 df=4	X^2<0.005	5.1%
+ Leaf Length	-20.3 df=1	X^2<0.005	5.9%

ii.) : Survivorship of Endoparasitism Mortality

Component	Deviance Explained	X^2 Test	% Change (Biol.Sig.)
Total Deviance in Mortality	12888.0 df=2493		
Complete model	75.0 df=2444		
- 3-Way Interaction (Month.Clutch Size. L.Length)	+306.0 df=16	X^2<0.005	3.2%
- 2-Way Interaction (Month.L.Length)	+241.0 df=4	X^2<0.005	2.5%
- 2-Way Interaction (Clutch Size.L Length)	+33.0 df=4	X^2<0.005	Negligible
- 2-Way Interaction (Month.Clutch Size)	+602.0 df=16	X^2<0.005	6.2%
3-Factor Model, No Interactions	10810.0 df=2484		
- Month	+957.0 df=4	X^2<0.005	8.9%
- Clutch Size	+1438.0 df=4	X^2<0.005	13.3%
- L.Length	+131.0 df=1	X^2<0.005	1.2%
Total Deviance in Mortality	12888.0 df=2493		
+ Month	-616.0 df=4	X^2<0.005	4.8%
+ Clutch Size	-904.0 df=4	X^2<0.005	7.0%
+ L.Length	-5.0 df=1	X^2=NS	Negligible

iii.) : Survivorship of All Mortalities

Component	Deviance Explained	X^2 test	% Change (Biol.Sig.)
Total Deviance in Mortality	5252.5 df=2493		
Complete model	4688.1 df=2444		
- 3-Way Interaction (Month.Clutch Size. L.Length)	+18.8 df=16	X^2=NS	4.0%
- 2-Way Interaction (Month.L.Length)	+37.9 df=4	X^2<0.005	0.8%
- 2-Way Interaction (Clutch Size.L Length)	+18.8 df=4	X^2<0.005	0.4%
- 2-Way Interaction (Month.ClutchSize)	+76.7 df=16	X^2<0.005	1.6%
3-Factor Model, No Interactions	4854.5 df=2484		
- Month	+119.7 df=4	X^2<0.005	2.5%
- Clutch Size	+76.5 df=4	X^2<0.005	1.6%
- L.Length	+28.2 df=1	X^2<0.005	0.6%
Total Deviance in Mortality	5252.5 df=2493		
+ Month	-272.4 df=4	X^2<0.005	5.2%
+ Clutch Size	-129.5 df=4	X^2<0.005	2.5%
+ L.Length	-98.2 df=1	X^2<0.005	1.9%

Table 3.3.6 : Mean Clutch Size Through The Season

Table of Mean Clutch Sizes on *R. obtusifolius* at Four Sites Through Season

	Month 1	Month 2	Month 3	Month 4	Month 5	Total
Site 1	3.432 ±0.139	-	2.849 ±0.065	2.804 ±0.088	2.542 ±0.351	2.915 ±1.03
Site 2	3.123 ±0.087	3.164 ±0.089	3.188 ±0.075	2.769 ±0.085	2.484 ±0.160	2.989 ±1.02
Site 3	3.587 ±0.010	2.932 ±0.088	3.000 ±0.059	2.847 ±0.081	2.564 ±0.132	3.053 ±1.02
Site 4	3.429 ±0.090	2.907 ±0.155	-	-	-	3.320 ±1.03

Table 3.3.7 : Clutch Size and Brood Fitness Through the Season

The index of brood fitness is calculated by multiplying egg fitness for each clutch size by the corresponding number of individuals.

	Clutch Size				
	1	2	3	4	>4
Month 1	0.200 ±0.053	0.265 ±0.058	0.466 ±0.061	0.647 ±0.078	0.108 ±0.090
Month 2	0.423 ±0.101	0.606 ±0.088	0.953 ±0.097	0.802 ±0.146	0.325 ±0.101
Month 3	0.292 ±0.095	0.462 ±0.049	0.753 ±0.062	0.629 ±0.087	0.790 ±0.172
Month 4	0.250 ±0.055	0.513 ±0.061	0.774 ±0.082	0.586 ±0.120	0.500 ±0.203

Figures

FIGURE 3.3.1 Relative Frequencies of Clutch Sizes Through the Season.

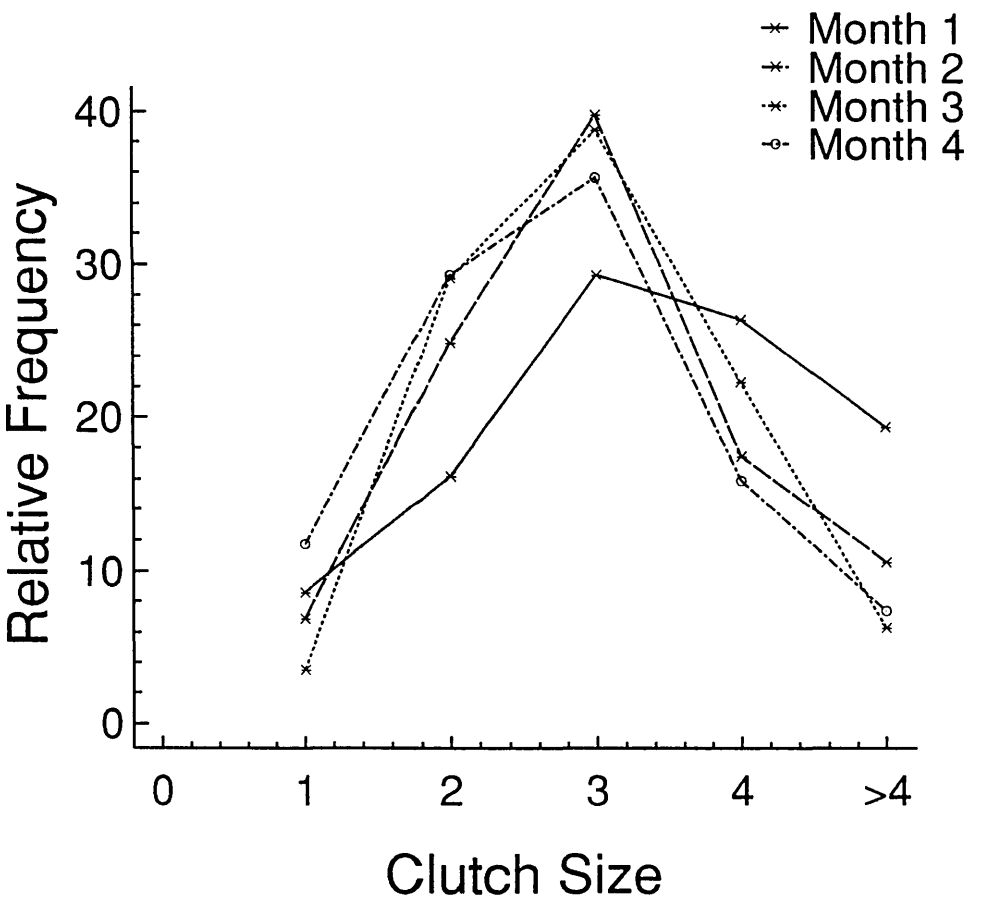


FIGURE 3.3.2 Mean Clutch Size Through the Season in Three Sites.

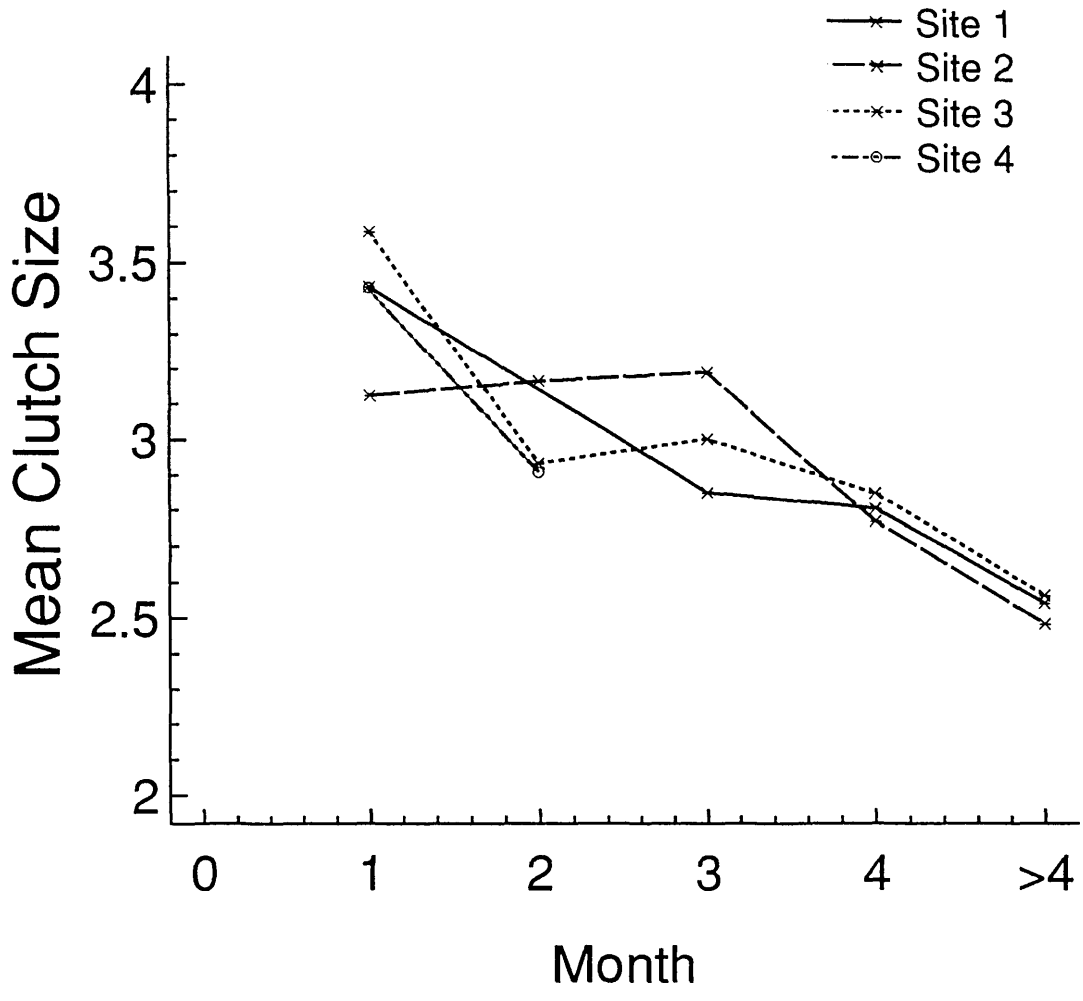
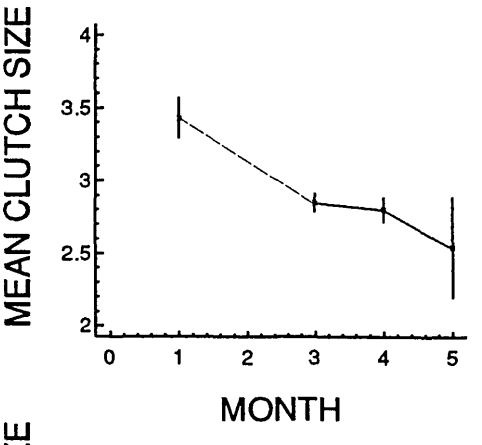
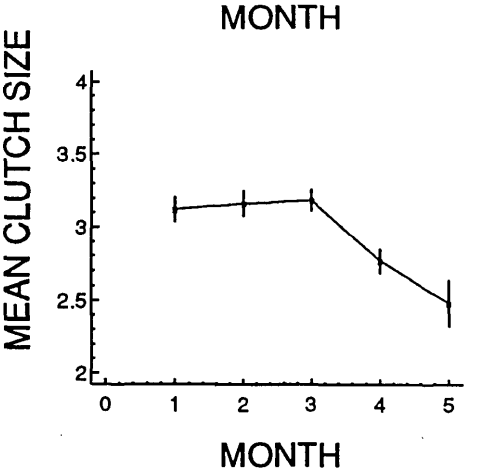


FIGURE 3.3.2 Continued. Individual Sites (With Standard Errors).

i.) Site 1.



ii.) Site 2.



iii.) Site 3.

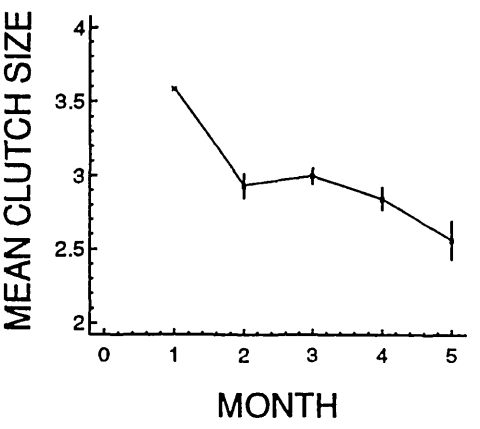


FIGURE 3.3.3 Probability of Larval Survival Versus Clutch Size Through the Season.

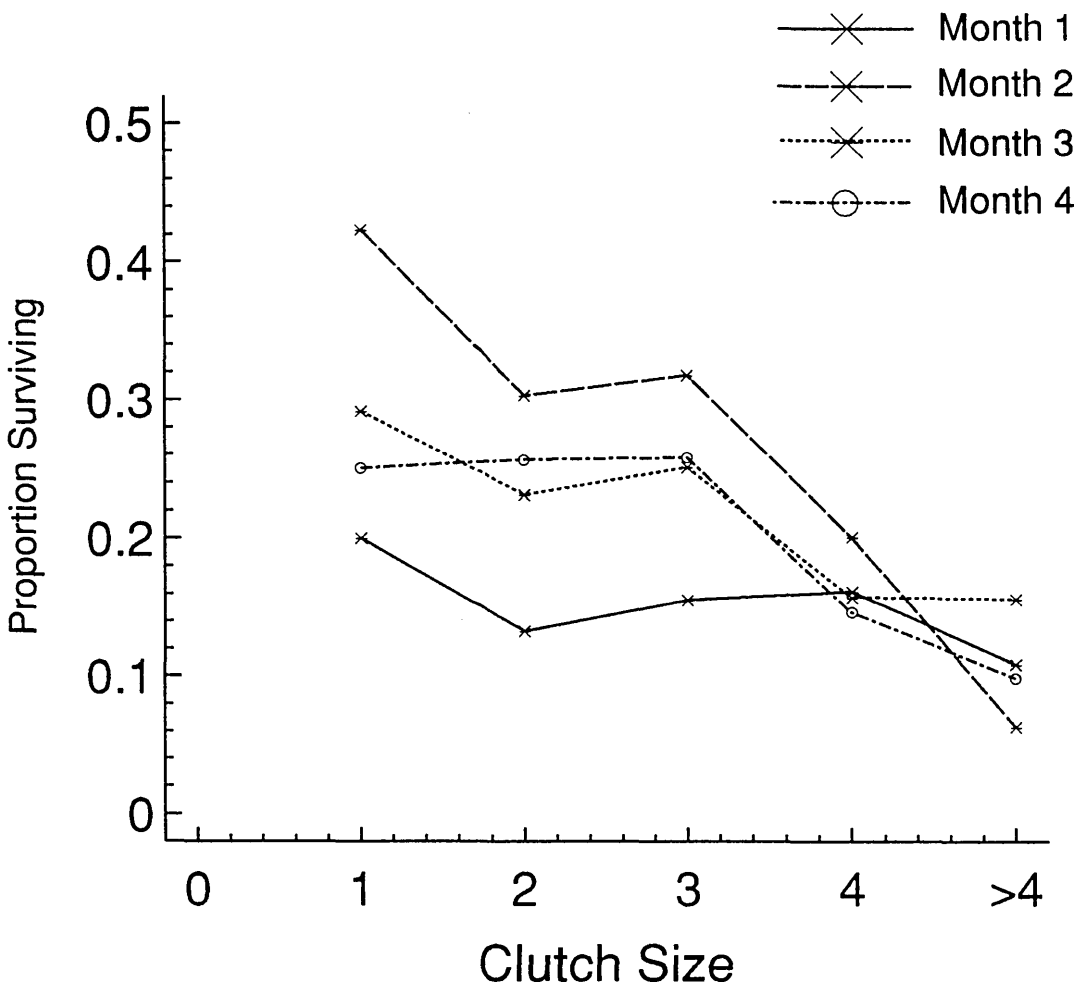


FIGURE 3.3.3 Continued. Individual Months (With Standard Errors).

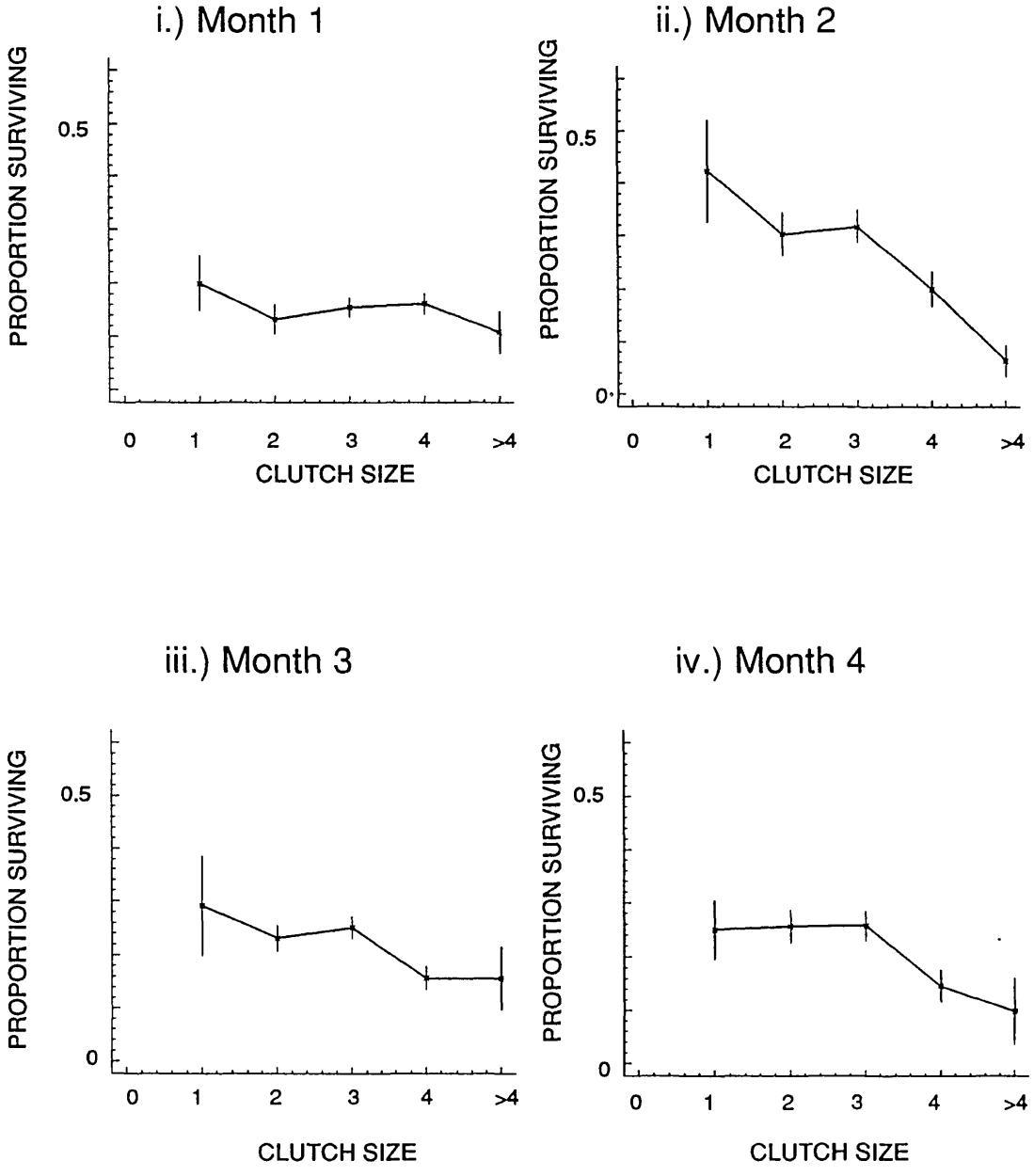


FIGURE 3.3.4 Probability of Individual Survivorship of the Larval Death Mortality Versus Clutch Size Through the Season.

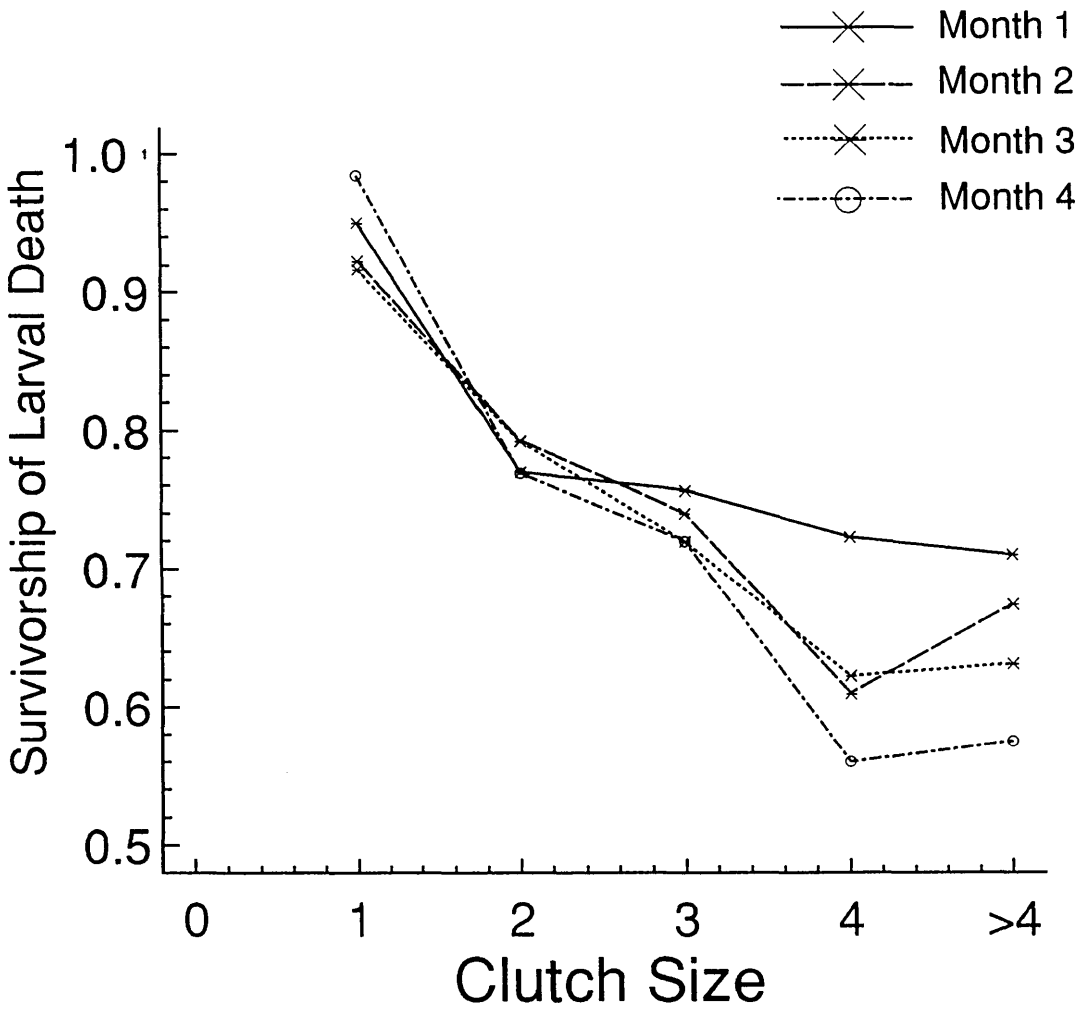


FIGURE 3.3.4 Continued. Individual Months (With Standard Errors).

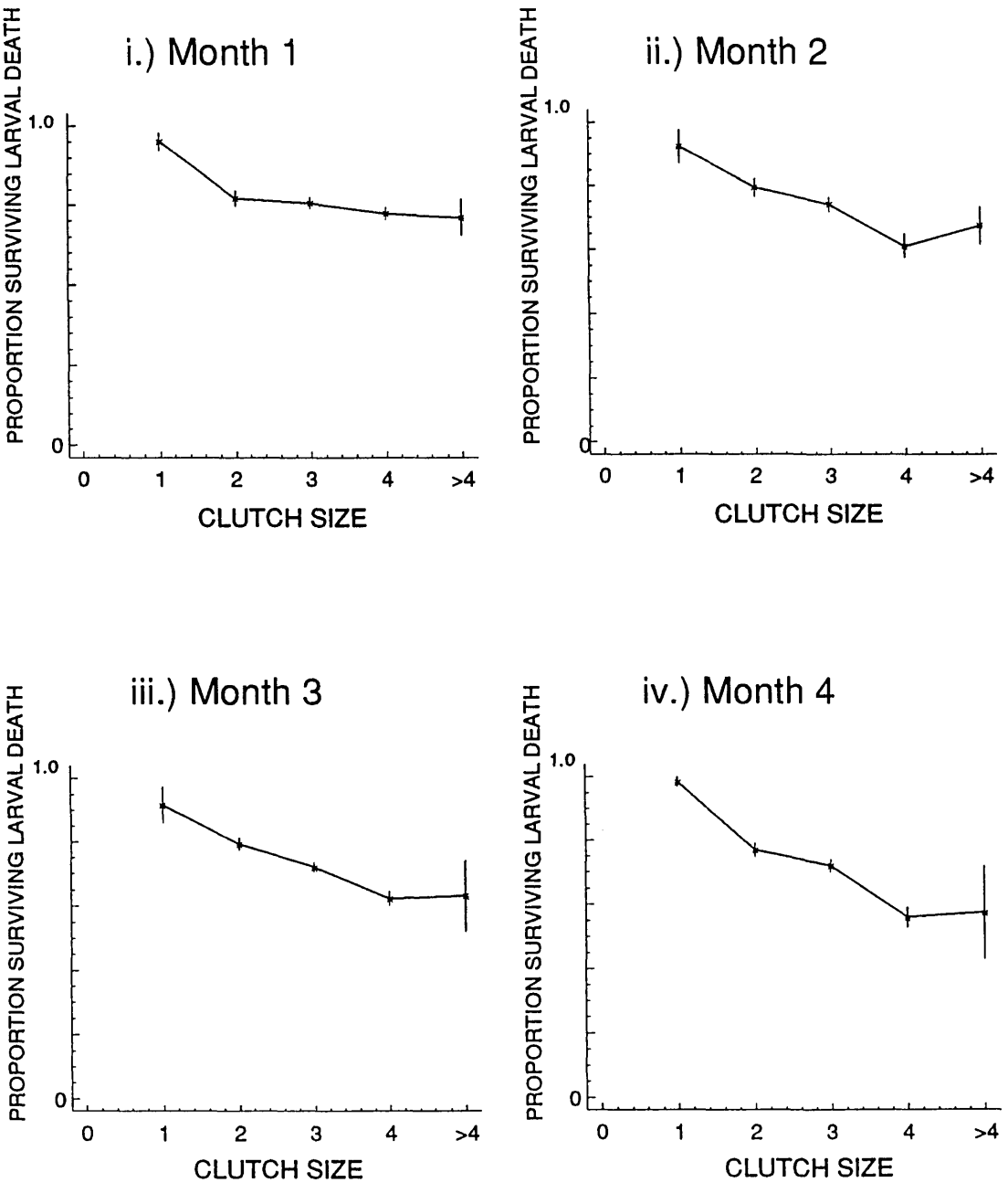


FIGURE 3.3.5 Probability of Individual Survivorship of Endoparasitism Versus Clutch Size Through the Season.

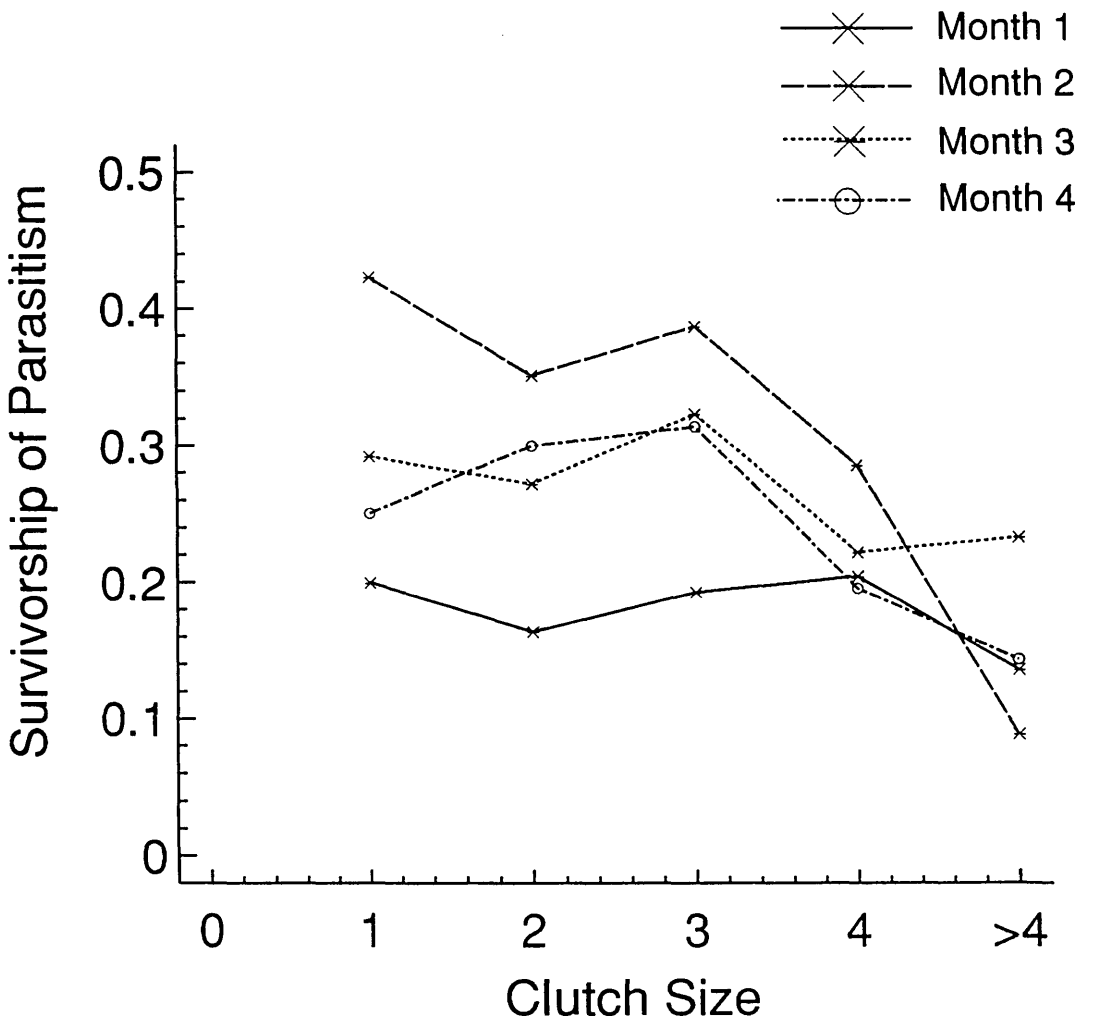


FIGURE 3.3.5 Continued. Individual Months (With Standard Errors).

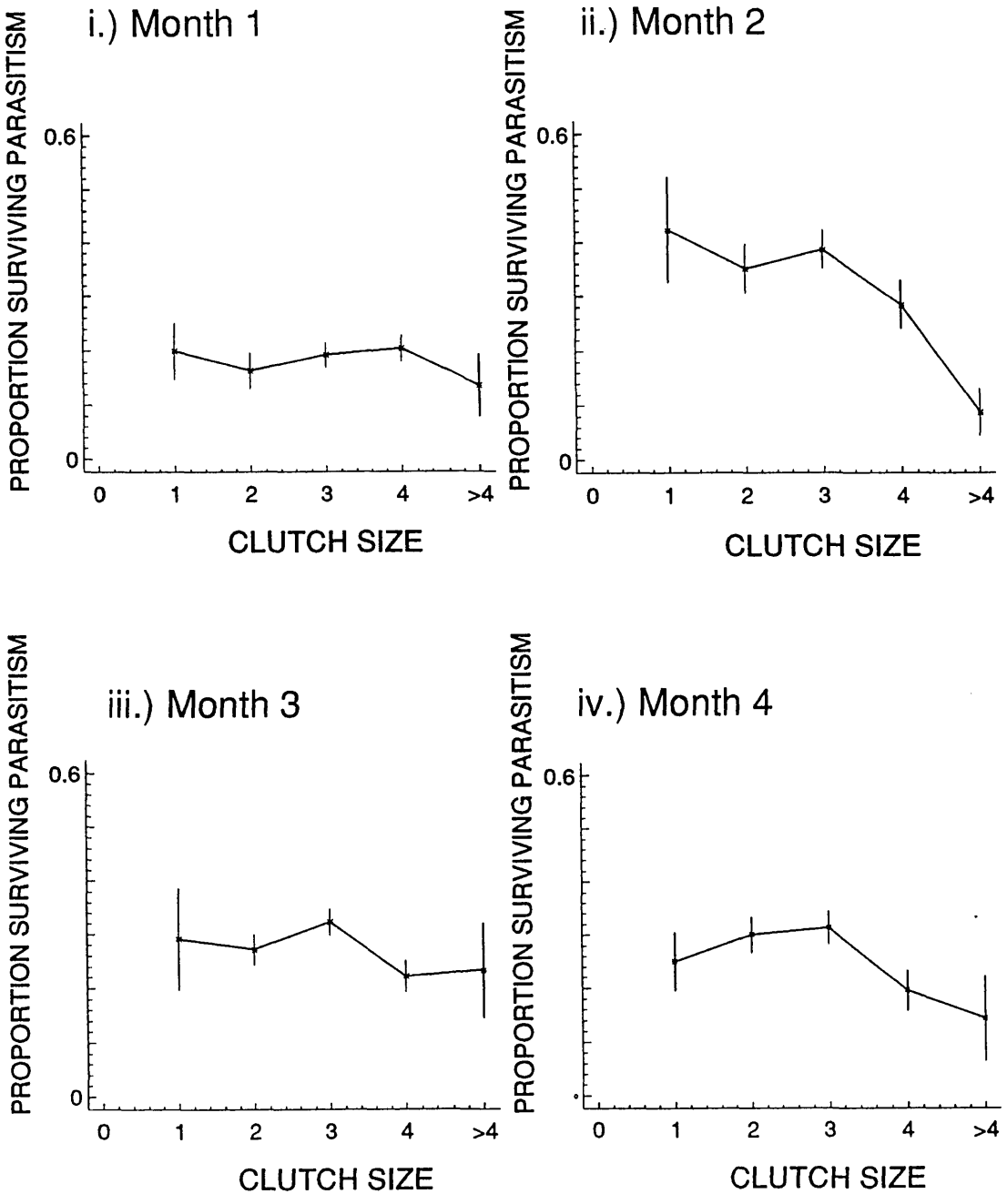


FIGURE 3.3.6 Projected Brood Fitness and Clutch Size Through the Season.

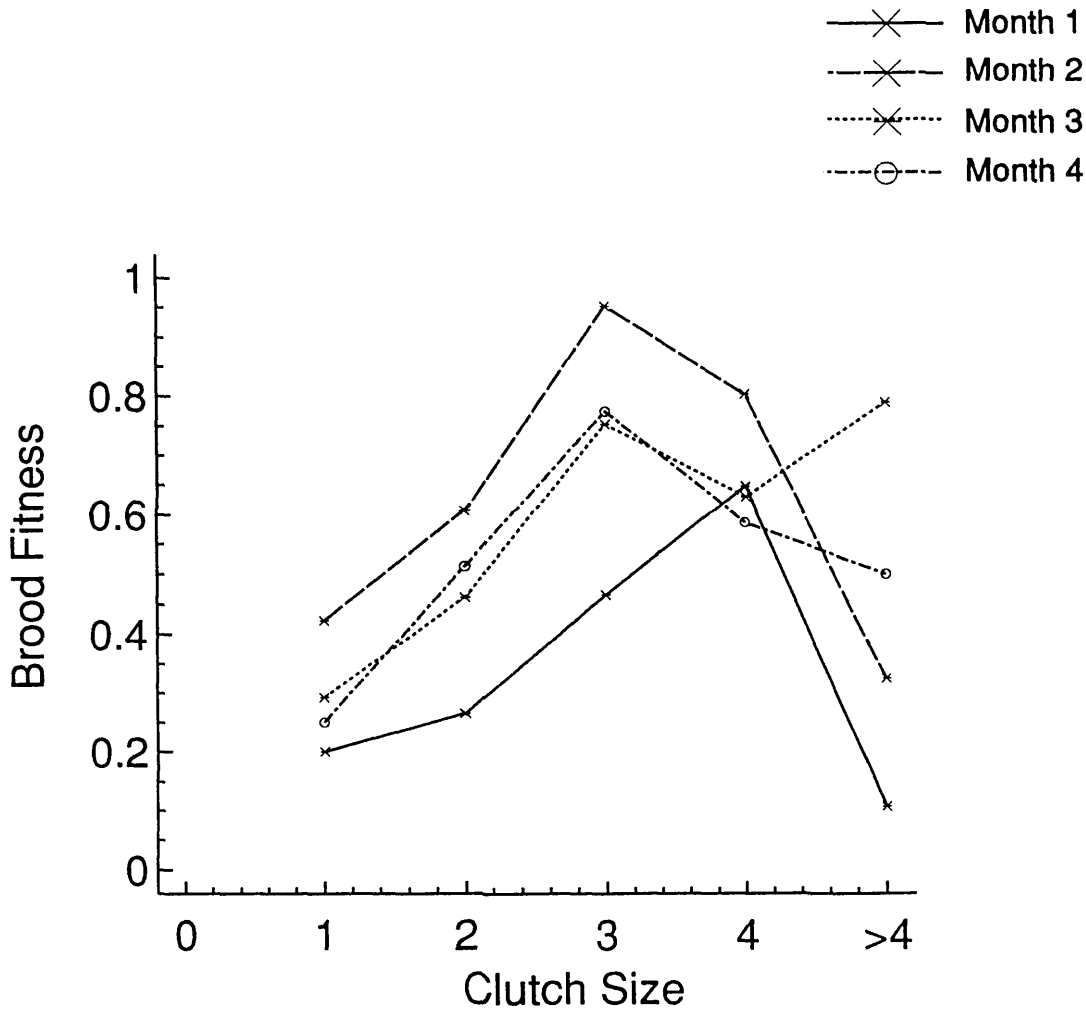


FIGURE 3.3.6 Continued. Individual Months (With Standard Errors).

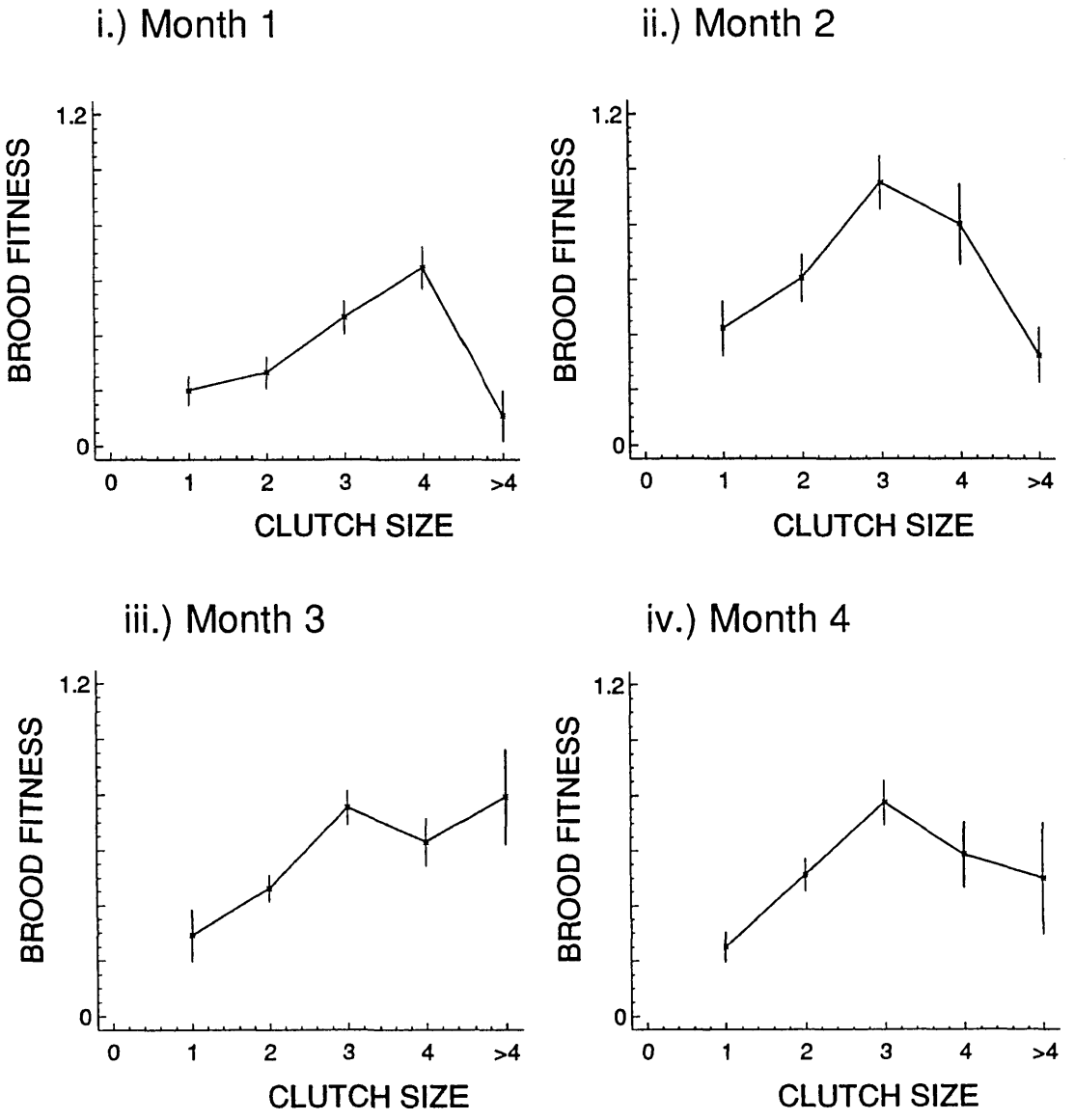


FIGURE 3.3.7 Probability of Survivorship of Parasitism by *Adelurola florimela* in Different Clutch Sizes Through the Season. (With Standard Error Bars.)

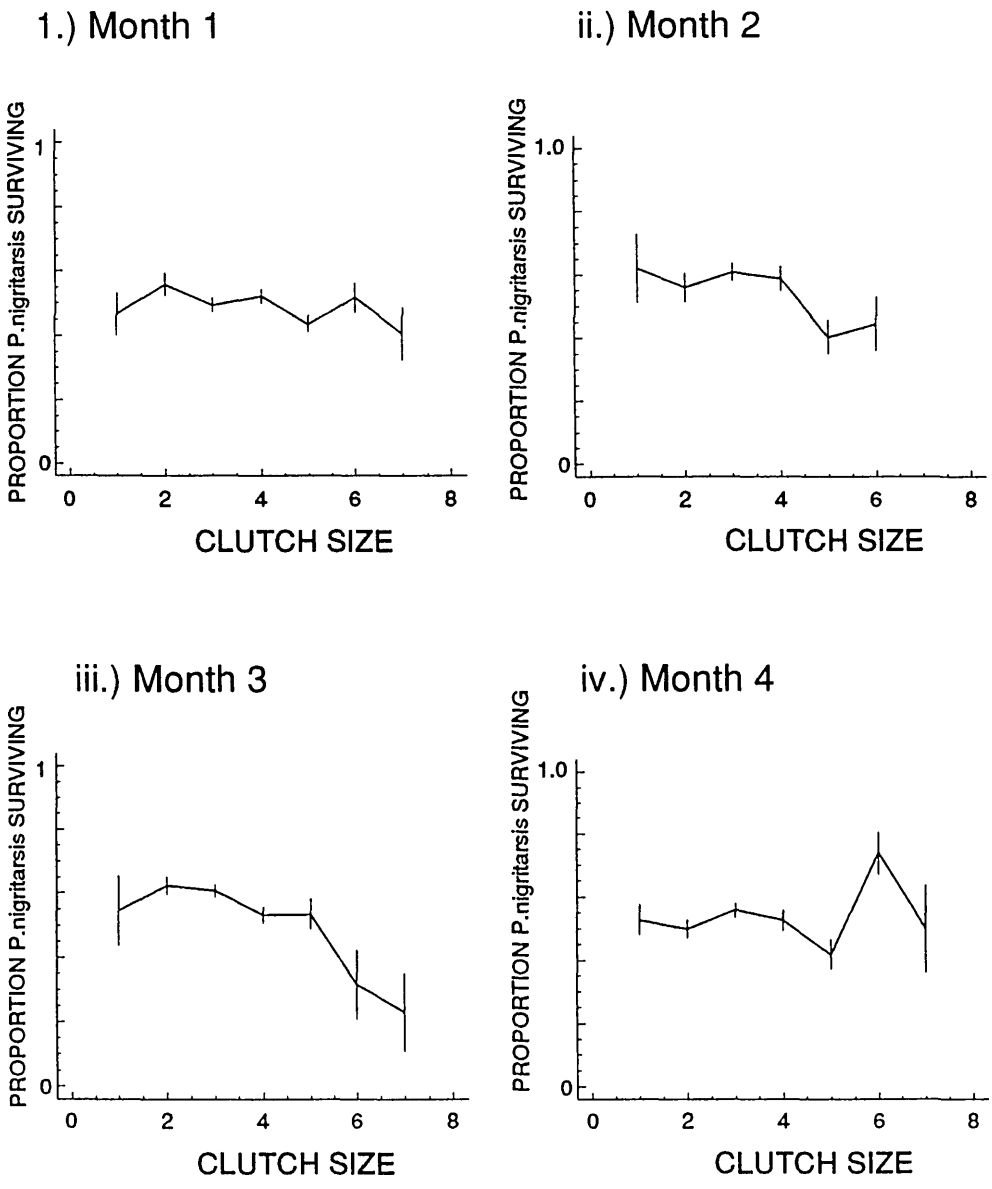


FIGURE 3.3.8 Probability of Survivorship of Parasitism by *Biosteres* spp. in Different Clutch Sizes Through the Season. (With Standard Error Bars.)

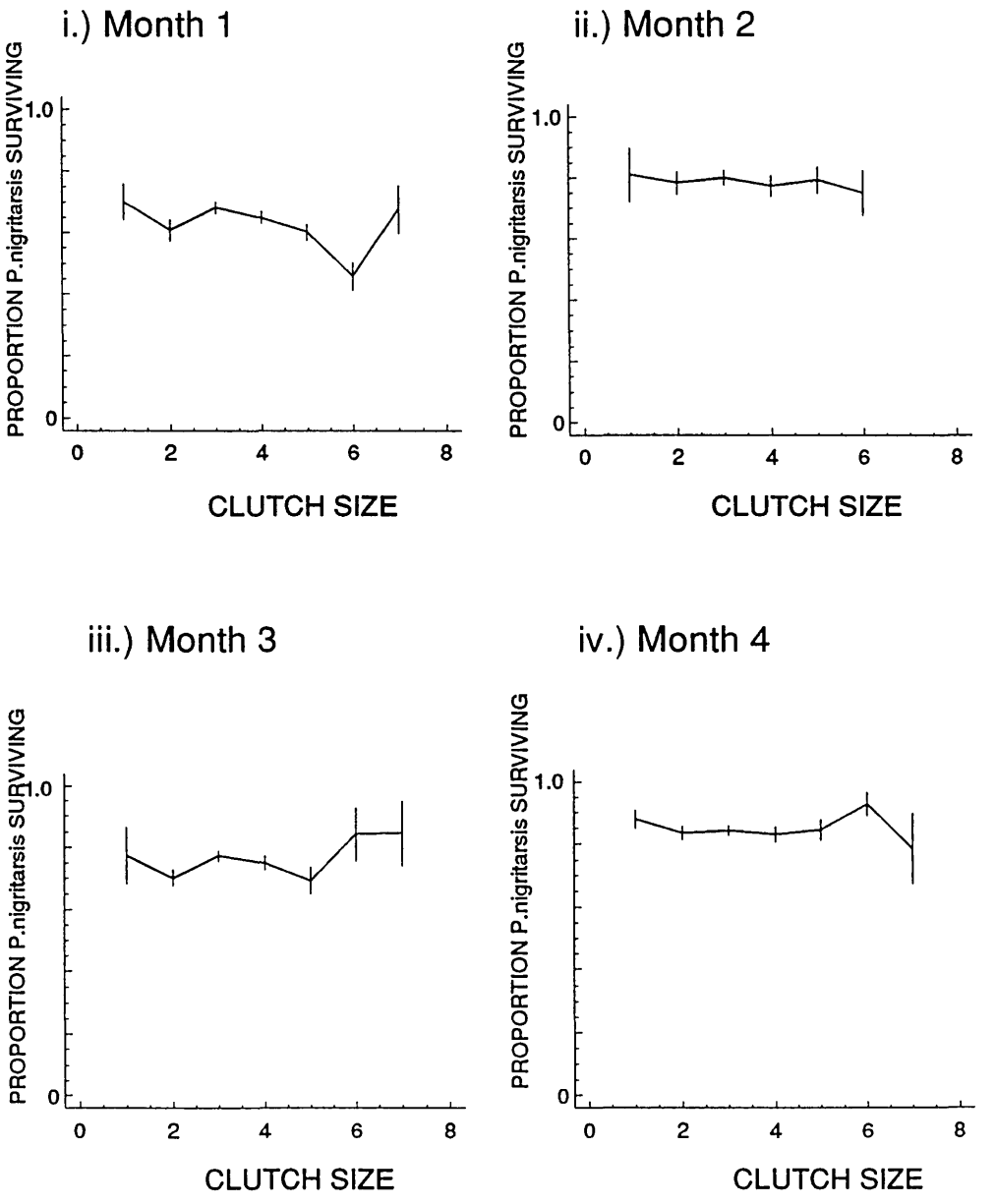


FIGURE 3.3.9 Probability of Survivorship of Parasitism by Minor Parasitoids in Different Clutch Sizes Through the Season. (With Standard Error Bars.)

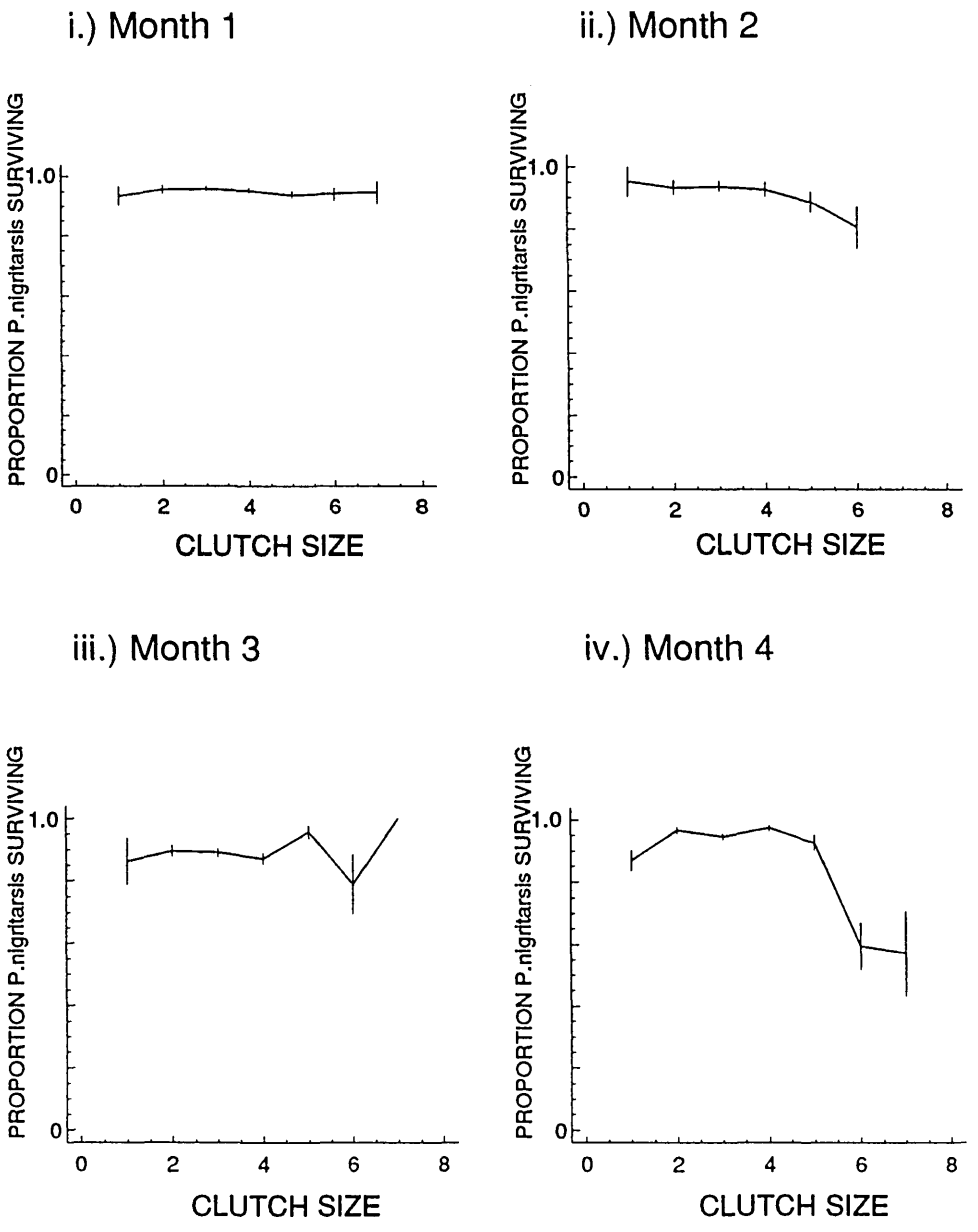
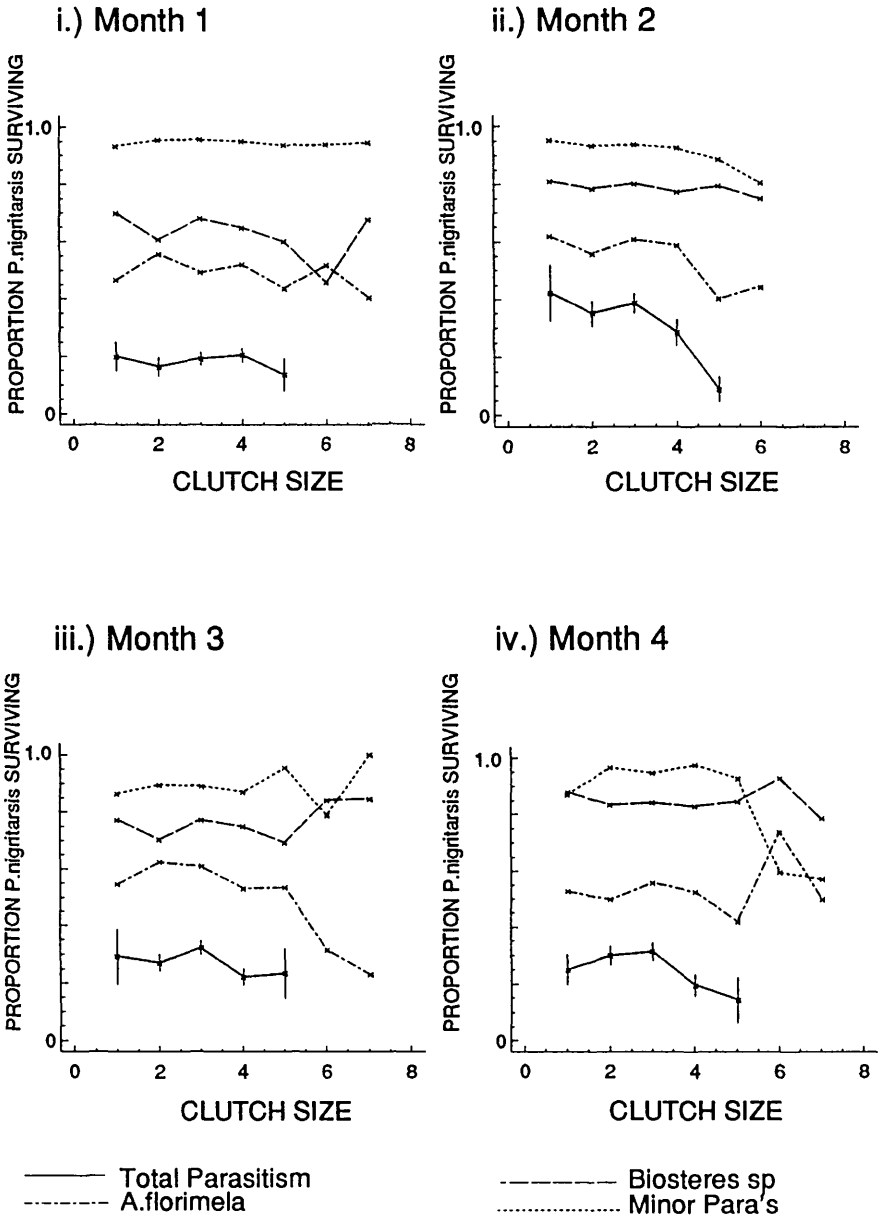


FIGURE 3.3.10 Comparison of the Profiles of the Different Parasitoid Species.

Each graph shows the monthly fitness profiles from each parasitoid category in a given month. Additionally the profile of survivorship of all parasitism in that month is given with standard errors (taken from Figure 3.3.5).



Chapter 3 : Section 4

The Frequency Distributions of Parasitoids in the Larvae of *Pegomya nigritarsis.*

Summary

1.) The behaviour of the two main parasitoids (and an amalgamation of minor species) attacking the leaf-miner *Pegomya nigritarsis* is considered with respect to their frequency distributions in individual host larvae.

2.) The high levels of superparasitism in the field imply that superparasitism is an adaptive strategy rather than a maladaptive behaviour to be avoided. Superparasitism could be the result of indiscriminate laying on hosts, when a female's fitness is limited chiefly by time and it does not pay to spend time discriminating. Alternatively superparasitism may be a deliberate strategy when unparasitised hosts are few, and it does not pay to reserve oviposition solely for unparasitised hosts.

3.) Whatever the density of a parasitoid species, the proportion of hosts escaping parasitism through the superparasitic behaviour of the parasitoid remained constant. i.e. The rate of superparasitism was constant with increasing parasitoid density. This implies that superparasitism occurs through overlap in host attack by non-discriminating females.

4.) Despite high numbers of parasitoids, a large number of unparasitised hosts were collected in the field and therefore it does not appear that ovipositing females lack free hosts altogether. Superparasitism probably occurs as females seek to maximise their rate of encounter with hosts, by not discriminating against parasitised hosts.

5.) *P. nigritarsis* broods are highly aggregated in the field, conforming very closely to a Negative Binomial distribution. The aggregated distribution of the host may lead to an aggregative response in the parasitoid populations as individuals concentrate on high density patches of hosts. This will result in a partial refuge for some of the host population who will escape parasitism. However it was not possible to show aggregation of parasitism with the data available.

Introduction

In the previous section I began to look at the behaviour of the parasitoids of *Pegomya nigritarsis* by studying levels of parasitism in different host clutch sizes. In this section I continue to consider the behaviour of the parasitoids of *P. nigritarsis* by looking at the frequency of parasitism in larval hosts in the field and in particular I look at the levels of superparasitism in the field.

For many years the traditional explanation for superparasitism has been either that it constitutes maladaptive behaviour (Howard 1897) or that it occurs through failure on the part of the female to identify the prior existence of conspecific parasitoids (Fiske 1910, Thompson 1929). However in recent years there has been both proof of discriminatory ability in superparasitising species (e.g. Salt 1961) and an increasing awareness of the adaptive value of superparasitism (van Alphen and Visser 1989). The arguments defending superparasitism as a deliberate and beneficial strategy all apply in situations where a parent's fitness gain per time unit is increased by accepting a previously parasitised host, even though the probability of survival of the egg may be much less than what it would be in an unparasitised host. This situation generally occurs either when unparasitised hosts are exceedingly limited or costly to find or when the cost of wasting an egg is low (Iwasa *et al.* 1984). Experimental measurement of the relative costs and benefits of superparasitism in the field would be extremely difficult and, as yet, there have been no such studies. Nonetheless it is possible to use field data to look at levels of parasitism and possibly infer whether or not parasitoids have the ability to discriminate against hosts previously parasitised by conspecifics by looking at their frequency distributions in host larvae (Salt 1932, 1934, van Lenteren *et al.* 1978, van Lenteren 1981).

Thus this section contains a study of the levels of superparasitism in hosts collected in the field. The quantitative examination of superparasitism involves the analysis of the frequency distributions of the parasitoid species. A useful test for non random responses of solitary parasitoids to host patchiness is to compare the frequencies of parasitoid eggs and larvae in hosts with a suitable random frequency distribution (Salt 1934). A further test is to see whether the distribution is aggregated and compare the frequencies of parasitoid eggs and larvae in hosts with an aggregated frequency distribution such as the negative binomial distribution.

The overall aim of the work in this section was to use a field sampling experiment to isolate the different host-attacking strategies of parasitoid species on a single host species and to get an impression of the levels of superparasitism in the natural environment. Admittedly this experiment was not expressly designed for a study of the frequency distributions of parasitoids in relation to host distribution in the field and it will not be possible to compare different spatial scales of host distribution. Nonetheless it should still be possible to look for general patterns in the distributions of hosts and parasitoids and to get an impression of the levels of superparasitism in the field.

Methods 1 : Practical

The data for the frequency distributions of different species of parasitoids were obtained from the original larval dissections which are described in Section 3 of this chapter. Thus monthly samples of approximately 200 mines on *Rumex obtusifolius* were taken from four different sites. The different parasitoid eggs and larvae in each host were identified and categorised into one of three groups as described in the Introduction to this Chapter; i.e. the braconid species *Adelurola florimela*, the

Biosteres spp. group - predominantly *Biosteres carbonarius* with three other closely-related species, and the group containing the remaining species of less abundant parasitoids.

Methods 2 : Analysing the Frequency Distributions of the Parasitoids

In order to study the occurrence of parasitism in host larvae, the frequency distributions of parasitoid eggs and larvae were compared with standard statistical distributions using X^2 Goodness of Fit tests. To test for conformity with a random egg-laying pattern, the Poisson distribution was used. Similarly, to test for an aggregated distribution of parasitoids in hosts, frequency distributions were compared with a negative binomial distribution. (For a full description see Methods of Section 2.)

I looked at the frequency distributions of attacks on individual hosts by each of the three parasitoid groups at different months through the season. The data from each host larva included a score for how many parasitoids (eggs plus larvae) of each type were present. All the parasitoids of *P. nigritarsis* are solitary with just one parasitoid emerging from each host pupa. I assumed that ovipositing parasitoids lay just one egg per host and do not self-superparasitise. Hence the total number of parasitoid eggs and larvae represents the total number of that type of parasitoid visiting that host larva. At this stage I have made no assumptions about the discriminatory ability of the parasitoids (i.e. whether the event of superparasitism is a deliberate strategy, or accidental, with certain types of hosts attracting more attacks than others.)

In addition to studying the frequency distributions of separate parasitoid species the data for all the parasitoids were pooled to give the monthly frequency distributions of the total numbers of parasitoids per host larva. These were then tested against the Poisson and Negative Binomial distributions as before.

Results

The frequency distributions for the three parasitoid groups, and the combined totals are shown in Tables 3.4.1 to 3.4.4. The total number of individuals is found by multiplying each frequency by its corresponding frequency size class and taking the sum total. The total number of hosts is simply the sum of the frequencies since each score represents the number of broods on a single host unit.

(Note that for the X^2 tests, the degrees of freedom are calculated by number of frequency classes (0, 1, 2, 3...etc.) minus 1. However some of the d.f. values for fits of the Poisson distribution may be less than this since it is occasionally necessary to pool data in the upper tail of the frequency distribution to give expected values greater than 5.)

In Table 3.4.1 the X^2 Tests against a Poisson distribution shows that the monthly frequency distributions of *A. florimela* parasitoids appear to be consistently non-random. However comparison with the negative binomial distribution was not possible because the variance of the data was always less than the mean suggesting a spaced or even distribution rather than an aggregated distribution.

The monthly frequency distributions of the *Biosteres* spp. group were also significantly different to the expected Poisson distributions (Table 3.4.2). But by

contrast to *A. florimela* the frequency distributions conformed closely to Negative Binomial distributions. This is evidence that these parasitoids are highly aggregated in their leaf-miner hosts.

The sample sizes for the frequency distributions of the minor parasitoids are much smaller (see Table 3.4.3). X^2 tests of Goodness of Fit generally suggested no significant difference between the frequency distribution and either the Poisson distribution or the negative binomial distribution. However the variance was always greater than the mean and low k-values in months 2 and 3 suggest high aggregation of parasitism. In Month 3 where the total number of parasitoids is higher (i.e. 146), the frequency distribution is significantly different to a Poisson distribution but not to the Negative Binomial. Hence I conclude that the data suggests (but does not prove) that the minor parasitoids tend to be aggregated in host larvae.

Finally the monthly frequency distributions of all parasitoid species in *P. nigritarsis* hosts are shown in Table 3.4.4. Despite the large total number of parasitoids, there were never more than 6 parasitoids in a single host. The frequency distributions were always significantly different to Poisson distributions (i.e. apparently non random) through the season (Table 3.4.4), but the variance was always less than the mean. Therefore it was not possible to fit the Negative Binomial distribution and it appears that parasitoids generally tend to spread themselves evenly in the field.

A visual summary of the relationship between mean number of individuals per host and the corresponding variances is shown in Figure 3.4.1. The mean number of parasitoids is essentially the ratio of total number of parasitoids to total number of hosts in a given month. While it indicates something of the relative numbers of parasitoids in different groups this is not to be confused with sample size since the

number of hosts (i.e. the sample size) is constant within each month. When one is comparing the behaviour of different species, the mean number of parasitoids per host represents a measure of relative abundance for the given month.

Two interesting patterns emerge from Tables 3.4.1 to 3.4.4 and Figure 3.4.1. There is a noticeable trend towards avoidance of previously parasitised hosts (i.e. mean increases relative to variance) as the number of parasitoids per host increases. Also there is remarkable within-group consistency in the pattern of dispersion (or aggregation) which arises (i.e. all datapoints for a particular parasitoid group lie on the same side of the line of Y = X) suggesting a consistent within-group behaviour of either aggregation (*Biosteres* and Minor Parasitoids) or dispersal (*A. florimela*). However there appears to be an anomalous situation whereby the data for Minor Parasitoids and *Biosteres* spp. show a variance greater than the mean, yet when the data for all parasitoids is pooled the mean is much greater than the variance. In other words while there appears to be aggregated parasitism within each of those two parasitoid groups, the pooled data implies a tendency towards dispersal and avoidance of superparasitism and multiparasitism.

Figure 3.4.2 shows an alternative view of the rate of exploitation of hosts by parasitoids. The proportion of hosts which are parasitised by a given group is plotted against the density of the parasitoids in the host (mean number of parasitoids per host as above). The line of Y = X represents 100% avoidance of conspecific superparasitism such that as parasitoid numbers increase, the numbers of parasitised hosts increase accordingly. All the data points for different parasitoid groups fall below this line, (which is not surprising since a situation where the proportion of parasitised hosts exceeds the number of parasitoids per host is clearly impossible.) The distance of a point below the line indicates the level of conspecific

superparasitism at the given parasitoid density since it shows the proportion of hosts which escape parasitism at that density through the parasitoids not laying exactly one egg per host. It appears here that the proportion of hosts escaping attack through superparasitism remains constant with parasitoid density. The curved line on the graph outlines the proportion of hosts parasitised with increasing parasitoid density if hosts are attacked purely at random as a Poisson process. There is greater avoidance of superparasitism than expected from purely random oviposition in *A. florimela* and when all parasitoid data are grouped together. However at the lower parasitoid densities there is evidence of slight aggregation with data points occurring below this line.

To summarise from Figures 3.4.1 and 3.4.2, it appears that the proportion of hosts escaping attack through superparasitism remains fairly constant with increasing parasitoid density. However parasitism appears to be aggregated when numbers of parasitoids are low. Aggregation in small samples and the assumption of the Poisson process as a model for random egg-laying will be discussed further.

Discussion

Superparasitism as an Adaptive Strategy.

The most instant and striking feature of the results of this study is the very high levels of superparasitism in the field (Tables 3.4.1 to 3.4.4). Ever since the earliest record of superparasitism (Howard 1897), the traditional view has been that superfluous parasitic eggs are wasted eggs and therefore that superparasitism is either maladaptive or the result of mistakes by imperfect animals (van Alphen and Visser 1990). There was disagreement on the issue of whether or not parasitoids have any ability to detect previously parasitised hosts, and hence whether they could avoid superparasitism. Salt

(1932, 1934, 1937) proved that parasitoids had the ability to avoid superparasitism and until recently it was believed that the avoidance of superparasitism was adaptive in all situations (van Lenteren 1981, Huffaker and Matsumoto 1982), despite the high levels of superparasitism recorded in field studies (e.g. this present one). But if it is possible for parasitoids to have the ability to discriminate against parasitised hosts, why should such a maladaptive behaviour persist so commonly?

Recently however, the adaptive value of the decision to superparasitise has been recognised and explored (van Alphen and Nell 1982, Charnov and Skinner 1984, Parker and Courtney 1984, Skinner 1985, van Dijken and Waage 1987, Strand and Godfray 1989), and a theory of the adaptive value of superparasitism has been developed.

In the case of solitary parasitoids it has been shown that the likelihood of superparasitism increases as the difference between oviposition time and rejection time decreases. i.e. Individuals fail to discriminate against hosts previously parasitised by conspecifics, because the process of discrimination is too costly, and the female's time would be better spent laying an egg on each host she encounters and resuming her search for hosts (van Alphen and Visser 1990). Thus when there is no discrimination against parasitised hosts, superparasitism will be a constant function of parasitoid density as random overlap between ovipositing females occurs.

Van Alphen and Visser (1990) have also shown that it can pay a female to superparasitise deliberately when the fraction of hosts parasitised by other females is large. Thus although the probability of an additional egg emerging as the successful parasitoid may be low it can still pay to superparasitise if the availability of free hosts is severely limited. The female may well be able to detect prior conspecific parasitoids in a host and deliberately oviposit nonetheless. In *Bracon hebetor* the rate

of encounter of the female with conspecific eggs or larvae affects the probability of ovicide and superparasitism - which increases with host density (Strand and Godfray 1989).

Whatever the conditions inducing a female to superparasitise, the decision should be made in terms of maximising lifetime fitness. Whether the female's fitness is limited by time spent handling hosts or by time spent searching for unparasitised hosts, superparasitic behaviour should occur when its resulting fitness pay-off exceeds the cost of avoiding it be this in added handling time or added host searching time. Or conversely, in either case, the behaviour of discrimination should only occur when the benefit of avoiding parasitised hosts exceeds the cost of searching for free hosts.

If nothing else, the extremely high levels of superparasitism do imply that there is not a universal avoidance of parasitised hosts as once thought. Van Alphen and Visser's review (1990) gives no quantitative indicators of what sort of levels of parasitoid density are required for their strategies of superparasitism (and self-superparasitism) to evolve but one can believe that the high numbers of parasitoids relative to hosts seen here might be conducive to more complex optimisation behaviour than simply avoiding parasitised hosts at all costs.

Is Occurrence of Parasitism in Hosts Random?

The most obvious test of whether the occurrence of parasitism in hosts is random is to compare the frequency distributions of parasitoids in hosts with a comparable random frequency distribution. From the tests of Goodness of Fit with Poisson distributions it appears that for all data sets (except the minor parasitoids), the occurrence of parasitism in hosts is non random.

The Poisson distribution has one parameter (the mean - which is equal to the variance) and describes the frequency distribution of an event which has an equal probability of occurring in all patches. Thus in this study where it has been used to describe the frequency of parasitoid eggs and larvae in larval hosts it has been assumed that each host larva has an equal proabability of being attacked by a parasitoid. However when one reflects that parasitoids encounter hosts in discrete broods, and that these broods themselves were shown to be unevenly distributed in the environment (see Section 2 of this Chapter), it is evident that the Poisson distribution is an inappropriate description of random selection of hosts by parasitoids. Unfortunately, owing to the complexity of the situation, it is difficult to think of an alternative model.

The problem of using a Poisson distribution as a model of random oviposition casts further doubts on the pattern of dispersion summarised in Figure 3.4.1. The underlying assumption of the graph is that when the variance is equal to the mean then parasitism is random (i.e. Poisson-distributed), while when the variance is less than the mean there must be a tendency toward an even distribution of parasitism. Likewise when the variance is greater than the mean, then the occurrence of parasitism has an aggregated frequency distribution. In reality parasitism is probably more complex than this. When it comes to inferring strategies of even egg-laying from variance to mean ratios a more conclusive test for active avoidance of conspecifics is required.

When host selection is considered in terms of the number of unparasitised hosts (Figure 3.4.2), the proportion of hosts escaping attack through overlapping of parasitism appears to be constant with increasing parasitoid density. It appears that parasitism is more evenly distributed than expected from a Poisson process but again, the adoption of the Poisson distribution as a model of random oviposition is inappropriate.

In both graphs and in the tests of fit of frequency distributions it appeared that the frequency distributions of *Biosteres* spp. and the minor parasitoids gave evidence of aggregative behaviour (insomuch as the negative binomial distribution describes a form of aggregation.) However the numbers of individuals in these species relative to their hosts are low, and apparent aggregation is a renowned artefact in such situations. Hence I would expect that the selection of hosts by these parasitoids is no more or less random than in *A. florimela* but that the fact that relatively few parasitoids occur in many hosts gives the appearance of aggregative behaviour.

It is difficult, therefore, to accurately divine the cause of superparasitism in these species. In particular it is hard to find a model of random selection of hosts against which to test the distribution of parasitism given the non-random distribution of host larvae and host broods in the field. Figure 3.4.2 illustrates that there is certainly not 100% avoidance of superparasitism and a (fairly constant) proportion of hosts escapes parasitism through the overlapping of parasitic attacks which suggests, but does not prove, random overlapping through indiscriminate host selection rather than either dispersal/avoidance or aggregation which might be related to parasitoid density.

The Host Distribution, Fitness Gain Rate and Aggregation.

Maximisation of fitness gain rate has already been used to justify superparasitism whether it be for time-limited females or egg-limited females. When one introduces

the concept of a patchy host distribution into the scenario the situation becomes more complex. As density of conspecifics on a patch increases, non-discriminating time-limited parasitoids will have to decide whether to leave a patch or discriminate against parasitised hosts more carefully. Similarly, discriminating, host-limited parasitoids will have to decide whether to remain on a patch of high parasitoid density or disperse in search of a patch with higher fitness returns.

In Section 2 of this chapter, the selection of oviposition sites by *Pegomya nigritarsis* (albeit in the previous field season in Wytham, Oxford) showed that the distribution of *P. nigritarsis* broods on leaves is highly aggregated. Unfortunately, since the leaf-tagging experiment involved just ten *R. obtusifolius* plants the data set was far too limited to see whether leaf-miner broods are also aggregated at the level of the host plant or host plant site. One can envisage that certain plants might be more preferable than others by virtue of their age, size, location etc., leading to host aggregation at the plant level and at the level of the site.

Whatever the level at which host aggregation were found, one might expect to find corresponding parasitoid aggregation as parasitoids maximise their fitness gain rate by searching in areas of high host density. Hence certain patches would have more superparasitism than expected while others will have less. Certainly it is conceivable that parasitoid eggs could be aggregated at the level of the leaf. i.e. Parasitoids concentrate their searching on leaves where host broods are likely to be concentrated. However there is no possibility of using the data from individual hosts to compare frequency distributions at the different spatial levels which might be relevant to the leaf-miner aggregation.

The apparent aggregation of parasitoids in individual host larvae could occur independently of the host distribution. It may be a result of a patchy distribution of a

parasitoid species in the field, for purely environmental reasons. Thus when all sites are grouped together, certain hosts appear to have attracted more parasitism than others even though the distribution within each of those sites may be even. Another possibility is that parasitoids maximise their success rate by responding to the number of eggs which a host lays. For example aggregation may occur on larger clutches, or modal clutches because once these are located, parasitoids can maximise their exploitation of the host in some way. However this assumes both that the leaf-miner's clutch size is under facultative control according to certain cues and that a parasitoid could use the same cues to concentrate its searching on that clutch size.

Finally, it is conceivable that aggregation might occur at the level of individual hosts, with certain types of individuals in each brood consistently attracting higher parasitism than others. For example in certain host-parasitoid interactions the rate of encapsulation as an immunological response of the host to endoparasitism may vary between hosts (Bouletreau 1986). Also the existence of more than one parasitoid in a host may reduce the probability of an individual being encapsulated (Askew 1968, DeLoach and Rabb 1972, Kitano 1986). However in view of the demonstrated aggregation of host broods one of the former explanations is probably more likely.

Multiparasitism.

Multiparasitism, the concurrence of parasitoids of different species in the same host larvae, was extremely common in this study. However the data shown in Table 3.4.4 simply outline total numbers of all parasitoids in hosts and may not be used to look for strategies of avoidance, discrimination etc. since the within-host variation in parasitoid species is not given.

In the literature, there is little documented evidence of interspecific recognition of parasitism (van Alphen and Visser 1990). Such examples that have been shown involve closely-related species which generally employ similar pheromonal markers that are recognised interspecifically. Considering that there are 9 parasitoid species from several genera involved in this study it would not be surprising if there were little or no recognition of interspecific oviposition cues. More significantly, the fact that there is no obvious discrimination against hosts parasitised by conspecifics, suggests that individuals in general do not value their eggs highly enough to show any discrimination against hosts.

In view of this I believe that the behaviours of the individual parasitoid species should be considered independently. One should assume that the adaptive considerations which predispose superparasitism only involve intraspecific effects.

Superparasitism and Population Stability.

Hassell, Lawton and Beddington (1976) defined three parasite responses which affect a parasitoid population's interaction with a host population and hence their foraging behaviour. First of all there is a response to host density whereby the rate of parasitism is influenced by host availability (the functional response).

The parasitoids' response may also be modified according to the distribution of the host population (the aggregative response) such that where there is aggregation of the host, parasitoids minimise their searching costs by concentrating their foraging in patches of high host density thereby maximising their rate of encounter with hosts. The concentration of parasitism in certain patches can provide a partial refuge for a sector of the host population which leads to stability in the host-parasitoid interaction (Hassell and May 1974, Way and Cammell 1970).

Aggregation of parasitoids may also result from a third host-parasitoid interaction - the interference effect. Interactions between conspecifics (for example discrimination against superparasitised hosts), may lead to longer patch times (van Alphen 1988), which will tend to lead to aggregation of the parasitoid population. Again, this will result in a partial refuge for a proportion of the host population and stabilise the host-parasitoid interaction.

Aggregation of *P. nigritarsis* broods was shown at the level of the leaf in Section 2. However it was not possible to directly compare the distribution of parasitoid broods because of the pooling of data for individual host larvae. Also there is no information available about time spent on host patches according to different parasitoid densities. In Figure 3.4.2, it can be seen that whatever the levels of parasitism, a sector of the host population escapes through overlapping of parasitism. Whether this represents a partial refuge due to an aggregative response to host density is not clear. One would first have to determine what rate of superparasitism would be expected by random overlap of parasitism which, as described above, would be difficult. Also there are no measurements of host density in the field through the season.

However since the proportion of hosts escaping parasitism does not increase with parasitoid density it appears that we can exclude the hypothesis that parasitoid interference affects the rates of parasitism in the field. In other words as parasitoid density increases there are no associated interference effects between parasitoids which reduce the rate of parasitism. This brings us back to the conclusion that the parasitoids do not spend time discriminating against parasitised hosts.

(It should be noted that one well-documented artefactual cause of aggregation is when Poisson-distributed samples with different means are pooled together. This may be the situation with the monthly samples for each species, if the data from each site were proved to be Poisson-distributed. Thus the data in Figure 3.4.2 may appear more aggregated than it really is and there may actually be greater active avoidance of superparasitism than is immediately apparent.)

Tables

3.4.1 : Frequency Distributions of *Adelurola florimela.*

i.) : The Frequency Distributions.

Mon.	No. of Individuals Per Host						Totals			
	0	1	2	3	4	5	P'site	Host	Mean	σ^2
1	991	752	62	10	1	-	910	1816	0.501	0.358
2	412	261	22	5	-	-	320	700	0.457	0.354
3	945	444	22	2	-	-	494	1413	0.350	0.267
4	707	491	71	6	2	1	664	1278	0.524	0.447

ii.) : Goodness of Fit With POISSON Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Conclusion About Distribution
1	134.8	3	$p < 0.001$ ***	Non Random
2	32.9	2	$p < 0.001$ ***	Non Random
3	58.1	2	$p < 0.001$ ***	Non Random
4	45.0	3	$p < 0.001$ ***	Non Random

iii.) : Goodness of Fit With NEGATIVE BINOMIAL Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Value k	Conclusion About Distribution
1	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated
2	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated
3	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated
4	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated

3.4.2 : Frequency Distributions of *Biosteres* spp.

i.) : The Frequency Distributions.

Mon.	No. of Individuals Per Host						Totals			
	0	1	2	3	4	5	P'site	Host	Mean	σ^2
1	1307	391	89	23	5	1	663	1816	0.365	0.450
2	583	95	17	4	1	-	145	700	0.207	0.265
3	1185	200	24	4	-	-	260	1413	0.184	0.201
4	1095	153	24	6	-	-	219	1278	0.171	0.208

ii.) : Goodness of Fit With POISSON Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Conclusion About Distribution
1	53.2	3	**$p < 0.001$ *****	Non Random
2	10.8	1	**$p < 0.001$ *****	Non Random
3	7.9	2	**$p < 0.025$ ****	Non Random
4	16.2	1	**$p < 0.001$ *****	Non Random

iii.) : Goodness of Fit With NEGATIVE BINOMIAL Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Value k	Conclusion About Distribution
1	0.547	5	P = NS	1.575	Aggregated
2	0.111	3	p = NS	0.747	Highly Aggregated
3	0.268	3	p = NS	1.966	Aggregated
4	0.837	4	p = NS	0.769	Highly Aggregated

3.4.3 : Frequency Distributions of Remaining Parasitoids.

i.) : The Frequency Distributions.

Mon.	No. of Individuals Per Host						Totals			
	0	1	2	3	4	5	P'site	Host	Mean	σ^2
1	1732	81	3	-	-	-	87	1816	0.048	0.049
2	672	21	6	1	-	-	36	700	0.051	0.075
3	1284	116	10	2	1	-	146	1413	0.103	0.124
4	1245	30	3	-	-	-	36	1278	0.028	0.032

ii.) : Goodness of Fit With POISSON Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Conclusion About Distribution
1	0.52	1	p = NS	Random
2	1.5	1	p = NS	Random
3	7.0	2	p < 0.01 **	Non Random
4	0.18	1	p = NS	Random

iii.) : Goodness of Fit With NEGATIVE BINOMIAL Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Value k	Conclusion About Distribution
1	0.007	2	p = NS	2.17	Aggregated
2	0.876	3	p = NS	0.093	Highly Aggregated
3	1.451	3	p = NS	0.602	Highly Aggregated
4	0.043	2	p = NS	0.181	Highly Aggregated

3.4.4 : Frequency Distributions of All Parasitoids Together.

i.) : The Frequency Distributions.

Mon	No. of Individuals Per Host							Total			
	0	1	2	3	4	5	6	P'site	Host	Mean	σ^2
1	393	937	379	91	12	4	-	1946	1816	1.121	0.706
2	217	389	73	18	3	-	-	671	700	0.859	0.537
3	429	767	182	27	7	1	-	1245	1413	0.881	0.551
4	443	652	153	25	4	1	1	1060	1279	0.829	0.576

ii.): Goodness of Fit With POISSON Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Conclusion About Distribution
1	219.4	5	$p < 0.001$ ***	Non Random
2	113.3	4	$p < 0.001$ ***	Non Random
3	202.2	4	$p < 0.001$ ***	Non Random
4	128.3	4	$p < 0.001$ ***	Non Random

iii.) : Goodness of Fit With NEGATIVE BINOMIAL Distribution.

Month	X^2 Value	D.F.	Significance of the Difference	Value k	Conclusion About Distribution
1	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated
2	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated
3	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated
4	NO FIT - VARIANCE IS LESS THAN MEAN				Not Aggregated

Figures

FIGURE 3.4.1 : Plot of Variance Versus Mean Number of Parasitoids Per Host.

The line of Y=X represents Poisson-distributed random host attack where the mean number of parasitoids per host is equal to the variance.

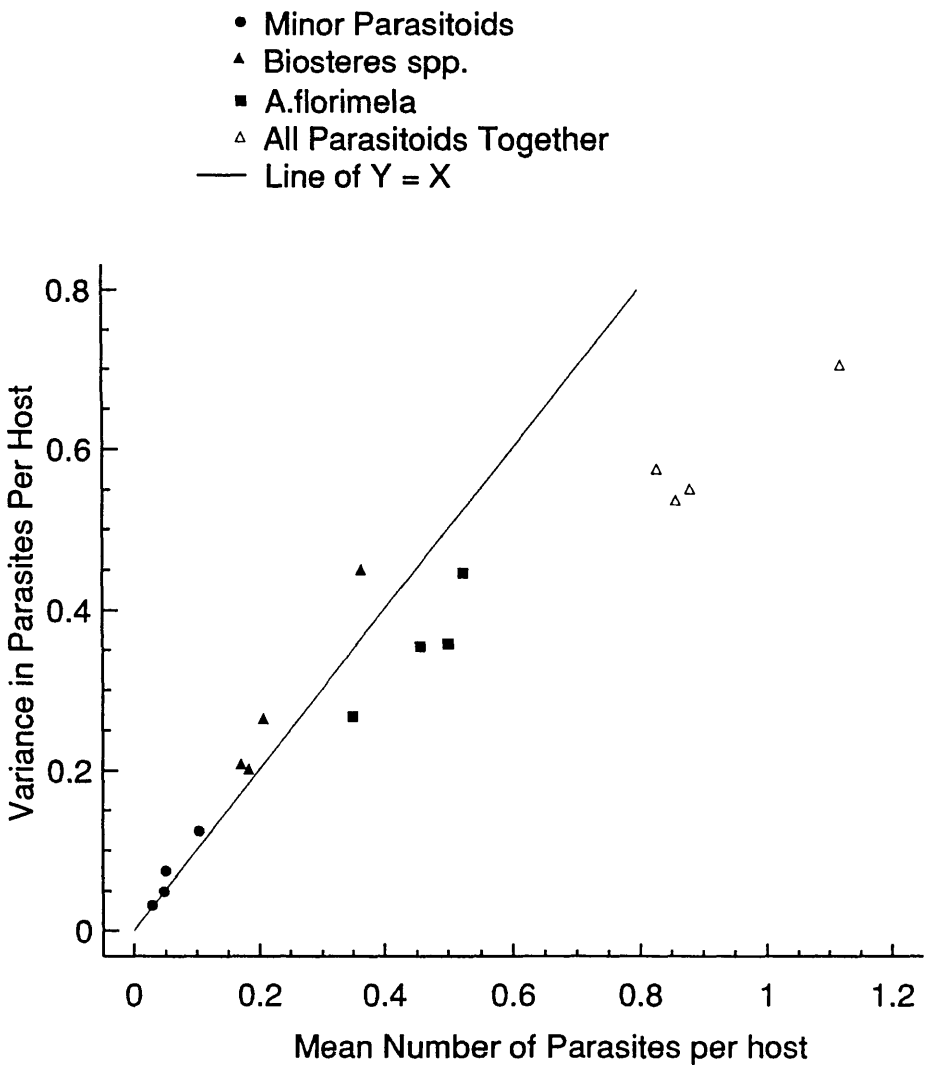
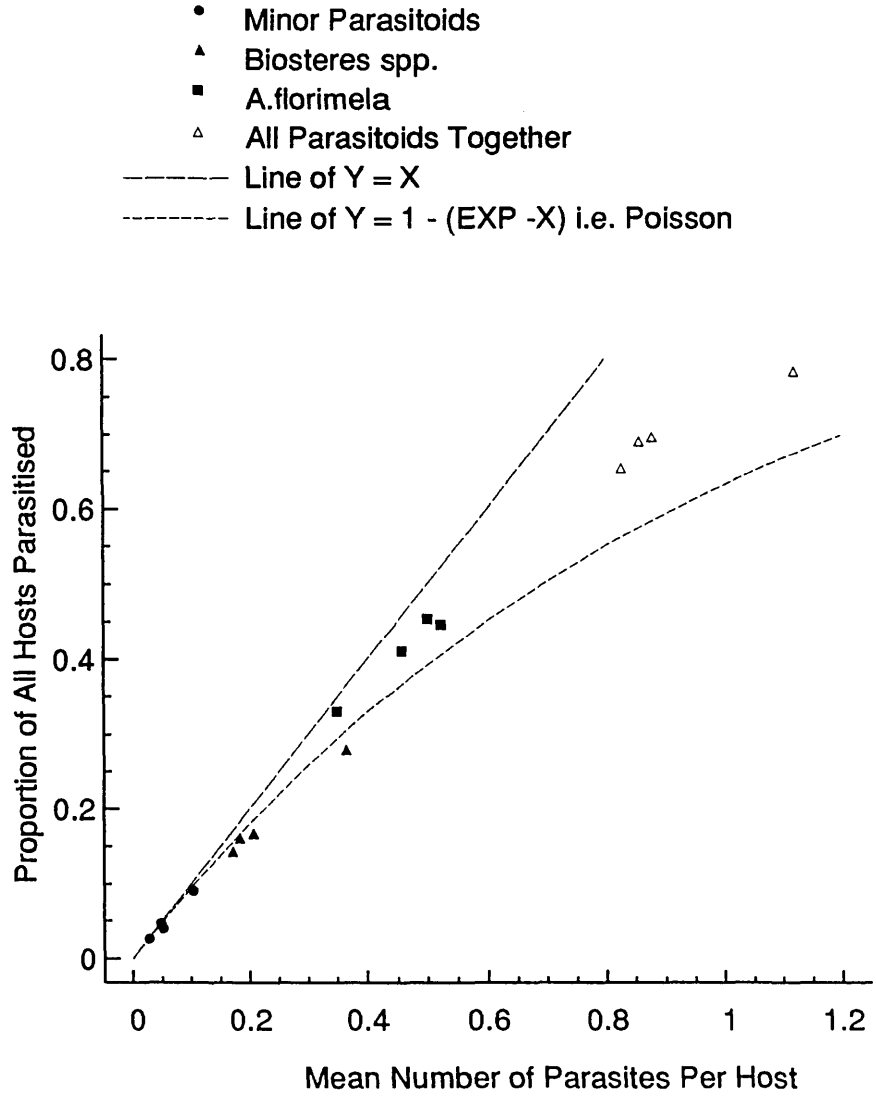


FIGURE 3.4.2 : Plot of Proportion of Hosts Parasitised Versus Mean Number of Parasitoids Per Host.

The line of Y=X indicates 100% dispersal of all parasitoids in their hosts while the line of Y=1 - (EXP -X) indicates Poisson-distributed parasitism. In low numbers of parasitoids, there is apparently non-random aggregated parasitism in hosts whereas in *A. florimela* and the data for all parasitoids together there is apparently less superparasitism than expected from random but not 100% avoidance.



CHAPTER 4

A COMPARATIVE STUDY OF SEX RATIO AND CLUTCH SIZE IN THE BETHYLIDAE - A FAMILY OF PARASITIC WASPS

Summary

1.) The Bethylidae are a family of aculeate parasitoid wasps which exhibit great diversity in certain aspects of their reproductive behaviour. However maternal care, the guarding of broods to pupation or emergence, is a widespread occurrence. Such behaviour constitutes a time investment which, it is argued, should lead to the maximisation of brood fitness in gregarious broods.

2.) Furthermore maternal care should exclude superparasitism in the field such that all mating on emergence occurs exclusively between siblings - a situation conducive to Local Mate Competition (Hamilton 1967).

3.) Since bethylid females are capable of exercising facultative control in the size of their broods and the sex of their offspring, optimal reproductive strategies can be expected in accordance with the size of the host attacked. Sex ratio theory predicts that females should only include as many males in a brood as are ncessary to ensure fertilisation of all daughters.

4.) A comparative study showed a strong negative relationship between clutch size and sex ratio in the Bethylidae in accordance with sex ratio theory. As the mean clutch size for a species increases the mean proportion of males per brood decreases. It appears that mothers include the number of males that will result in the greatest number of fertilised daughters emerging from the given brood.

Introduction and Aims

In the Bethylidae the widespread behaviour of maternal care comprises an enormous time investment for a mother. Females invest so much time in guarding their developing brood against conspecifics and other parasites that they should be expected to maximise the fitness of each brood. Thus we can argue that the primary constraint to brood fitness is the size of the limited resource, namely the host. Consequently a bethylid female has an adaptive decision to make concerning the number of offspring to allocate to a host that maximises the fitness of the brood.

A second allocation decision which arises as a consequence of the brood size is what ratio of male to female offspring in the brood to produce. Since bethylids are all haplodiploid and almost exclusively arrhenotokous, females have the capacity for facultative control of the sex of each offspring. In the case of arrhenotoky, fertilised offspring are female while unfertilised eggs develop into males.

Hamilton (1967) predicted that as the level of inbreeding experienced by emerging siblings increases females should bias their investment to the sex that competes less. He predicted that where a female parasitoid's offspring are under the highest degree of Local Mate Competition she should lay only enough sons to fertilise all her daughters in order to minimise competition between her sons and maximise the reproductive potential of her brood. Thus where there is exclusive sibling mating the ideal number of males is effectively one per brood.

It is now more than twenty years since Hamilton's theoretical predictions were made yet their intuitive appeal has been such that tests of it have been few and limited for the most part to overviews, modelling and studies on individual species (Hamilton 1979, Werren 1980, 1983, Taylor and Bulmer 1980, Taylor 1981, Charnov 1982, Waage and Ng 1984, Waage and Lane 1984). The first study to examine and

compare LMC effects on the sex ratio between taxonomically related species was on the Scelionidae by Waage (1982). Apart from this there have been few, if any, comparative tests. Yet the approach lends itself particularly well to studies of this sort by virtue of its ability to explain taxonomic patterns in the light of the predictive theory (see Pagel and Harvey (in press) for a review of the comparative method in ecology). Furthermore, the economic importance of certain parasitic wasp families leads to a large body of field and laboratory data which can be utilised in comparative analyses.

In the Bethylidae females will have these twin considerations on encountering a host; how many eggs to lay and what sex ratio to produce (i.e. the reproductive strategy) (Waage and Lane 1984). Since maternal care of gregarious broods minimises the probability of superparasitism there is obviously enormous potential for LMC as emerging siblings mate exclusively with each other. Thus ideally a single male should be laid in each clutch (provided of course that this male is capable of fertilising all his sisters). In terms of the overall population sex ratio this will lead to a female-bias. Comparing between species, differences in average clutch size might lead to differences in the overall ratio of females to males in that the relative proportion of males in large clutches may be much lower than that in smaller clutches. Thus an inverse relationship between clutch size and sex ratio might be expected.

The aim of this study is to examine and compare reproductive strategies in the Bethylidae in order to explain the patterns found by the different pressures and constraints acting on different species across the family. A general account of the biology of the family is given followed by a brief description of the methods used in conducting the survey of the literature. Methods are also described for a more

quantitative analysis of sex ratio and clutch size using data from the literature. The results are grouped and presented within topics which are discussed in sequence in order to understand the considerations a female has leading up to oviposition and brood allocation. Finally there is a broader discussion, particularly on the findings of the quantitative study and more general implications of the comparative analysis. (Part of this Chapter has already been published in Behavioural Ecology and Sociobiology 22: 211-217 by Griffiths and Godfray 1988.)

Biology of the Bethylidae

The Bethylidae are a diverse family of small wasps which originated from an early aculeate group. Though relatively primitive in development with respect to other Aculeata, they often have quite advanced behaviour patterns, comparable to those of the Parasitica or higher Aculeata, including such habits as host paralysis, external parasitic development, maternal care of eggs and subsocial behaviour.

In recent years several species have been employed with varying degrees of success in biological control programmes where their longevity, and efficiency of seeking and parasitising coleopterous or lepidopterous larval hosts makes them highly suitable, despite a low reproductive potential. However, while the biology of particular species has often been closely studied, knowledge of the biology and taxonomy of the family as a whole is still limited.

Furthermore a full phylogeny has yet to be worked out. Evans (1964) tentatively proposed a plan for the North American Bethylidae which is summarised in Figure 4.1 to cover the main genera for which there is literature on biology.

Evans proposed that the Bethylidae arose from a large Tiphiid-like ancestor, fully winged in both sexes and with 13 antennal segments. This basic form has

undergone a reduction in body size and in development of certain structures such as the antennal segments and the wings. However development from this form has also involved the evolution of more elaborate and advanced behaviour patterns. While other Aculeata exploit free-living hosts, the Bethylidae have evolved to exploit small larval hosts in concealed or cryptic situations. Thus they have remained relatively small with adaptations for locating and subduing their hosts in their hosts' habitats. In instances where species have adapted to parasitising larger hosts they themselves have remained small.

There is a great diversity of behaviour within the Bethylidae although general patterns still exist. Typically, females subdue host larvae by repeated stinging leading to partial or complete paralysis. Most bethylid species attack Coleoptera species though one subfamily, the Bethylinae, has evolved to exploit Lepidoptera. The host is then left *in situ* (normally concealed) or, in some genera it is dragged, to a suitable place of concealment, whereupon the female lays from one to several eggs on the host, which will develop externally as larvae. Final instar larvae drop off the host and pupate within a cocoon in the immediate vicinity. Adults from a brood tend to hatch more or less simultaneously and mate on emergence with other members of the same brood before dispersing. Commonly males emerge first and chew their way into females' cocoons to copulate.

One of the earliest attempts to classify the Bethylidae was based on behaviour (Bridwell 1920), using such features as degree of maternal care, feeding habit and extent of host paralysis. However while Bridwell's observations may be helpful in grouping behaviour patterns they have no significance in terms of adaptation in different subfamilies. For example the genera *Epyris*, *Laelius* and *Scleroderma* fall into separate behavioural categories under Bridwell's system despite being members

of the same subfamily according to both Evans (1964) and Krombein (1979). Similarly taxonomically unrelated genera (e.g. *Goniozus* and *Laelius*) are categorised together by Bridwell. Overall the emergence of behavioural similarities in unrelated genera implies that certain habits must have evolved more than once through evolutionary convergence.

Thus there is great diversity among species in form and behaviour as they have adapted to a range of hosts and environments. A study of this diversity and the environmental constraints which lead to it should tell us more about the development of the reproductive strategy as a component of a bethylid female's life history.

Methods

A Comparative Survey of Bethylid Reproductive Strategies

As the majority of field studies on Bethylidae has been on economically significant species, the entire series of Review of Applied Entomology (dating back to 1913) was surveyed (both by family and genus) for relevant studies and any further studies referenced therein. All relevant information concerning life history, and particularly reproductive strategy, was collected and organised under subject headings. (e.g. Records of superparasitism, records of winglessness, records of host preference).

The Relationship Between Clutch Size and Sex Ratio

A quantitative comparative study on the relationship between clutch size and sex ratio was conducted using field and lab. data from the literature. Data was only accepted from studies which included estimates of both mean emergent sex ratio and mean clutch size for a given species. All sex ratios were converted to proportion of males,

(which may lead to a slight rounding error in this variable).

Where clutch size ranges, as opposed to specific mean clutch size values were given it was possible to employ a weight against this data point in the analysis which reflected the size of the range. It was assumed that the larger the range of typical clutch sizes given for a particular species, then the less reliable the value of any mean calculated from this would be. The General Linear Interactive Modelling system, GLIM (McCullagh and Nelder 1983) can treat differences due to points with large weights as more important than differences corresponding to points with a small weight. The weight was calculated by the formula;

$$\text{Weight} = 1 / (1 + \text{Log}(1 + \text{Range}))$$

A regression analysis was performed both with and without the weighting system. GLIM fits a model using a maximum likelihood method which is equivalent to a least squares procedure when the errors are normally distributed.

Since sex ratios were obtained from the literature as percentage males on the dependent variable axis these values were arcsine square-root transformed. This serves to normalise the error distribution. Clutch size and log clutch size were compared as independent variables to see which explained more of the variance of errors from the model.

The effect of subfamily and genus as explanatory factors of variance was investigated by including the eight genera and three subfamilies as factors in the analysis.

Results

A total of more than 200 references was found of which at least 50% were taxonomic or descriptive and of no use for this study. The remaining references varied in their

style, content and relevance. The body of literature concerning reproductive strategies could be divided into five topics for comparative analysis and discussion. They are presented in sequence to help understand the conditions and considerations leading up to oviposition and the corresponding decisions governing brood allocation; namely how many eggs and what sex ratio to produce.

Oviposition Preferences and Behaviour.

The first task of any ovipositing female is location and selection of a suitable host. In this section we draw together observations on maternal behaviour leading up to host location, selection and oviposition and look at the patterns in the different species.

Female Aptery and Location of a Host.

Searching behaviour for hosts is obviously affected by female mobility and by the habits and lifestyle of the host. For example in the Pristocerinae where hosts are located under bark, in wood,or in soil,females are wingless (Evans 1964). It appears that the ability to fly has been lost as females have become adapted to this mode of searching. (The less likely alternative is that females in the subfamily lost the ability to fly and have subsequently adapted their host preferences.)

Host Preferences

While behaviour in the Bethylidae is often diverse, there is considerable uniformity in host preferences. All hosts are coleopterous except in the Bethylinae and a few instances in the genus *Epyris* where lepidopterous hosts are attacked. Individual species tend also to be specific with regard to the genus and often the species of host attacked. Finlayson (1952) showed that although *Cephalonomia waterstoni* can be induced to attack three separate *Laemophloeus* (Cucujidae) species, there is a marked

order of preference. Indeed the least preferred species was only attacked by adults late in life who had failed until then to find hosts. However the preferred species was immediately attacked and oviposited on when presented to adults of any age.

Avoidance of Superparasitism and Multiparasitism.

Having located a larva of the right species a female must assess its suitability as a host. Females tend to discriminate against previously parasitised hosts and superparasitism is rare (see Table 4.1.3). The widespread practice of maternal care and egg-guarding presumably discourages such a thing occurring in the field (see Table 4.1.1). However superparasitism has been shown occurring in the lab. (Cherian & Israel 1942, Finlayson 1950). There are also a few records of ovicide and larvicide on previously parasitised hosts prior to egg-laying by a female (Goertzen & Doutt 1975, Venkatraman & Chacko 1961; Griffiths pers.obs. *Goniozus nephantidis* (and see Table 4.1.2)). However this type of behaviour is likely to be rare in the field and limited to those situations where eggs are left unguarded.

Another aspect of host choice is the phenomenon of multi-parasitism (i.e. oviposition on hosts previously parasitised by other species). For example Venkatraman & Chacko (1961) found that when *Goniozus marasimi* eggs occur on the same host as *Bracon* (a braconid) the latter's eggs develop to adulthood but the *Goniozus* eggs do not. This leads to a pattern where braconid females are not loath to multi-parasitise but *Goniozus* females avoid previously parasitised hosts.

Paralysis of Host.

Once a host has been selected and attacked the resulting paralysis tends to be permanent (i.e. until the host's tissues are exhausted) and the host larva fails to develop any further. Only in the genus *Goniozus* are there records of host recovery,

(although the host's movements and further development are restricted). The mobility of the host obviously affects the nature of post-ovipositional maternal care which can be offered. Another consequence of temporary paralysis is that eggs are affixed more strongly and have thicker chorionic shells than eggs laid on permanently paralysed hosts (Gordh & Hawkins 1981).

The Relationship Between Clutch Size and Host Size.

Once a suitable host has been found and paralysed, an ovipositing female must then decide how many eggs to lay on the resource. Her decision will be affected primarily by the size of the host and by whether she maximises brood or individual fitness of offspring. In this section these issues are presented under three questions concerning the relationship between clutch size and host size.

Within Species Are Larger Clutches Laid on Larger Hosts?

There are a great many records for individual species stating that females regulate their clutch size according to the size of the host being oviposited on (including Yamada 1955, Gordh 1976, Mertins 1980 and see Table 4.1.4). Yamada (1955) records that a single egg is laid by *Goniozus claripennis* larvae of 6 mm length and up to eight eggs are laid on hosts of 20 mm length. In fact it is a fairly universal observation in the literature. The most comprehensive study is by Kishitani (1965) who used larval head width as an index to show a clear correlation with number of eggs laid per host.

Within Species is Clutch Size Always Optimal?

Obviously ovipositing females want to maximise the number of individuals that can survive on a given host. However there may well be a cost to exceeding the optimal,

most productive solution. Busck (1917), Rilett (1949) and Schaefer (1962) looking at different genera record lower survivorship and reduced adult size in large clutches. Commonly (e.g. Jayaratnam 1941) females refuse to lay more eggs on a host and only lay again when a fresh host is supplied. Wheeler (1928) suggested that observed cases of ovicide were brood regulatory. However Goertzen & Doutt (1975) studying *Goniozus platynotae* believe ovicide has more of a territorial and nutritional significance.

However clutch size and host size may not be so closely related. For example in some species an abundance of hosts may lead to less than the expected optimal clutch size being laid. Parker and Courtney (1984) identified a range of different considerations which may preclude maximal use of the host resource such as the risk of mortality between oviposition events, maximisation of the fitness gain rate and the probability of superparasitism.

Kearns (1934) looking at *Cephalonomia gallicola* actually states: "The number of eggs laid upon a single host appears to be correlated to a certain extent with the abundance of food material. A scarcity of the host seems to encourage the female to lay a greater number of eggs per individual host and often to return to the host upon which she has previously oviposited to lay additional eggs". However this was the only record of self-superparasitism found in this study. It seems likely that having located and paralysed a host, females generally tend to lay the most productive clutch size given the amount of resource offered by the host before then seeking the next host, however near.

Across Species Are Larger Clutches Laid on Larger Hosts?

When studying average clutch size and average host size between species one might again expect some noticeable trend, with species that attack larger host species laying larger broods on average than species attacking smaller host species. However there is great diversity in the literature due to differing preferences for host genera (and even orders), and different life histories. While *Prorops nasuta* lays one egg and parasitises a relatively small scolytid (2-10 mm long), there are records of *Epyris* species, also solitary ectoparasitoids, attacking tenebrionid larvae which may be up to 40 mm in length (which is much larger than the larvae attacked by many gregarious bethylids). Similarly while *Scleroderma immigrans* lays up to 40 eggs on cerambycids which may be up to 60 mm in length, clutches of up to 40 have been recorded for *Goniozus indicus* on the sugar cane white moth (approximately 10-25 mm).

Clearly however, for a more satisfactory insight into the relationship, much more relevant information is required. Sadly despite the number of host records given, there are very few records of actual size of host, and such studies that gave data were not consistent enough in their choice of parameters to warrant a quantitative comparative analysis.

Adaptive Sex Ratio Control.

Once a female has assessed the quality of her host and determined the optimal number of eggs to lay she then has the further consideration of what ratio of male and female offspring to lay in the brood. In the Bethylidae it is argued that the behaviour of maternal brood care virtually guarantees the highest level of Local Mate Competition; the one-female-per-host situation. When maternal care takes the form of active host defense, conspecific intruders of either sex are excluded from the brood. Male

offspring hatch first and chew their way into their sisters' coccoons to fertilise them, hence outbreeding will be exceptional. Even where maternal care consists solely of host concealment this will make superparasitism on the host very unlikely. Furthermore haplodiploidy will enable the fertilised mother to exercise facultative control of the sex of her offspring (see Table 4.1.5). Thus a mother should be expected to include only as many males as necessary to fertilise the females in the brood (Hamilton 1967).

Hartl (1971) predicted that if only one male were laid in each brood, then should male mortality occur, there could be penalties in terms of the consequent female infertility. Mothers should be selected to produce the mean number of males that maximises the number of inseminated females emerging from each brood, and he assumed that this number of males per brood should have a binomial distribution. Green, Gordh & Hawkins (1982) tested Hartl's prediction of binomial sex ratio alongside a model of their own for precise sex ratios (i.e. less than binomial variance). They found that in the bethylids *Goniozus gordhi* and *Goniozus emigratus*, precise sex ratios were laid. They concluded that selection must favour precise sex ratios because they result in a higher mean number of inseminated females emerging from each brood of a given size. Precise sex ratios have also been shown in *Goniozus legneri* by Gordh, Woolley & Medved (1983). Furthermore Gordh (1976) and Green et al. (1982) state that within species there is a facultative increase in number of males with increasing clutch size.

One implication of precise sex ratios is that the allocation of sexes in a clutch is non-random (i.e. the number of males in a brood is carefully controlled) and there is probably a sequential pattern in the allocation of sex to eggs. Mertins (1980) looked at oviposition sequences in *Laelius pedatus* and found that typical clutches of 2, 3 or 4

eggs each included a single male egg. Typically the male was always the last egg laid in each brood. Powell (1938) states that *Cephalonomia tarsalis* always lays two eggs per host and that these are invariably male and female.

Thus, having decided how many eggs to lay, it appears that a female maximises the number of inseminated daughters in the brood by the laying of precise sex ratios. Facultative variation of the sex ratio is made possible through the haplodiploid condition of arrhenotoky.

Finally, the prior experience of a female appears to be of some importance in determining her patterns of progeny allocation. For example Gordh, Woolley & Medved (1983) used a stepwise regression analysis to show that the sex ratio (i.e. proportion of males) decreased as the number of days since the last oviposition increased. Thus as the interval since their last host attack increased, females increased their investment in daughters. It has also been shown that the sex ratio of consecutive broods tends to move towards a male bias (Gordh & Hawkins 1981, Gordh, Woolley & Medved 1983). This occurs as females exhaust their sperm supplies and fewer and fewer fertilised eggs are included in each brood. Similarly later broods will often tend to decrease in size as egg and sperm supplies become limited (Gordh & Evans 1976).

Oviposition and Solitary Bethylidae.

Thus far this study of reproductive strategies has assumed that all species in the study are gregarious. However when females lay solitary broods then several of the important conditions leading to optimal clutch size and sex ratio strategies may no longer apply (e.g. maximisation of brood fitness, LMC, maternal care). In this section the predicament of solitary bethylids is studied and compared with the gregarious habit.

The Evolutionary Relationship Between Solitary and Gregarious Habits.

Godfray (1987b) argues that the solitary and gregarious habits are separate and distinct phenotypes which arise through the presence or absence of fighting mandibles in larvae. A gene for fighting leads to solitary broods and can invade a population of non-fighting, gregarious individuals but it is much harder for a gene for tolerance leading to gregariousness to invade a population of fighting, solitary individuals. However this theory applies mainly to endoparasitic species and its relevance to the situation of the ectoparasitic Bethylidae is questionable. Certainly there is no documented evidence either for fighting mandibles in the larvae or for any aggression between larvae in solitary bethylid species.

Evans (1964) speculates that the Bethylidae originated from a Tiphiid-like form which laid solitary eggs on hosts. Thus while gregariousness has evolved in certain bethylid lines, the solitary habit may be constrained in certain genera or has not had to evolve to meet environmental pressures. Thus a lower level of maternal care and the maximisation of offspring fitness has been favoured by selection in these genera.

Alternatively, the solitary habit may have re-evolved from gregarious species where the probability of the female locating another host is very high. Thus selection favoured females who laid suboptimal broods which maximised individual offspring fitness. Certainly, under Evan's phylogeny (see Fig. 4.1), the solitary genera *Prorops*, *Epyris* and *Holepyris* do not appear to be close relatives, although they are all in the subfamily Epyrinae. Neither does *Prorops* seem to be more Tiphiid-like than any of the gregarious genera.

The Effect of Aggregation of Hosts on the Sex Ratio.

In solitary species where a single egg is laid on each host the amount of inbreeding will depend on the aggregation of the hosts on which siblings are laid. Where hosts are aggregated and it is likely that siblings may mate with each other a female-biassed sex ratio may still be expected. But if hosts are widely dispersed and there is much outbreeding in emerging parasites, a species should tend to lay closer to a 0.5 "Fisherian" ratio as sons and daughters are likely to make a more equal contribution to a female's future generations.

However there are few biological records in the literature for solitary Bethylidae and there is very little information in the literature to test this. One of the solitary species extensively studied is *Prorops nasuta*, the coffee berry borer. The sex ratio is recorded as 1:3 (males to females) which is still strongly biased. The wasp enters individual coffee beans where several host larvae are found at a time, thus potentially providing a situation for LMC. However isolated records such as this obviously do not constitute satisfactory support for a model.

Individual egg fitness and reduced maternal care.

Another implication of solitary behaviour is that the potential of a host as a resource for rearing offspring may not be maximised. As discussed in the section on clutch size and host size solitary wasps do not necessarily attack small hosts. Some wasps in the genus *Epyris* have been observed to attack tenebrionid hosts up to 40mm in length (Bridwell 1917). Clearly the whole host is not consumed by the single larva and the principal of maximum host exploitation for maximal brood fitness does not apply here. Instead it is likely that individual offspring fitness is maximised. One can predict

there should be very little maternal care as the female will disperse her eggs to maximise their individual fitness and not expend such a large time investment on each solitary brood.

In *Epyris extraneus* maternal care is limited to carriage and cryptic concealment of the host before paralysis (Bridwell 1917, Williams 1919), while in *Prorops nasuta* the female remains in a coffee berry until her offspring emerge as adults, although it must be remembered that there is often more than one host in each berry which the female may attack. Ticheler (1961) records up to 22 progeny of one female found in a single berry.

The Relationship Between Sex Ratio and Clutch Size : a Test of LMC Theory.

The specific data points and the breakdown of genera and species are summarised in Table 4.2. From 9 genera there were 23 species, 5 of which had 2 studies giving different estimates of sex ratio and clutch size, and one species with three different estimates. In 3 cases identical values were recorded for a given species. Since it was not clear whether these were the result of separate studies or whether field observations were just being replicated only a single value was taken. The other 3 species had slightly different estimates. Initially these were included separately in the analysis, giving a total of 27 data points. Later these were converted into an overall mean value for the species to see the effect on the overall relationship.

The regression analysis is summarised in Tables 4.3.1 and 4.3.2. The unweighted regression showed a significant negative relationship between the transformed mean emergent sex ratio (Arc(SR)) and clutch size; $r^2 = 29.5\%$, although

the log of clutch size actually accounts for much more of the variance from the grand mean than clutch size itself; $r^2 = 46.6\%$. Figure 4.2 shows the relationship with the fitted regression line and a second line T which shall be referred to later.

Where more than one pair of values were found for a particular species the overall mean was taken as the value for the species, but this made little difference to the model fit. When the independent variable was clutch size; $r^2 = 28.3\%$, and where it was log clutch size; $r^2 = 44.9\%$.

The weighted analysis gave similar results. For clutch size; $r^2 = 26.5\%$. For log clutch size as the independent variable; $r^2 = 44.8\%$.

In one species, *Prorops nasuta*, a clutch size of one is typically laid. Obviously there is no within brood adaptive control of sex ratio in response to clutch size so in one analysis the effect of excluding this point from the model was investigated. However this made little difference to the overall fit. (With log clutch size as the independent variable; $r^2 = 52.2\%$.)

Taxonomic effects were studied by including subfamily and genus as explanatory factors (omitting the clutch size of one). Adding subfamily as a factor explains 37.1% of the variance, which is significant. Now log clutch size explains an additional 29.8% of the remaining variance, which is still significant in itself. However if genus is added as a nested factor after subfamily it explains 33.3% of the variance. Log clutch size now only explains an extra 5% of the variance from the grand mean, which is no longer significant. Thus after the variance due to subfamily and genus have been accounted for, clutch size itself explains little of the remaining variance.

Discussion

Whatever the treatment of variables employed in the analysis it is clear that there is a significant negative relationship between clutch size and sex ratio in the Bethylidae. As predicted at the outset, the percentage of males included in a brood decreases as species lay larger clutches. This can be compared with Hamilton's (1967) predictions for the extreme conditions of LMC, that only as many males as necessary to ensure fertilisation of the mother's daughters should be included. On Figure 4.2 the line T represents the curve of 1/Log Clutch Size which would be expected if all bethylids included just one male per brood regardless of clutch size. It can be seen that while there is some conformity to this in smaller broods, as clutch size increases the mean sex ratio diverges away from this line and more than one male is being laid per brood.

One reason for this may be simply that in larger broods, a single male is incapable of fertilising all of his sisters, therefore more than one male is included in each brood. There are laboratory records of females exhausting their sperm supplies such that any further eggs they lay are unfertilised hence male (Kearns 1934, Mamaev 1973, Gordh et al. 1983, Gordh and Hawkins 1981) although this does not necessarily preclude a female to laying male eggs for the remainder of her life; females have been recorded to gain fertilisation from their male offspring (VanEmden 1931, deToledo 1942, Rilett 1949).

A second possible reason for increasing male bias in larger broods is that as clutch size increases the penalties of mortality of the single male and the consequent infertility of the brood increases. (This assumes that larger brood laying species invest a greater proportion of their lifetime egg production in each brood than smaller brood laying species.) As discussed in the section on Adaptive Sex Ratio Control, Hartl (1971) predicted that females should produce the number of males that maximises the

number of fertile females emerging from each brood with binomial variance around the mean. Green et al.(1982) have shown that females would be selected to produce males with less than binomial variance and actually lay precise sex ratios in the two species which they studied. Hence in the situation of the Bethylidae it may be correct to say that rather than simply including as many males as necessary to ensure fertilised females in a ratio of (n-1)/2n as Hamilton (1967) would have it, bethylid mothers include the number of males that maximises the number of fertile females on average in each brood.

When subfamily and genus were entered into the analysis to explain the variance from the grand mean, clutch size no longer explained a significant amount of the remaining variance. This could be taken to mean that the relationship between clutch size and sex ratio is simply a taxonomic artefact and an effect of related species with similar clutch sizes habitually laying similar sex ratios. Ridley (in press) queries the validity of using taxonomically related species as independent data points. However personal experience with *Goniozus nephantidis* and the work of Gordh (1976) and Green et al. (1982) suggest that sex ratio is an evolutionarily labile character and subject to facultative within-species variation. The fact that taxonomy accounts for so much of the variance is due to host ranges being evolutionarily conserved. Hence closer related species tend to lay more similar ranges of clutch size and it is clutch size that is the primary determinant of sex ratio.

It is debatable whether the data from solitary species should be included in this analysis since the argument has been that brood fitness should be maximised for every host and this does not apply for solitary species. Furthermore there is the obvious point that solitary laying females are not optimising brood sex ratio according to clutch size. However in the end valid data was only obtained for one solitary species

and the exclusion of this data point made little difference to the overall fit.

One area of study which would be interesting to look at further would be the relative sizes of males and females in different species. Unfortunately such data is difficult to obtain for a comparative study and insufficient data was found. It would be interesting to see whether the ratio of male size to female size is related to mean clutch size for a given species. For example as mean clutch size increases do mothers decrease or increase their relative investment in sons? Where species lay a similar clutch size but include different proportions of males is there a significant difference in male:female size ratio?

Further study could also focus on the winged/wingless polymorphisms which appear in the Bethylidae (see Table 4.1.6). In his phylogeny of the Bethylidae, Evans (1964) drew out three main types of polymorphism in relation to subfamily; sexual dimorphism with apterous females in the Pristocerinae, polymorphism in both sexes in some genera of the Epyrinae, and no polymorphisms (genera from all three subfamilies). Hamilton (1967) recognised the significance of wing polymorphism to inbreeding of haplodiploid species. Winglessness in males may have implications for dispersal and consequently the level of outbreeding in the species while winglessness in females may affect female host searching strategies. Thus Hamilton (pers.comm.) queried whether species diverging upwards of line T on Figure 4.2 had more than one male per brood because there was greater outbreeding which might be reflected in a high proportion of winged males whereas species that tend to have one male per brood may have largely wingless males. However this was not supported here, due probably to exclusive sibling breeding being guaranteed by maternal care. Also the majority of data points come from the uniformly winged Goniozus genus which is the most reported genus in the applied literature.

Post Script : A Response to Ridley (1989)

The study on the effect of clutch size on sex ratio was presented as a paper in Behavioural Ecology and Sociobiology (Griffiths and Godfray 1988; and see Appendix). It subsequently came under criticism from Ridley (1989) because we justified the treatment of related species as separate data points by evidence of within-species adaptive variation. He cites two types of situation where the use of the comparative method may be justified and then queries the validity of our approach.

Firstly, as in any significance test of a hypothesis, trials should be completely independent. But he points out that even if a character is free of phylogenetic constraint it is still likely to be associated with other factors within that taxon, i.e. even if within-species adaptive variation in sex ratio with clutch size can be shown, there are probably many other 'third variables' (aspects associated with clutch size in the Bethylidae) which are constrained and thus do not allow it to be treated as an independent character in different species.

The second situation that Ridley offers is when convergent evolution and taxonomic similarities are caused by separate mechanisms - respectively, adaptation and phylogenetic constraint. The comparative method can be employed to determine the relative importance of the different causal processes. (Steams 1983, Cheverud *et al.* 1985). Thus under general conditions, natural selection is constrained from effecting evolutionary change and related species are similar. Adaptive change occurs occasionally when natural selection 'breaks through'. Our approach would be justified then if natural selection had 'broken through' to solve the optimal clutch size problem independently in each species. Ridley concludes that while the argument may work against constraints it is not valid to apply it for calculating significances of non-independent trials as we were.

It has to be said that Ridley's criticism is probably valid in strict terms and undermines the statistical validity of the test. Indeed the likelihood that host size is taxonomically constrained to some degree has already been acknowledged. Furthermore no attempt was ever made to actually relate "degree of relatedness" to reproductive strategy in quantitative terms. However surely as a general pattern within and between species indicating the allocation of males to broods of increasing size it is still a useful guide.

Tables

Table 4.1 : Reference Tables

4.1.1 : Maternal Care in the Bethylidae.

Reference	Species	Observation
Baker 1976	*Pristocera rufa*	Brood guarding and cleaning
Williams 1919	*Epyris extraneus*	Host carriage, concealment
Howard 1920	*Laelius trogadermatis*	Depilation of host
Ayyappa & Cheema 1952	*Laelius voracis*	Depilation of host
Mertins 1980	*Laelius pedatus*	Depilation of host
Yamada 1955	*Laelius sp.*	Depilation of host
Powell 1938	*Cephalonomia tarsalis*	Host carriage, concealment
Rilett 1949	*Cephalonomia waterstoni*	Host carriage, concealment
Kuhne & Becker 1974	*Scleroderma domesticum*	Brood guarding
Bridwell 1920	*Scleroderma immigrans*	Brood guarding
Chatterjee 1941	*Bethylus distigma*	Brood guarding
Doutt 1973	*Prosierola bicarinata*	Aggressive brood guarding
Avasthy & Chaudhary 1963	*Parasierola sp.*	Brood guarding
Girling 1979	*Parasierola sp.*	Brood guarding
Jayaratnam 1941	*Parasierola nephantidis*	Brood guarding
Rude 1937	*Parasierola cellularis*	Host carriage
Gordh 1976	*Goniozus gallicola*	Aggressive host guarding

Table 4.1 : Reference Tables (Continued)

4.1.2 : Intraspecific Ovicide in the Bethylidae.

Reference	Species	Observation
Remadevi et al.1978	*Parasierola nephantidis*	Rarely female eats own eggs
Goertzen and Doutt 1975	*Goniozus platynotae*	Female eats eggs less than 72hrs old of other females
Venkatraman and Chacko 1961a,b	*Goniozus marasimi*	Females regulate own brood if hosts scarce and always destroy other female's eggs
Bridwell 1920	*Scleroderma immigrans*	If host drys out females may consume own eggs
Malyshev 1968	*Scleroderma sp.*	Females continue ovipositing until host tissues dry up, then consume remaining eggs

4.1.3 : Superparasitism in the Bethylidae.

Reference	Species	Observation
Samad 1973	*Goniozus rugosus*	Never attacks previously parasitised larvae
Venkatraman & Chacko 1961	*Goniozus marasimi*	Female eats eggs off previously parasitised larvae before laying own eggs
Gordh 1976	*Goniozus gallicola*	When two females on one host only one oviposits. Females aggressive towards other intruding females
Cherian & Israel 1942	*Goniozus indicus*	Only one female per host unless simultaneous attack and oviposit
Goertzen & Doutt 1975	*Goniozus platynota*	Female eats eggs off previously parasitised host before laying own eggs
Finlayson 1950	*Cephalonomia waterstoni*	Can be induced to oviposit on previously attacked hosts

Table 4.1 : Reference Tables (Continued)

4.1.4 : Facultative variation of Clutch Size in the Bethylidae.

Reference	Species	Observation
Kearns 1934	*Cephalonomia gallicola*	Clutch size correlated to abundance of hosts
Ayyappa and Cheema 1952	*Laelius voracis*	Clutch size regulated to host size
Mertins 1980	*Laelius pedatus*	Clutch size regulated to host size
Mertins 1985	*Laelius utilis*	Clutch size regulated to host size
Antony and Kurian 1960	*Perisierola nephantidis*	Clutch size regulated to host size
Rao et al.1948	*Perisierola nephantidis*	Clutch size regulated to host size
Bridwell 1919	*Perisierola sp.*	Clutch size regulated to host size
Gordh et al.1983	*Goniozus legneri*	Clutch size regulated to host size
Gordh and Hawkins 1981	*Goniozus emigratus*	Clutch size regulated to host size
Gordh 1976	*Goniozus gallicola*	Clutch size regulated to host size
Kishitani 1965	*Goniozus japonicus*	Clutch size regulated to host size
Legner et al.1982	*Goniozus legneri*	Clutch size regulated to host size
Yamada 1955	*Goniozus claripennis*	Clutch size regulated to host size and species

Table 4.1 : Reference Tables (Continued)

4.1.5 : Control of Sex of offspring in the Bethylidae.

Reference	Species	Observation
Mertins 1985	*Laelius utilis*	Male egg always laid last temporally and positionally
Gordh et al.1983	*Goniozus legneri*	Lays precise sex ratios
Green et al.1982	*Goniozus gordhi*	Lays precise sex ratios
	Goniozus emigratus	Lays precise sex ratios
Gordh 1976	*Goniozus gallicola*	Larger broods have more males
Powell 1938	*Cephalonomia tarsalis*	Always lays one male and one female egg on each host
Finlayson 1950	*Cephalonomia waterstoni*	Second egg laid usually male

4.1.6 : Records of Wing Polymorphisms in the Bethylidae.

Reference	Subfamily/Genus/Species	Observation
Kearns 1934b	*Cephalonomia gallicola*	Males dimorphic, Females wingless
Bridwell 1920	*Scleroderma sp.*	Males and Females dimorphic
Keeler 1929	*Scleroderma immigrans*	Males and Females dimorphic
Malyshev 1968	*Scleroderma sp.*	Males and Females dimorphic but winged males and wingless females are much rarer
Baker 1976	*Pristocera rufa*	Males winged, Females wingless
Bognar 1957	*Pristocera depressa*	Males winged, Females wingless
Chatterjee 1941	*Bethylus distigma*	Males and Females wingless
Evans 1964	*Pristocerinae*	Tendency to dimorphism; males winged, Females wingless
	Scleroderma, Bethylus, Cephalonomia	Tendency to winglessness and wing reduction in both sexes

Table 4.2 : Records of Bethylid Clutch Size and Sex Ratio.

Species	Sex Ratio	Clutch Size	Reference
Pristocera rufa	0.17	15.9	Baker 1976
Laelius pedatus	0.33	3.0	Mertins 1980
Laelius utilis	0.29	3.4	Mertins 1985
Laelius voracis	0.33	4.0	Ayyappa and Cheema 1952
Prorops nasuta	0.25	1.0	Hargreaves 1926; deToledo 1942
Cephalonomia tarsalis	0.50	2.0	Powell 1938
Cephalonomia waterstoni	0.33	2.0	Finlayson 1950; Rilett 1949
Scleroderma immigrans	0.17	10-40	Bridwell 1920
Scleroderma turkmenicus	0.04	100-110	Mamaev 1973
Prosierola bicarinata	0.17	4.0	Doutt 1973
Goniozus sp.	0.12	11.7	Girling 1979
Goniozus sp.	0.25	1-25	Avasthy and Chaudhary 1966
Goniozus sp.	0.20	4.0	Taylor 1933
Goniozus aethiops	0.14	7.0	Gordh and Evans 1976
Goniozus cellularis	0.20	5.0	Nickels et al. 1950
Goniozus claripennis	0.17	3.0	Voukassovitch 1924
Goniozus emigratus	0.20	14.8	Willard 1927
Goniozus emigratus	0.03	4-17	Busck 1917
Goniozus emigratus	0.18	15.6	Green et al. 1982
Goniozus gallicola	0.17	6.5	Gordh 1976; Green et al.1982
Goniozus indicus	0.09	9-40	Cherian and Israel 1942
Goniozus indicus	0.09	1-24	Gifford 1965
Goniozus jacinctae	0.25	1.8	Danthanarayana 1980
Goniozus nephantidis	0.25	1-15	Jayaratnam 1941
Goniozus nephantidis	0.17	10-18	Antony and Kurian 1960
Goniozus platynotae	0.25	3.4	Goertzen and Doutt 1975
Goniozus procerae	0.20	10.0	Coquard 1984

Table 4.3 : Results of Regression Analysis.

4.3.1 : Overall Results of Regression Analysis.
1.) Unweighted regression, independent variable Clutch Size; $Arc(SR) = 0.5 - 0.022\ CS;\ F_{(1,25)} = 10.88,\ p < 0.01\ ;\ r^2 = 29.5\%$
2.) Unweighted regression, independent variable Log Clutch Size; $Arc(SR) = 0.63 - 0.23 Log(CS);\ F_{(1,25)} = 22.7,\ p < 0.01\ ;\ r^2 = 46.6\%$
3.) Weighted regression, independent variable Clutch Size; $Arc(SR) = 0.52 - 0.026\ CS;\ F_{(1,25)} = 9.4,\ p < 0.01;\ r^2 = 26.5\%$
4.) Weighted regression, independent variable Log Clutch Size; $Arc(SR) = 0.63 - 0.23\ Log(CS);\ F_{(1,25)} = 21.1,\ p < 0.01;\ r^2 = 44.8\%$
5.) Weighted regression, independent variable Log Clutch Size, exclude *Prorops nasuta*; $F_{(1,24)} = 27.3,\ p < 0.01;\ r^2 = 52.2\%$.)

4.3.2 : Subfamily and Genus as Explanatory Factors of Variance.		
1.) The effect of subfamily;		
Subfamily;	$F_{(2,23)} = 7.1,\ p < 0.01.$);	$r^2 = 37.1\%$
Log Clutch Size;	$F_{(1,22)} = 20.7,\ p < 0.01.$);	$r^2 = 29.8\%$
2.) The effect of genus;		
Subfamily;	$F_{(2,23)} = 7.10,\ p < 0.01.$);	$r^2 = 37.1\%$
Genus;	$F_{(4,19)} = 5.60,\ p < 0.01.$);	$r^2 = 33.3\%$
Log Clutch Size;	$F_{(1,18)} = 3.82,\ p < 0.1)$;	$r^2 = 5.0\%$

Figures

Figure 4.1 : A Phylogeny of the Bethylidae (Evans 1964).

A summary of Evans' (1964) proposed phylogeny of the sub-families in the Bethylidae summarised for the genera which occur in this study.

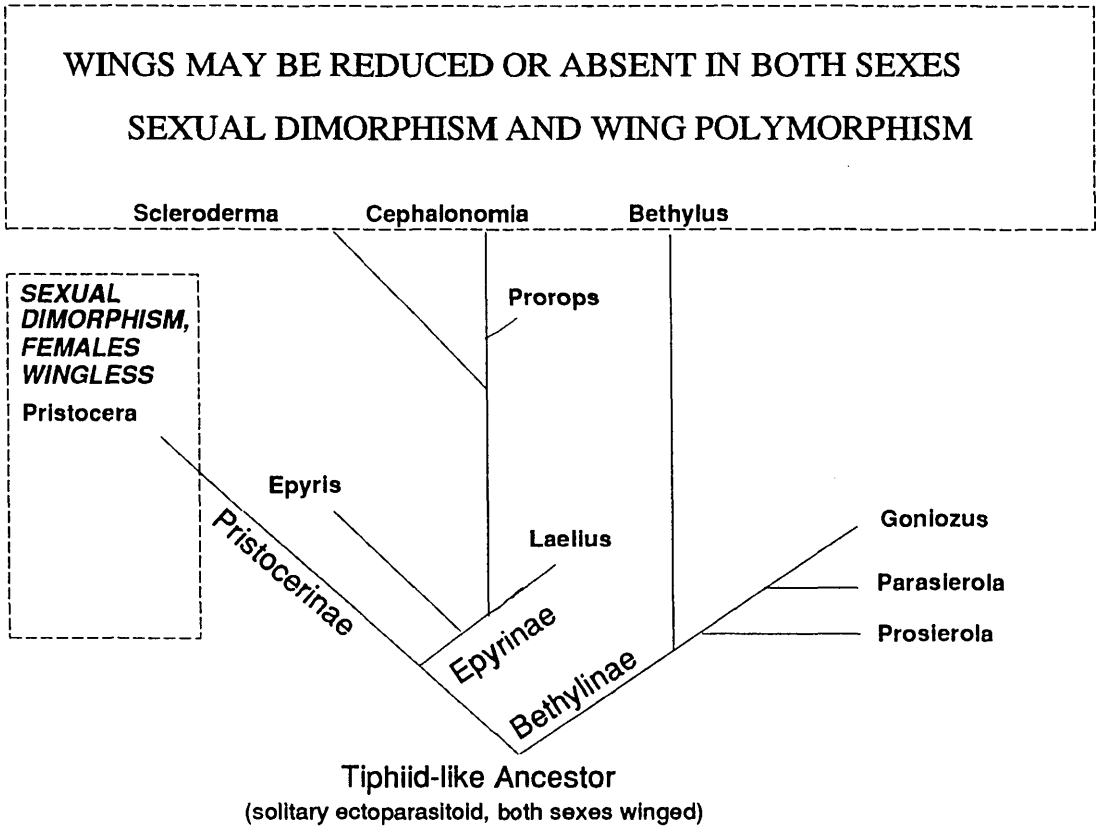
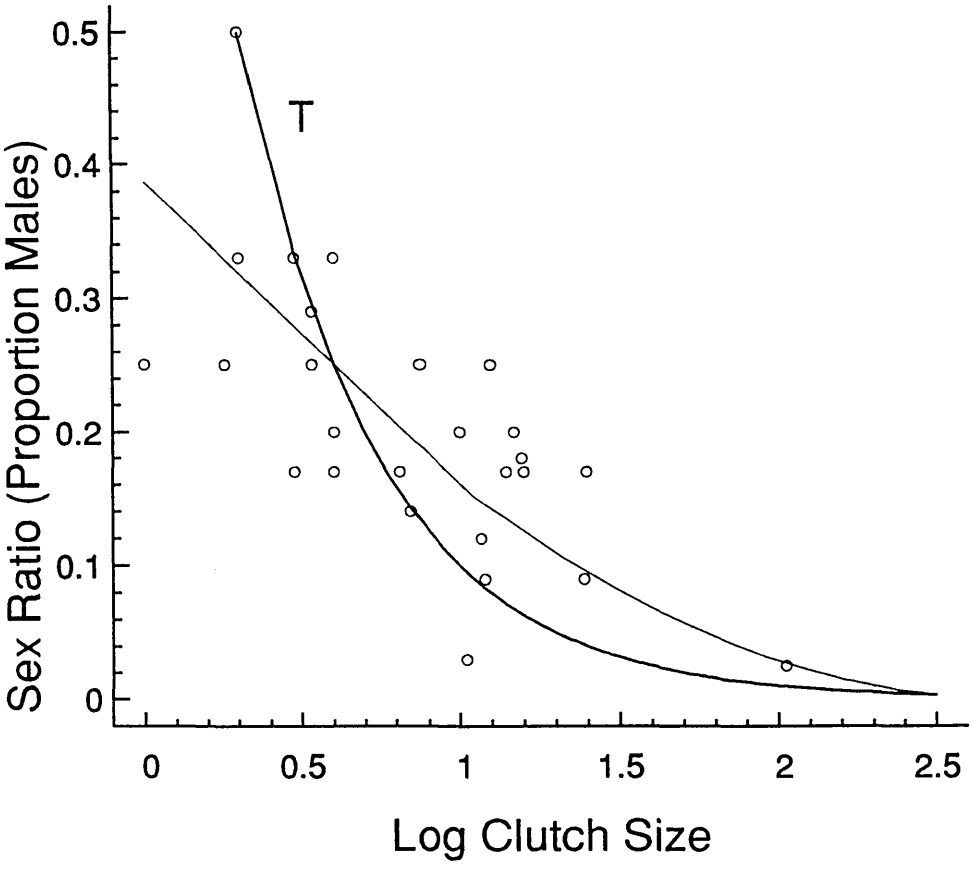


Figure 4.2 : The Relationship Between Clutch Size and Sex Ratio in the Bethylidae.

The line T indicates where sex ratio is the reciprocal of clutch size, i.e. one male per brood. The second line is a line of best fit, calculated with arcsine transformed data and then converted back (hence the curvature.) It describes a regression of Arc (Sex Ratio) = 0.63 - 0.23.Log (Clutch Size), $r^2 = 44.8\%$.



CHAPTER 5

SUMMARY AND CONCLUSIONS

The main objective of this thesis was to contribute to the body of experimental tests of insect clutch size. The core of the work was based around laboratory and library work during the winter, and field work during the summer.

In Chapter 1, I reviewed the development of clutch size theory and the concept of brood laying as a means of maximising one's fitness in certain situations. There are many benefits to laying eggs in groups rather than singly (Table 1.1) but by far the most universal and hence best explored benefit is that in certain situations a female can maximise her rate of gain of fitness by laying her eggs in groups. This occurs typically when a female's fitness is limited chiefly by time or opportunities to oviposit. Having decided to lay eggs in batches a female must also decide how many eggs to include. The fitness of a female's offspring may decrease with increasing brood size for a number of reasons (Table 1.2) and this in turn can lead to an optimum number which maximises the total fitness returns to the female from the brood. The over-riding conclusion gained from this chapter was that there is a huge diversity of reproductive strategies shown by different species within the Insect class by which parental fitness is maximised. The strategy of laying eggs in broods occurs throughout many of the insect orders including the Coleoptera, Lepidoptera, Hymenoptera and Diptera.

In Chapter 2, I described an experimental system which I worked on in the laboratory using the gregarious ectoparasitoid, *Goniozus nephantidis*. I quantified the relationships between clutch size and offspring size and offspring size and fitness and used these to predict the clutch size which should result in the highest total fitness returns to the parent. However this prediction proved to be far in excess of the clutch sizes typically found on the given host size range. One reason for this could have been

that females were not seeking to maximise their fitness with each brood although there is a strong case for saying that females of this species should. An alternative and more likely explanation is that females do indeed lay broods that maximise their total fitness returns from the brood but that this experiment failed to determine the full extent of these fitness returns. Since all work occurred in the laboratory it was not possible to quantify offspring size and fitness in the field. I drew a distinction between species where the effects of clutch size on offspring fitness are experienced immediately (e.g. through mortality or reduced size and fecundity), and those such as *G. nephantidis* where offspring fitness may be indirectly expressed through behaviour in the field (e.g. searching ability.) A notable observation from this work was that there was enormous variation in clutch size even within a narrow range of host sizes.

In the leaf-miner *Pegomya nigritarsis* (Chapter 3) the distribution of broods on leaves in the field is highly aggregated. Clutches tended to be laid on younger leaves, which were larger than average and the coexistence of more than one brood on a leaf occurred more often than expected by chance overlap. (Although it was not clear whether such superoviposition was a choice on the part of ovipositing females or the effect of certain leaves being more favourable sites and attracting greater attention.) However, once a site has been selected, there is no obvious determinant of the clutch size which is laid. Clutches ranged in size from 1 to 8 eggs but the only factor which explained any of this variation significantly was the plant itself, i.e. there was greater variation in clutch sizes between plants than from leaf to leaf on the same plant. Thus the clutch size decision may be a response to some aspect of the whole plant such as anticipated parasitism according to its location. I found that the probability of larvae being parasitised increased with increasing clutch size (although there was great variation between broods of the same size in the parasitism which they experienced.) Thus there is an optimal brood size at which the proportion of offspring surviving to

pupation is maximised. This was also found to be the modal clutch size collected from the field. Thus it appears that ovipositing females tend to lay the clutch size which maximises brood fitness rather than individual fitness. At the end of Chapter 3 I looked at the frequency distributions of the parasitoid eggs and larvae in individual hosts. I found that superparasitism occurred more frequently than expected by random host encounters and argued that even in solitary endoparasitoids the laying of additional eggs in hosts could have an adaptive value. In this case it appears to occur as parasitoids maximise their rate of encounter with hosts rather than spending time discriminating against parasitised hosts.

Finally, in Chapter 4 I made a comparative study of the reproductive behaviour in the hymenopteran sub-family the Bethylidae. In particular I looked at a situation in which clutch size itself determines another aspect of an insect's reproductive ecology, namely the sex ratio. Local Mate Competition results in highly female-biassed sex ratios as individuals possess the ability to lay only as many males as necessary to fertilise their whole brood. A general trend occurs both within and between species such that as clutch size increases, the relative proportion of males required for fertilisation of all female offspring decreases.

All three experimental chapters on clutch size theory (Chapters 2 to 4) involved systems where the parent's rate of gain of fitness was increased by laying eggs in broods rather than singly. In each case individual *per capita* fitness decreases with increasing clutch size resulting in a calculable brood fitness optimum. In the Bethylidae, (Chapters 2 and 4), offspring fitness declines with larval competition. In *P. nigritarsis* there is both larval competition and an increase in parasitism with increasing clutch size. In all cases it is possible to calculate clear brood fitness optima

and in terms of the list of advantages of gregarious laying given in Figure 1.1, it is the maximisation of rate of gain in fitness which seems to be the paramount benefit in each of the systems investigated.

The most universal observation to emerge from the studies of clutch sizes in *G. nephantidis*, clutch sizes in *P. nigritarsis* and survivorship of *P. nigritarsis* larvae in different-sized clutches is the very large amount of within-species variability which occurs. In Chapter 3, Section 3 I note that natural selection will favour even the slightest of trends which improve fitness, but nonetheless the maintenance of such high variance in behaviour does suggest either that females are imprecise layers, or that optimal reproductive strategies in insects may be rather more complex than in the conventional examples which are often cited for mammals or birds.

Variability may arise through disruptions, random quirks and inconsistencies in the environment (Klomp and Teerink 1962). Another possible explanation of high variance may be that females are incapable of consistent, accurate assessment and oviposition but show stochastic variance about the actual optimum (Godfray and Ives 1988).

It is very possible that alternative optimal strategies are adopted, either by different individuals, or by the same individuals through their life. One obvious difference between the life-histories of insects and vertebrates is that insect females generally produce many more offspring (and many more broods) in a lifetime. Certainly in the cases which I have studied, oviposition behaviour constitutes a potentially on-going process through the female's life. Hence a dynamic optimisation approach (as opposed to strictly deterministic assumptions) may be more appropriate in studying insect oviposition strategies since the optimal strategy for lifetime fitness may depend more on the state of the female at the given time (e.g. hunger, egg

availability, risk of mortality). i.e. Their priorities for maximising their lifetime fitness may be subject to greater change (Iwasa *et al.* 1984, Mangel 1989). One can envisage that this will lead to much greater variation in clutch sizes observed in the population compared with less fecund avian or mammalian systems where reproduction constitutes a greater component investment in lifetime fitness.

Thus I would suggest that future work on insect clutch sizes should pay more attention to the variability in oviposition behaviour, both between individuals and in the same individuals. In particular I believe an approach using predictive dynamic optimisation models backed up by controlled laboratory experiments would be particularly rewarding. More emphasis should be placed on following females throughout their adult life to see whether clutch size variation can be explained in terms of previous (or future) reproduction.

In addition to studies of the decision of individual insects, the variation itself should be studied more closely. The possible components should be quantified, and rationalised in terms of their selective significance. For example Godfray and Ives (1988) have incorporated environmental and individual stochasticity into simple invertebrate clutch size models and shown that the predicted optimal clutch size can be substantially different to deterministic predictions. As yet, many of the models which they introduced have not been practically tested.

I observed in the introductory literature review to the thesis that studies on insect clutch sizes have been confined mainly to the Hymenoptera, Coleoptera and Lepidoptera. A widening of scope from the insect orders which have traditionally been studied would certainly be welcome.

Furthermore most manipulative studies have concentrated on systems where the *per capita* effect on individual fitness has been expressed in terms of increasing larval competition for limited resources. However in Table 1.2 I list seven other documented *per capita* effects some of which are somewhat speculative but others of which might be of interest for comparing some of the selective pressures on the clutch size decision.

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APPENDIX 1

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Local mate competition, sex ratio and clutch size in bethylid wasps

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Summary. Parasitoid wasps of the family Bethylidae lay groups of eggs on the larvae of beetles and moths. The larvae develop together and pupate in the vicinity of the host. On hatching, the majority of copulations are between siblings leading to a high degree of local mate competition. Sex ratio theory predicts that as the number of individuals developing on a host increases, the population sex ratio will become progressively more female biassed. A comparative study of sex ratios across the family supports this prediction.

Introduction

Gregarious parasitoid wasps lay clutches of eggs on, or in, the bodies of their insect hosts. The eggs hatch and the larvae feed together on the host until pupation. The host is a fixed resource and there will be an optimum clutch size for the mother to ensure the maximum number of good quality offspring. In the last few years there has been much interest in factors affecting the optimum clutch size both in invertebrates in general (Parker and Courtney 1984; Parker and Begon 1986; Begon and Parker 1986; Godfray 1987a; Mangel 1987; Ives 1988) and in parasitic wasps in particular (Charnov and Skinner 1984, 1985; Skinner 1985; Iwasa et al. 1984; Waage and Godfray 1985; Waage 1986; Godfray 1987b; Godfray and Ives 1987).

Parasitoid wasps, like other Hymenoptera, are haplodiploid with males developing from unfertilized eggs and females from fertilized eggs. An ovipositing female can thus determine the sex of a particular offspring by whether or not she fertilizes the egg. There is now a large body of evidence showing that parasitoids make adaptive decisions about sex allocation (reviews in Charnov 1982; Waage 1986). The most often studied factor biassing the sex ratio is Local Mate Competition (LMC: Hamilton 1967). If offspring of one sex compete for a limiting resource more strongly than offspring of the other sex, natural selection will favour the mother to bias her sex ratio towards the sex that competes less (Taylor 1981). In the case of LMC, sons, but not daughters, compete for mates leading to a female biassed sex ratio. Alternative descriptions of LMC are given by Colwell (1981), Nunney (1985) and Frank (1986).

The majority of studies of reproductive strategies in parasitoids have examined clutch size and sex ratio independently (for exceptions see Werren 1980; Waage and Ng 1984; Frank 1985; Godfray 1986). However, in species where pupation and mating occur around the remains of the host, the level of LMC will be influenced by the number of parasitoids developing on the host. In other words, optimal sex ratio will be influenced by clutch size. In this paper we perform a comparative analysis of clutch size and sex ratio in the parasitoid wasp family Bethylidae. Our aim is to determine the relationship between sex ratio and clutch size and to compare this with the predictions of LMC theory. Before proceeding to the methods section, we briefly describe the biology of Bethylidae.

Together with the families Dryinidae and Chrysididae, the Bethylidae make up the hymenopteran superfamily Bethyloidea. Evans (1964) divides the family into three subfamilies and his classification is followed here. The genera *Goniozus sensu stricto* and *Parasierola* are today treated as *Goniozus* (Gordh and Evans 1976) though in the analysis we retain them as separate taxonomic units.

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Bethylid females typically subdue their hosts, the larvae of beetles and moths, by repeated stinging which leads to partial or complete paralysis. The host may then be left in situ or dragged to a suitable place of concealment (Bridwell 1917; Williams 1919; Rude 1937; Powell 1938; Rilett 1949), where the female places from one to a large number of eggs externally on the host's body. If the host is hairy, the female may remove some of the hairs, presumably to facilitate larval feeding (Howard 1926; Vance and Parker 1932; Kearns 1934a; Ayyappa and Cheema 1952; Mertens 1980, 1985). A number of authors have noted that bethylids typically lay larger clutches on larger hosts (Ayyappa and Cheema 1952; Mertens 1980, 1985; Bridwell 1919; von Kuhne and Becker 1974; Antony and Kurian 1960; Yamada 1955; Kishitani 1965; Legner 1982; Gordh et al. 1983; Gordh 1976; Gordh and Hawkins 1981; Green et al. 1982; Ramachandra Rao et al. 1948; Samad 1973). The parasitoid larvae develop as ectoparasites and eventually pupate around the host remains. Mating normally takes place at the pupation site and males frequently chew into female cocoons in order to copulate (see Discussion).

Bethylids have been used in a number of biological control programmes (Antony and Kurian 1960; le Pelley 1968; Gordh et al. 1983; Gordh and Evans 1976). An understanding of their behavioural ecology may help in the design of mass-rearing programs and in their evaluation as biological control agents.

Methods

We surveyed the literature for accounts of bethylid biology that mentioned both the clutch size and sex ratio. The majority of biological work on this family has concerned economically significant species so we searched the Review of Applied Entomology from the first volume (1913) until the present and looked for further studies referenced in the papers we found. In addition to information on clutch size and sex ratio, we recorded any other aspects of the biology of the wasps relevant to reproductive strategies.

Our basic data consisted of a list of sex ratios and clutch sizes. The majority of studies gave sex ratios in the form males: females and these were converted to proportion males. Ratios were typically expressed in the original papers as whole numbers: 1:2, 1:3 etc., and this approximation leads to some rounding error in this variable. Clutch sizes were normally given as means and/or ranges. Where ranges alone were given, we calculated a mean value as the average of the limits of the range. Two analyses of the relationship between sex ratio and clutch size were performed. In the first, all clutch size means were treated identically while in the second analysis, the different mean clutch sizes were weighted in inverse proportion to their range. The reason for this was that it was assumed that values of mean clutch size calculated from large ranges were more unreliable than those calculated from small ranges. The formula used for calculating the weight was

$$\text{Weight} = 1/(1 + \text{Log}(1 + \text{Range})).$$

To examine the influence of taxonomy, we included the genus and subfamily of the wasps in the analysis. In part of the analysis we exclude species with a clutch size of one. The reason for this is that sibling mating cannot occur in solitary species where larvae develop alone on a host (unless hosts attacked by the same parent are in close proximity: see Discussion).

Analysis was performed using the GLIM general linear modelling program (McCullagh and Nelder 1983). Normal error distributions were assumed and so the response variable, sex ratio, was arcsine square-root transformed. The effects of clutch size and the logarithm of clutch size were explored as explanatory variables. GLIM fits a model by using a maximum likelihood method which is equivalent to a least squares procedure when normal error distributions are assumed. The data are compared with values predicted by the model and the differences minimised by adjusting the parameters. When a weight is specified, the program treats differences corresponding to data points with large weights as more important than those with small weights. Taxonomic effects were examined by including the three subfamilies and eight genera as factors in the equivalent of an analysis of covariance.

Results

Thirty studies recording both the sex ratio and clutch size in 23 species were found (Table 1). There were 6 species with two or more estimates of clutch size and sex ratio. In 3 species, the multiple records were identical and only a single record has been included, as it was not clear if one author was reproducing the results of the other. The records for the other three species were different (though generally similar) and in the initial analysis have been included separately giving 27 records of clutch size and sex ratio. All species except one, *Prorops nasuta*, were gregarious though the recorded range of clutch size in some other species included one.

In the unweighted regression, clutch size (CS) was significantly negatively related to the transformed values of sex ratio (Arc(SR)) ($\text{Arc(SR)} = 0.50 - 0.022\text{CS}$; $F_{1,25} = 10.88$, $P < 0.01$; $r^2 = 0.295$). However, the logarithm of clutch size (Log(CS)) was a considerably better explanatory variable ($\text{Arc(SR)} = 0.63 - 0.23\,\text{Log(CS)}$; $F_{1,25} = 22.7$, $P < 0.01$; $r^2 = 0.466$): Figure 1 illustrates this relationship. Replacing the two pairs of values referring to the same species by their averages makes little difference to the model fit: the values of r^2 for clutch size and the logarithm of clutch size become 0.283 and 0.449 respectively.

The weighted regressions give broadly similar results: (Clutch size: $\text{Arc(SR)} = 0.52 - 0.026\text{CS}$; $F_{1,25} = 9.4$, $P < 0.01$; $r^2 = 0.265$. Log clutch size: $\text{Arc(SR)} = 0.63 - 0.23\,\text{Log(CS)}$; $F_{1,25} = 21.1$, $P < 0.01$; $r^2 = 0.448$). Omitting the single species with

Table 1. Records of bethylid clutch size and sex ratio

Wasp species	Sex ratio	Clutch size	Reference
Pristocera rufa	0.17	15.9	Baker (1976)
Laelius pedatus	0.33	3.0	Mertens (1980)
Laelius utilis	0.29	3.4	Mertens (1985)
Laelius voracis	0.33	4.0	Ayyappa and Cheema (1952)
Prorops nasuta	0.25	1.0	Hargreaves (1926); de Toledo (1942)
Cephalonmia tarsalis	0.50	2.0	Powell (1938)
Cephalonmia waterstoni	0.33	2.0	Finlayson (1950); Rilett (1949)
Scleroderma immigrans	0.17	10–40	Bridwell (1920)
Scleroderma turkmenicus	0.04	100–110	Mamaev (1973)
Prosierola bicarinata	0.17	4.0	Doutt (1973)
Goniozus sp.	0.12	11.7	Girling (1979)
Goniozus sp.	0.25	1–25	Avasthy and Chaudhary (1966)
Goniozus sp.	0.20	4.0	Taylor (1933)
Goniozus aethiops	0.14	7.0	Gordh and Evans (1976)
Goniozus cellularis	0.20	5.0	Nickels et al. (1950)
Goniozus claripennis	0.17	3.0	Voukassovitch (1924)
Goniozus emigratus	0.20	14.8	Willard (1927)
Goniozus emigratus	0.03	4–17	Busck (1917)
Goniozus emigratus	0.18	15.6	Green et al. (1982)
Goniozus gallicola	0.17	6.5	Gordh (1976); Green et al. (1982)
Goniozus indicus	0.09	9–40	Cherian and Isreal (1942)
Goniozus indicus	0.09	1–24	Gifford (1965)
Goniozus jacinctae	0.25	1.8	Danthanarayana (1980)
Goniozus nephantidis	0.25	1–15	Jayaratnam (1941)
Goniozus nephantidis	0.17	10–18	Antony and Kurian (1960)
Goniozus platynotae	0.25	3.4	Goertzen and Doutt (1975)
Goniozus procerae	0.20	10.0	Coquard (1984)

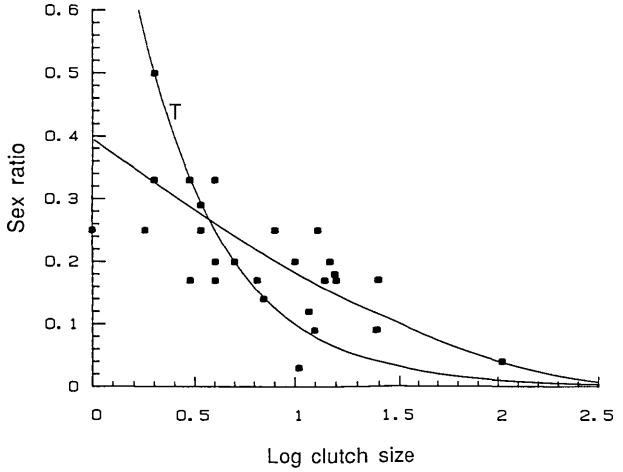


Fig. 1. The relationship between sex ratio and clutch size in Bethylidae. The line marked *T* is the theoretical relationship assuming sex ratio is the reciprocal of clutch size. A *fitted line* (see text) is also illustrated

a clutch size of one also makes only a small difference to the fit (e.g. Log clutch size: $F_{1,24}=27.3$, $P<0.01$; $r^2=0.522$).

The effects of parasitoid taxonomy were included in an analysis using the weighted regression and omitting the species with a clutch size of one. Adding subfamilies as factors explains 37.1% of the variance, which is significant ($F_{2,23}=7.1$, $P<0.01$). Adding the logarithm of clutch size after subfamilies explains a further 29.8% variance, which is also significant ($F_{1,22}=20.7$, $P<0.01$). Thus clutch size has a marked effect on sex ratio even after subfamily effects have been removed. If genus is added after subfamily as a nested factor, an additional 33.3% of the variance is explained ($F_{4,19}=5.6$, $P<0.01$). Now if the logarithm of clutch size is added after both subfamily and genus are included in the analysis, an extra 5.0% of the variance is explained ($F_{1,18}=3.82$, $P<0.1$). Thus including genera in the analysis markedly decreases the explanatory power of clutch size.

Discussion

The analysis presented here demonstrates a significant negative relationship between clutch size and sex ratio. This relationship can be compared with the prediction of LMC theory. Hamilton (1967) showed that if n females laid eggs on a patch and their progeny mated among themselves, the optimal sex ratio for each parent would be $(n-1)/2n$. More recently, Taylor and Bulmer (1980) pointed out that with a haplodiploid genetic system the optimal sex ratio is $(n-1)(2n-1)/n(4n-1)$, al-

though this results in quite similar predictions to Hamilton's expression. When $n=1$, a sex ratio of zero is predicted and this is interpreted to mean that a female lays only enough males to fertilize all her daughters. Where clutch sizes are small enough that a single male can inseminate all his sisters, the predicted sex ratio is then the reciprocal of clutch size. We first discuss the evidence that in bethylids, the majority of matings take place between siblings (the assumption of $n=1$) and then compare our observed sex ratios with the prediction of 1/(clutch size).

As discussed in the section on bethylid biology, pupation invariably occurs around the host remains. Males normally hatch before females (Bridwell 1920; Powell 1938; Avasthy and Chaudhary 1966; Baker 1976; Gordh 1976; Remadevi et al. 1981; Gordh et al. 1983) and mating occurs at the pupation site. Frequently, males chew open the cocoons of their sisters in order to mate with them (Bridwell 1920; Willard 1927; Taylor 1933; Kearns 1934a; Powell 1938; Gordh 1976; Mertens 1980; Remadevi et al. 1981; Gordh et al. 1983). A common consequence of exclusive sibling mating noted by Hamilton (1967) is the evolution of male aptery: wings are superfluous for a male if dispersal results in no further matings. Male aptery has been recorded in a number of bethylids (Hamilton 1967), although the picture is rather complex as examples of both female aptery, and winglessness in both sexes, have also been recorded (Evans 1964).

One factor that leads to non-sibling mating in many groups of parasitoids, superparasitism, is probably relatively unimportant in bethylids. Superparasitism is said to occur when a female lays her eggs on a host which has been previously parasitised. The offspring of both females develop together and may ultimately mate among themselves (Werren 1980). Female bethylids, however, normally guard their eggs from predators and other parasitoids, including conspecifics (Bridwell 1918; Chatterjee 1941; de Toledo 1942; Pruthi and Mani 1942; Avasthy and Chaudhary 1963; Malyshev 1968; Doutt 1973; von Kuhne and Becker 1974; Backer 1976; Gordh 1976; Remadevi et al. 1978; Girling 1979) thus reducing the likelihood of superparasitism. Even if a female becomes separated from her eggs, mixed clutches from more than one female are unlikely as the typical behavior of many females on discovering a previously parasitised host is to destroy any eggs present (Goertzen and Doutt 1975; Venkatramen and Chacko 1961; pers. obs.: *Goniozus nephantidis*). In conclusion, while it is obviously impossible to make an accurate generalisation about the biology of a whole family of wasps, exclusive sibling mating seems to be the predominant mating system in the Bethylidae.

A line describing sex ratio as the reciprocal of clutch size has been included in Fig. 1. The chief difference between the data and the predicted relationship is that the sex ratio is more male biassed than expected in the larger clutches. There are at least two possible reasons for this. First, a single male may not be able to inseminate all the females in a large clutch. At least in laboratory studies, females are recorded as running out of sperm and producing highly male-biassed or completely male broods (Kearns 1934b; Mamaev 1973; Gordh et al. 1983; Gordh and Hawkins 1981). Running out of sperm may not, however, preclude a female from producing further daughters as there are several records of an unfertilised female producing male eggs and then remaining with her offspring and eventually mating with them (van Emden 1931; de Toledo 1942; Rilett 1949). A second reason that large clutches may have more males is the risk that all the males in a clutch die before emergence leaving their sisters unfertilized. This risk would be of greater significance in a large clutch, as a greater number of daughters would then be unfertilized. Hartl (1971) was the first to discuss this problem and predicted that the mother should lay the number of males that resulted in the maximum number of fertilized females. Hartl suggested that sex ratios considerably larger than 1/(clutch size) should be found, though his predictions were based on the assumption that a female could only produce a sex ratio with binomial variance. Green et al. (1982) have since shown that sex ratios with less than binomial variances should normally be selected and that the variance in the sex ratio of the bethylids *Goniozus gordhi* and *G. emigratus* is less than binomial (see also Mertens 1980, 1985; Gordh et al. 1983). Gordh (1976) and Green et al. (1982) note that within a species the number of males per clutch increases as clutch size increases, a pattern that parallels our interspecific pattern.

We found a single species with a clutch size of one, and this was excluded from the analysis illustrated in Fig. 1. All else being equal, we would expect a sex ratio of 0.5 in this species, as there would be no possibility of sibling mating between members of the same brood. In fact a sex ratio of 0.25 is recorded. However, this species, *Prorops nasuta*, is a parasitoid of the beetle *Hypothenenus hampei*, the coffee berry borer. The female beetle burrows into a coffee berry and lays a clutch of eggs. The female bethylid also enters the berry and though she only lays a single egg per host, she

attacks several hosts (Hargreaves 1926; de Toledo 1942; le Pelley 1978). Her young (and the young of any other parasitoid in the bean) eventually mate among themselves resulting in LMC and selection for a sex ratio less than equality.

The relationship between sex ratio and clutch size remained after subfamily had been entered as a factor but was much weaker after the entry of genus as a factor. There are two possible interpretations of this. First, if sex ratio were an evolutionarily conserved character, the fact that taxonomic relatedness explained so much of the variation would indicate that it is wrong to treat each species as an independent data point. Our observed significant relationship between sex ratio and clutch size might then be a taxonomic artefact (Ridley 1983). However, we believe sex ratio to be an evolutionary labile character and this is supported by the observation that sex ratio and clutch size are correlated intraspecifically (Green et al. 1982). If this is correct then the values of sex ratio and clutch size for the individual species are statistically independent. We believe that the reason why taxonomy explains so much of the variance in sex ratio is that host range is evolutionary conserved and host range determines host size and hence parasitoid clutch size. In this view, taxonomy is only correlated with clutch size which is the primary determinant of sex ratio.

In conclusion, this study shows observed sex ratios to be in broad agreement with the predictions of LMC theory. This is particularly encouraging considering that the data used here come from a large number of studies, that sex ratio was typically not recorded very accurately and that intraspecific variation in clutch size and sex ratio was ignored. The original evidence for LMC came from a comparison of sex ratios in highly inbred organisms (Hamilton 1967). Since then most quantitative tests of LMC have been experimental (Werren 1980; Herre 1985; Frank 1985) with the exception of Waage's (1982) comparative study of Scelionidae. The large body of literature that has accumulated on parasitoids due to their economic importance represents a valuable source of data for comparative sex ratio studies.

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