# Clutch size in an obligately siblicidal parasitoid wasp

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**Abstract.** Insect parasitoids can be grouped by their pattern of brood production: 'gregarious' parasitoids can produce multiple offspring per host, but 'solitary' parasitoids can produce only a single offspring per host because their larval stages engage in lethal contest competition to monopolize the host resource. Theoretical and empirical studies have largely overlooked the possibility that solitary parasitoids may lay clutches of more than a single egg, leading to obligate siblicide. When this possibility has been recognized at all, it has been treated under the rubric of self-superparasitism (in which a parasitoid deposits eggs on the same host during two or more distinct host encounters). It is argued here, however, that multiple-egg clutches and self-superparasitism are fundamentally different behaviour patterns, entailing different potential costs and benefits. These differences are linked to variation in age structure within broods (self-superparasitism can produce mixed-age offspring within broods) and host quality assessment problems that are specific to self-superparasitism (during a second encounter, the parasitoid may not be able to assess its prior oviposition behaviour on the host). Clutch size was investigated in a solitary parasitoid wasp, *Compieriella bifasciata* (Howard) (Hymenoptera: Encyrtidae), that commonly produces multiple-egg clutches. Clutch size varied in response to aspects of the environment that were likely to shape the costs and benefits of multiple-egg clutches: larger clutches were produced when the parasitoid (1) had no contact with high-quality hosts and (2) experienced a high density of conspecific female parasitoids. Two types of costs are associated with depositing multiple-egg clutches: the opportunity cost of the extra egg(s) and the direct cost of the time required to deposit them. Multiple-egg clutches may represent a conditional strategy that increases reproductive success. A review of the literature shows that multiple-egg clutches are a widespread feature of the reproductive behaviour of solitary parasitoids.

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Obligate siblicide occurs in a wide variety of invertebrate and vertebrate taxa (Polis 1981; Anderson 1990; Anderson & Ricklefs 1992; Elgar & Crespi 1992). When obligate siblicide is a prelude to sibling cannibalism, it may be, at least in part, a means by which parents provide food to developing offspring (Polis 1981). In this case, production of excess offspring may be viewed as an extension of parental investment in the surviving offspring. When obligate siblicide is not coupled with sibling cannibalism, as has been observed in birds and some embryonic salamanders and sharks (Polis 1981), the production of multiple offspring within a brood appears to function primarily to reduce the probability that the

brood yields zero offspring (the 'insurance egg' hypothesis; Dorward 1962; Anderson 1990).

Insect parasitoids are free-living as adults but develop through their immature stages by feeding upon, and generally killing, a single host. Insect parasitoids can be grouped by their pattern of brood production: 'gregarious' parasitoids can produce multiple offspring per host, but 'solitary' parasitoids can produce only a single offspring per host (Godfray 1987; Rosenheim 1993). When more than one offspring of a solitary parasitoid species initiates development within a single host, physical combat or one of various types of physiological suppression occurs such that at most one parasitoid survives (Salt 1961; Strand 1986; Mackauer 1990; Godfray 1994).

Largely distinct theories have been developed for the oviposition behaviour of gregarious and solitary parasitoids. Models have focused on the

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question of clutch size for gregarious parasitoids (Godfray 1994; Mangel et al. 1994) and for host acceptance by solitary parasitoids (Godfray 1994). Most recently, much theoretical attention has been devoted to understanding the conditions under which a parasitoid that encounters an already parasitized host should deposit additional eggs ('superparasitism'; Charnov & Skinner 1984; Iwasa et al. 1984; Hubbard et al. 1987; Ives 1989; Strand & Godfray 1989; Mangel 1992; Roitberg et al. 1992; Visser et al. 1992a; Weisser & Houston 1993). Many parasitoid species are less likely to superparasitize a host previously parasitized by themselves ('self superparasitism') than those previously parasitized by a conspecific parasitoid ('conspecific superparasitism'; Gates 1993; Visser 1993; Godfray 1994). Of the various forms of superparasitism examined, the one requiring the most stringent conditions to be favoured by natural selection is self superparasitism by solitary parasitoids (Hubbard et al. 1987; Visser et al. 1992a). This result is intuitive because self superparasitism by solitary parasitoids leads ineluctably to siblicide.

What conditions are predicted to favour self superparasitism by solitary parasitoids? First, self superparasitism is more likely to be favoured when the costs are low. Costs may include both (1) the direct cost of the time required to superparasitize and (2) the opportunity cost of the additional egg(s) deposited in the host. Both costs will be lessened when opportunities to reproduce on other hosts in the environment are limited, e.g. when high-quality hosts are rare (Roitberg et al. 1992; Visser et al. 1992a, b; Visser 1993; Weisser & Houston 1993) or when the risk of mortality for the adult parasitoid is great (Roitberg et al. 1992; Weisser & Houston 1993). Second, self superparasitism is more likely to be favoured when the potential benefits are high. Benefits are most likely to be high when there is a high risk that a single egg deposited in the host will fail to develop; i.e. when (1) the host has effective defensive responses that can kill parasitoid eggs or larvae, or (2) competing conspecific parasitoids are present and may also oviposit in a host, leading to contest competition between offspring from several mothers to monopolize the host resources (Visser et al. 1990, 1992a, b; van Alphen et al. 1992; Visser 1993).

What has largely been overlooked in the development of theory for parasitoid oviposition is the possibility that solitary parasitoids may lay clutches of more than a single egg (Godfray 1994). When this possibility has been recognized at all, it has been treated under the rubric of self superparasitism. Thus, the aim of this study was to investigate the production of multiple-egg clutches by solitary, obligately siblicidal parasitoids. Specifically, we attempted first to propose and justify the usage of an appropriate terminology that distinguishes between self superparasitism and multiple-egg clutches, second, to test the hypothesis that clutch size 'decisions' made by solitary parasitoids respond to local environmental conditions (the density of hosts and the presence of potentially competing conspecific female parasitoids), third, to synthesize the existing hypotheses under which multiple-egg clutches can function as part of a flexible reproductive strategy, and fourth, to demonstrate through a literature review that multiple-egg clutches are a strategy widely used by solitary insect parasitoids.

We begin by describing the traits that distinguish self superparasitism from multiple-egg clutches in solitary parasitoids. The most widely adopted definition of superparasitism, which we accept, is the parasitism of a host that was parasitized during a prior encounter by the same or a conspecific female (van Dijken & Waage 1987; Godfray 1994). We furthermore propose that solitary parasitoids be considered to deposit a 'multiple-egg clutch' when a single female deposits more than one egg during a single host encounter. The current practice of extending the term 'self superparasitism' to encompass multiple-egg clutches deposited during a single host encounter is, we suggest, counterproductive, because multiple-egg clutches and self superparasitism by solitary parasitoids (as we are currently proposing its usage) are fundamentally different for at least two reasons. First, a female encountering a host that she previously parasitized may not be able to make key assessments of the host's condition that are a direct result of her prior interaction with the host. Specifically, a female may not be able to (1) recognize that a host is indeed parasitized ('host discrimination' ability; Rosenheim & Mangel 1994), (2) determine whether the previous egg was laid by herself or some other conspecific parasitoid (Godfray 1994), (3) determine the number of eggs laid previously, (4) determine the sex of eggs previously laid and (5) recognize any other aspects of the previous encounter that

might influence host quality, such as possible direct feeding on the host ('host feeding'; see Jervis & Kidd 1986). A solitary parasitoid that deposits a multiple-egg clutch presumably has a relatively complete 'knowledge' of events occurring during the complete encounter. Second, the distinct host encounters that comprise self superparasitism are separated by some time interval; for multiple-egg clutches, the 'inter-oviposition interval' will usually be approximately zero. (Some rare exceptions occur for parasitoids that remain with a host for a period of hours, depositing eggs at intervals; e.g. Hagstrum & Smittle 1978; in this case a single clutch may contain some minimal amount of age structure.) The inter-oviposition interval is important, because age differences between parasitoid larvae are frequently the key determinant of which offspring will prevail in lethal contest competitions (Strand 1986; Mackauer 1990; Visser et al. 1992c; Godfray 1994; see also van Alphen et al. 1992). Each of these differences means that multiple-egg clutches and self superparasitism are functionally different behaviour patterns for solitary parasitoids, associated with different potential costs and benefits. As we discuss below, imprecise terminology has been a barrier to progress in our understanding, and indeed has rendered much of the older literature nearly impossible to interpret.

Here we investigated clutch size in a solitary parasitoid wasp, *Comperiella bifasciata* (Howard) (Hymenoptera: Encyrtidae), that commonly produces multiple-egg clutches, as defined above (Blumberg & Luck 1990). We showed that clutch sizes varied in response to aspects of the environment that are likely to shape the costs and benefits of obligate siblicide: the parasitoid's history of contact with high quality hosts and the density of conspecific female parasitoids. We discuss several hypotheses under which multiple-egg clutches can function as a conditional reproductive strategy, and demonstrate with a literature review that multiple-egg clutches are a widespread feature of the reproductive behaviour of solitary parasitoids.

## Biology of *Comperiella bifasciata*

*Comperiella bifasciata* is a solitary parasitoid that lays its eggs within the bodies of armoured scale insects (family Diaspididae). Both the parasitoid and the host scale insect studied here are native to east Asia; the parasitoid was purposely

introduced to California as a biological control agent (Rosen & DeBach 1978). The oviposition behaviour of *C. bifasciata* consists of drumming the host with the antennae, drilling into the host, probing with the ovipositor and depositing an egg (Blumberg & Luck 1990); with rare exceptions (4 of 178 ovipositions=2.2%), not more than one egg is laid per probe (D. Hongkham & J. A. Rosenheim, unpublished data). Parasitoid larvae lack the large, piercing mandibles that typify fighting species, and do not appear to engage in physical combat (Compere & Smith 1927). Rather, siblicide apparently occurs through some form of physiological suppression; in some cases multiple larvae may reach the second or third of the five developmental instars before being eliminated (Compere & Smith 1927; Flanders 1944).

The California red scale, *Aonidiella aurantii* (Maskell), continues to feed and develop after being parasitized, and some individuals are able to kill eggs and young larvae of *C. bifasciata* through the formation of a surrounding hemocytic capsule (Compere & Smith 1927; Brewer 1971; Blumberg & Luck 1990). Encapsulation rates can be high, and for some populations there is evidence that encapsulation rates are reduced when multiple parasitoid eggs are present per host (Brewer 1971; Blumberg & Luck 1990). Finally, Blumberg & Luck (1990) observed that some females lay multiple-egg clutches (18% for a California population and 6% for an Israeli population); this observation led to the current study.

## METHODS

### Insect Cultures

The parasitoid culture was initiated in April 1992 from a field collection of parasitized California red scale, *Aonidiella aurantii* (Maskell), growing on orange trees, *Citrus sinensis*, at the University of California Lindcove Field Station, Tulare County, California. Parasitoids were reared at $26+2°C$ on *A. aurantii* growing on butternut squash, *Cucurbita moschata*. The *A. aurantii* population was derived from a laboratory culture that had been maintained at the University of California, Riverside for many years and then at the University of California Kearney Agricultural Center for additional years. Scale insects were reared on butternut squash in a growth chamber at 24–27°C. Host scale insects

used in the assays were newly moulted third instar female *A. aurantii* that had mated but had not yet matured sufficiently to have the scale insect cover become affixed to the scale insect body. We used a series of seven squash fruits sequentially through the experiment and recorded squash number to test for effects of variable squash quality.

## Behaviour Assay

Our objective was to assess the influences of two factors on clutch sizes deposited by *C. bifasciata*. The first factor was the density of conspecific female parasitoids, which we manipulated by holding females before the assay either singly or in groups of three. This experimental manipulation has been successfully used by other workers to investigate the role of intraspecific competition (Visser et al. 1990, 1992b; van Alphen et al. 1992; Visser 1993, in press; Michaud & Mackauer 1995). This treatment produces the female–female contacts that can occur in nature when parasitoid densities are high; it is not uncommon to observe several *C. bifasciata* females foraging for hosts on a single citrus fruit in the field (Rosenheim, personal observation). The second factor was host availability, which we manipulated by first depriving females of host contacts and then giving females access to a rapid sequence of three high quality hosts. The first host in the sequence of three mimics what a wasp might experience if she encountered a host in an environment with low host density, while the second and third hosts mimic what a wasp might experience in environments with progressively higher host densities. This manipulation has also been previously used, with large shifts in clutch size observed over the first two or three hosts encountered as female parasitoids change their perception of host availability (van Lenteren & DeBach 1981; Ikawa & Suzuki 1982; Rosenheim & Rosen 1991; Visser, in press). In the field, host densities for *C. bifasciata* can vary over several orders of magnitude, and host distributions can be very patchy (Rosenheim, personal observation); thus, parasitoids can experience widely variable rates of host encounter in nature. Finally, because parasitoid oviposition behaviour is also predicted to be sensitive to the number of mature oocytes present in the ovaries ('egg load'; reviewed by Minkenberg et al. 1992), we quantified egg load so that we could statistically control for its effect.

We isolated parasitoid pupae from hosts, held them singly in gelatin capsules (diameter=7 mm; length=21 mm) with small droplets of honey at 24–28°C, and checked them daily for emergence. Newly emerged females were placed into one of two treatments: (1) in the 'solitary' treatment we placed a single female with a single male in a gelatin capsule with honey for one day; (2) in the 'grouped' treatment, we placed three females with a single male in a gelatin capsule with honey for one day.

Behaviour assays were conducted during 1993 from 1000 to 2000 hours on the day after the treatments were established; thus, parasitoids were 24–48 h old (at 27°C females live about 10 d on average; Flanders 1944). On most of the days when we conducted assays, we alternated tests of females from the two treatment groups. Each assay consisted of three stages. In each stage, we isolated a single female parasitoid with a single host insect under the top of a gelatin capsule resting on the squash surface and allowed it to oviposit during a single host encounter. We placed the squash under a stereomicroscope, illuminated it with fibre optic lighting, and observed parasitoid behaviour to record the total time spent on the host. We terminated each stage of the assay, comprising one host encounter, when the wasp first walked off the host after the initial encounter. The completion of an oviposition bout was obvious: instead of remaining on the scale insect cover to re-initiate host inspection with the antennae ('antennal drumming'), the parasitoid simply walked off the host and began searching behaviour on the squash surface (and often thence onto the gelatin capsule). There was always adequate room between the host insect and the gelatin capsule (diameter=7 mm) for the minute wasp (length$\simeq$1 mm) to walk off the host; thus the capsule did not 'force' the parasitoids to remain on the host. To begin the second stage, we moved parasitoids to a second host insect in one of two ways: (1) if the parasitoid walked onto the gelatin capsule after abandoning the first host, we simply positioned the capsule over a new host; (2) if the parasitoid remained on the squash surface after abandoning the first host, we slid the gelatin capsule slowly over the squash surface, occasionally nudging the parasitoid, to position the capsule over a fresh host. We allowed parasitoids to oviposit on the second and third hosts in exactly the same way to complete the replicate. If at any

stage the parasitoid did not locate a host within 10 minutes, we halted the replicate and presented no additional hosts.

Immediately following the behaviour assay, we used an ocular micrometer at $12 \times$ magnification to measure the longest diameter of each scale insect cover ($d_1$), if one was apparent, and the bisecting perpendicular diameter ($d_2$); we calculated scale cover area as $d_1 d_2 \pi / 4$ (the area of an ellipse). To determine the number of eggs laid per host, we immediately dissected the host in a drop of water under a stereomicroscope. A small tear was made in the scale insect's exoskeleton, and the fat body and internal organs were gently teased out to locate all the parasitoid eggs. The female parasitoid was frozen and held for dissection later the same day. During dissection, we slidemounted one hind tibia and measured it with an ocular micrometer at $100 \times$ magnification. We dissected the female in a drop of water under a stereomicroscope. We removed the ovaries, teased the oocytes from the ovarioles, and counted the number of mature, full-sized oocytes. We summed the number of eggs found in dissections plus the number deposited during the assay to obtain estimates of egg load.

We tested 119 females (57 in the solitary treatment, 62 in the grouped treatment). Due to the rejection of some hosts by parasitoids and the loss of some parasitoids during transfer, the total number of replicates declined to 116 on the second host and 110 on the third host. We used step-wise polychotomous logistic regression to test the influence of all treatment variables and covariates on clutch size decisions (Dixon 1990). We coded clutch size as an ordered variable with four discrete states for clutches of size 1, 2, 3 or $\geq 4$. We coded treatments as 0 for females held singly and 1 for females held in groups of three. The first set of analyses treated oviposition on each host separately. The final analysis considered oviposition on all three hosts together, with an additional variable introduced to test the importance of successive host encounters (the number of hosts previously encountered was coded as an ordered variable with three states: 0, 1 or 2).

## RESULTS

Parasitoids had large egg loads at the beginning of the behaviour assay (mean $\pm$ SD $= 37.4 \pm 5.7$;

range: 24–55); thus parasitoids were free to deposit more eggs than they actually did during the assay. Parasitoids deposited both single and multiple-egg clutches on all three of the sequentially encountered hosts (Fig. 1). Females held in groups of three prior to the assay deposited significantly larger clutches on the first host (logistic regression coefficient $= 1.73 \pm 0.39$, $\chi^2 = 22.0$, $P < 0.001$) and the second host (coefficient $= 1.08 \pm 0.45$, $\chi^2 = 6.1$, $P = 0.014$) than did females held singly (Fig. 1). The trend remained on the third host, but the difference was not significant ($\chi^2 = 2.1$, $P = 0.16$; Fig. 1). None of the other variables, including parasitoid size, egg load, host size or squash number, had a significant influence on clutch size for any of the hosts ($P > 0.05$). Mean clutch size was largest on the first host, and became progressively smaller on the second and third hosts as females experienced contacts with the high quality hosts (coefficient $= -0.98 \pm 0.16$, $\chi^2 = 37.9$, $P < 0.001$).

Parasitoids depositing multiple-egg clutches repeated the entire sequence of behaviour patterns (one to several bouts of antennal drumming, drilling and probing) between each oviposition. Thus, the time required to lay multiple-egg clutches was well described by a simple linear relationship with clutch size (Fig. 2); females required approximately 1.6 minutes to deposit each additional egg in multiple-egg clutches. In multiple regression analyses, none of the other variables tested (solitary versus grouped treatment, parasitoid size, parasitoid egg load or host size) had a significant effect on total handling time for any of the three hosts ($P > 0.10$). Thus, each egg in multiple-egg clutches was associated with a similar cost in terms of time required for oviposition.

## DISCUSSION

*Comperiella bifasciata* females may lay either single or multiple-egg clutches in their scale insect hosts; because larvae engage in lethal physiological suppression of conspecific larvae, multiple-egg clutches lead to obligate siblicide (Compere & Smith 1927; Flanders 1944). Two costs are associated with depositing multiple-egg clutches: the opportunity costs of the extra egg(s) and the direct cost of the time required to deposit them. Other costs (borne most immediately by the offspring)

(a) First host

(b) Second host

(c) Third host
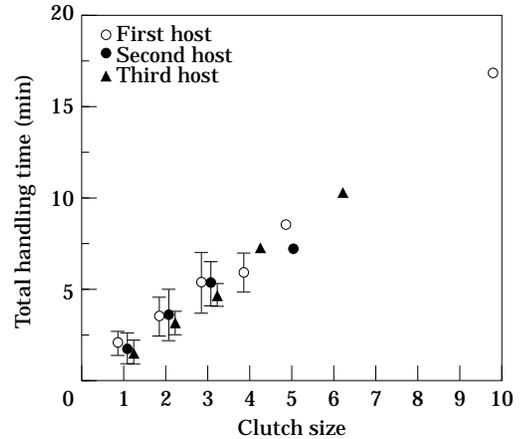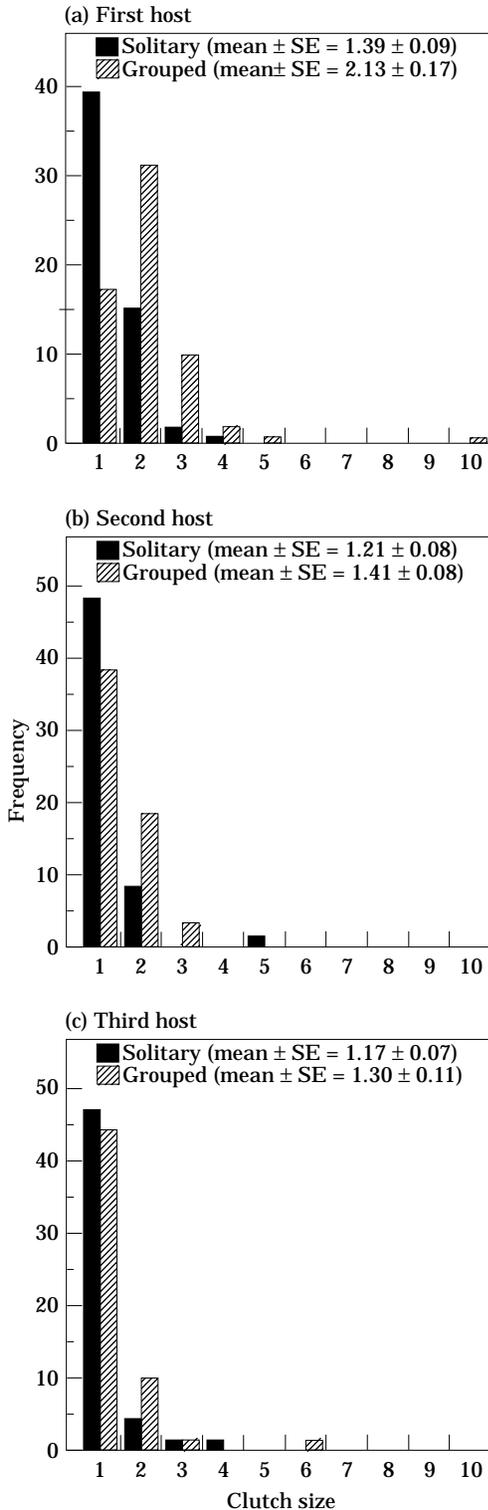


**Figure 2.** Total time required by the parasitoid *Comperiella bifasciata* to deposit clutches of eggs on the scale insect host, *Aonidiella aurantii*. Shown are means ± SD. Data are plotted with a slight offset on the x-axis to avoid superimposing the symbols. For the first host: total handling time=0.36+1.58 (clutch size), $F_{1,117}$=415, $r^2$=0.78, $P$<0.001; for the second host: total handling time=0.10+1.64 (clutch size), $F_{1,115}$=120, $r^2$=0.51, $P$<0.001; for the third host: total handling time=−0.08+1.62 (clutch size), $F_{1,112}$=324, $r^2$=0.74, $P$<0.001.

are possible, including the costs of competition between siblings prior to the elimination of supernumerary larvae and the possibility that both sibs will be killed during combat (Salt 1961; Lawrence 1988; Harvey et al. 1993). The total composite cost is apparently balanced against some benefits (as yet uncharacterized) to produce the patterns of variable clutch size observed here: larger clutches are produced when parasitoids have not contacted hosts and when the density of competing conspecific females is high.

How might we interpret the progressively smaller clutch sizes observed as parasitoids contacted the second and third hosts? Female parasitoids were 24–48 h old at the time of the assay,

**Figure 1** Clutch sizes deposited by the obligately siblicidal parasitoid *Comperiella bifasciata* on three sequentially encountered host scale insects, *Aonidiella aurantii*. Parasitoid females were held either singly ('solitary') or in groups of three ('grouped') for one day prior to the behaviour assay.

and had been held from eclosion without access to hosts. Thus, parasitoids may initially have estimated that their environment had few hosts. With rapid successive contacts with high quality hosts, parasitoids apparently adjusted their estimates of host availability upward. Greater host density represents an enhanced opportunity for future reproduction, an increased risk of lifetime reproductive success becoming limited by the finite egg supply (Rosenheim, in press), and thus greater costs of allocating extra eggs and time to multiple-egg clutches. Clutch sizes therefore apparently decreased to minimize the costs of depositing additional eggs on an already exploited host. Similar patterns of decreasing clutch sizes with successive host encounters have been observed in gregarious parasitoids (van Lenteren & DeBach 1981; Ikawa & Suzuki 1982; Rosenheim & Rosen 1991; Visser, in press).

The interpretation of the parasitoid density treatment is slightly more complex. Previous workers have used manipulations of parasitoid density to study oviposition by solitary parasitoids, and have suggested two explanations for why parasitoids held singly or in groups might differ in behaviour. The first explanation treats a possible benefit of depositing more than one egg per host. Grouped females may estimate that there is a greater likelihood of superparasitism and the associated contest competition between larvae from different mothers. If each additional egg increases the likelihood that one of the siblings will be the eventual winner in competitions with offspring from other females, then depositing more than one egg per host may be beneficial (Visser et al. 1990; van Alphen et al. 1992; Visser 1993). The second explanation treats a possible cost of having more than one egg per host. Grouped females may estimate that host availability will be depressed as hosts are exploited by conspecific females. Thus, future opportunities for reproduction may be reduced, and the opportunity cost of depositing additional eggs may be reduced (Visser et al. 1992b). We suggest that these two explanations are not mutually exclusive; *C. bifasciata* held in groups may deposit multiple-egg clutches because of enhanced benefits and/or reduced costs. Under any combination of these effects, multiple-egg clutches can be viewed as a response to intensified intraspecific competition.

## Benefits of Multiple-egg Clutches

Several general hypotheses have been proposed under which multiple-egg clutches represent a conditional strategy that increases the reproductive success of solitary parasitoids. Here we review these hypotheses and examine their potential applicability to *C. bifasciata*.

Parasitoid eggs are generally negligible in size compared to the size of the host insect; thus, multiple-egg clutches are unlikely to function as a means of providing additional food to a siblicidal offspring. Multiple-egg clutches may also be accidentally produced (Godfray 1987). This is likely for some parasitoids that lay multiple eggs in a single, very rapid probe because of proximate constraints on the precise regulation of oocyte release (e.g. Schlinger & Hall 1961). Because *C. bifasciata* lays eggs one at a time in separate probes, multiple-egg clutches are unlikely to be an accident produced by the mechanics of oviposition; some of the exceptionally large clutches may, however, be a product of accidental oviposition in those rare cases where several eggs are laid in a single probe (Fig. 1; D. Hongkham & J. A. Rosenheim, unpublished data). Our results also suggest that multiple-egg clutches in *C. bifasciata* are not a result of other types of accidental oviposition; accidental oviposition would not be expected to produce the associations between clutch size and conspecific female density or history of host encounters that we have documented (Fig. 1).

The 'insurance egg hypothesis' (Dorward 1962) proposes that the benefit of multiple-egg clutches is an increased likelihood of successfully producing a single offspring. Four variants of this hypothesis have been proposed for parasitoids. First, multiple-egg clutches may provide insurance against a single egg dying due to inviability or host defensive reactions (Parker & Courtney 1984; Godfray 1987). Under this hypothesis, the addition of supplementary eggs does not change the per-egg probability of death. Second, multiple-egg clutches may be a means of overwhelming the host's defences, thereby decreasing the per egg probability of death and producing a large increase in the likelihood of successfully producing an offspring (van Alphen & Visser 1990). Evidence from both Californian and Australian populations of *C. bifasciata* suggests that the host's ability to encapsulate eggs decreases as

clutch size increases (Brewer 1971; Blumberg & Luck 1990). In both cases, however, the effect was small; furthermore, an Israeli population did not show decreased encapsulation rates in multiple-egg clutches (Blumberg & Luck 1990). Third, when more than one female oviposits in the same host (superparasitism), multiple-egg clutches may increase the probability that one member of the sibship will be the winner in contest competitions (Parker & Mock 1987; van Alphen & Visser 1990; Mackauer et al. 1992). Fourth, depositing multiple-egg clutches may decrease the likelihood that the host will subsequently be superparasitized when females can 'count' the number of parasitoid eggs already present (Bakker et al. 1990; van Alphen & Visser 1990). Finally, we propose a fifth hypothesis. Many insect parasitoids are themselves attacked by parasitoids, termed 'hyperparasitoids' (Sullivan 1987). If hyperparasitoid attack occurs before the elimination of supernumerary parasitoid larvae, and if an unparasitized sib is able to eliminate its parasitized sibling(s), then multiple-egg clutches could increase survival of one member of the brood. Hyperparasitoids might be unable or unwilling to attack all members of a group of siblings. *Comperiella bifasciata* is subject to hyperparasitism by wasps in the genus *Marietta* (Hayat 1986); however, further study is needed to assess the possibility that hyperparasitism favours multiple-egg clutches.

To summarize, there are a number of hypothetical means by which multiple-egg clutches can increase the likelihood that a female will successfully produce an offspring from a host. All five variants of the insurance egg hypothesis are potentially applicable to *C. bifasciata*, and a primary goal of ongoing work is to evaluate these possible benefits of supernumerary eggs.

## Multiple-egg Clutches in Obligately Siblicidal Parasitoids

Although conventional wisdom holds that solitary parasitoids lay one egg per host encounter, multiple-egg clutches deposited during a single host encounter are known from a taxonomically diverse array of solitary parasitoids (Table I). The actual proportion of solitary parasitoid species that produces multiple-egg clutches is likely to be greater than that indicated by Table I. Until recently, even conspecific superparasitism was

viewed as a non-adaptive behaviour, leading to a 'waste' of eggs (reviewed by van Alphen & Visser 1990). The potential function of multiple-egg clutches produced by solitary parasitoids was even less likely to be recognized. We suspect that behaviour that was not understood, or thought to be a non-functional artefact of a laboratory environment (where most studies have been conducted), was unlikely to be reported.

Even more important, published descriptions of parasitoid oviposition often make it impossible to determine whether multiple eggs are deposited as a result of self-superparasitism or multiple-egg clutches. Many experimenters confine individual parasitoids with hosts for an extended period of time and subsequently dissect hosts to count the number of eggs deposited, which cannot distinguish self-superparasitism from multiple-egg clutches (e.g. Cloutier 1984). Even when researchers observed parasitoid behaviour directly, they often failed to report whether eggs were laid in one or several host encounters, and applied the term superparasitism to both behaviour patterns. As we have argued, we hope that future workers will restrict the term self-superparasitism to cases where eggs are deposited in more than one host encounter.

Some studies have examined determinants of multiple-egg clutches in solitary parasitoids. Employing 'solitary' versus 'grouped' treatments similar to those employed in this study, Michaud & Mackauer (1995) demonstrated that *Monoctonus paulensis* lays larger clutches (1) when the density of conspecific parasitoids is higher, (2) when deprived of hosts and (3) when mated (as opposed to virgin). Visser (in press) showed that *Asobara tabida* deposits single-egg clutches when foraging in a patch containing only unparasitized hosts, but lays some two-egg clutches in unparasitized hosts when 75% of the hosts in a patch are parasitized. Finally, a decrease in clutch size with successive host contacts has been found in the two other species in which this has been examined, albeit informally: *Venturia canescens* (Beling 1932) and *A. tabida* (van Alphen & Nell 1982). Additional work on the benefits of multiple-egg clutches is needed to further develop the hypothesis that multiple-egg clutches are a conditional strategy which increases reproductive success.

Our results suggest that current models of reproductive behaviour for solitary parasitoids are incomplete because none of them recognizes the

**Table I.** Solitary parasitoids (i.e. species with siblicidal offspring) observed to deposit multiple-egg clutches during a single host encounter

| Species (Family) | Clutch sizes observed | Oviposition in single versus multiple probes | Reference |
|---|---|---|---|
| Dendrocerus carpenteri (Ceraphronidae) | 1–2 or more | ? | Bennett & Sullivan (1978) |
| Comperiella bifasciata (Encyrtidae) | 1–10 | Multiple (very rarely single) | Blumberg & Luck (1990): current study |
| Asaphes lucens (Pteromalidae) | 1–3 | Multiple | Keller & Sullivan (1976); D. J. Sullivan, unpublished data |
| Asaphes californicus (Pteromalidae) | 1–3 | ? | Sullivan (1972); D. J. Sullivan, unpublished data |
| Cotesia plutellae (Braconidae) | 1–3 | Single | C. F. Chilcutt, unpublished data |
| Opius dimidiatus (Braconidae) | 1–2 | Single | J. M. Nelson, unpublished data |
| Asobara tabida (Braconidae) | 1–2 | Single | van Alphen & Nell (1982); Mollema (1988); Visser, in press |
| Monoctonus paulensis (Braconidae) | 1–6 | Multiple | Michaud & Mackauer (1995), unpublished data |
| Trioxys utilis (Braconidae) | 1–10 | Single | Schlinger & Hall (1961) |
| Xenarcha lustrator (Braconidae) | 1–3 | Multiple | Shaw (1983) |
| Rhysipolis decorator (Braconidae) | 1–2 | Multiple* | Shaw (1983, unpublished data) |
| Rhysipolis hariolator (Braconidae) | 1–2 | Multiple* | Shaw (1983, unpublished data) |
| Rhysipolis ?meditator (Braconidae) | 1–2 | Multiple* | Shaw (1983, unpublished data) |
| Aleiodes assimilis (Braconidae) | 1–2 | Multiple | M. R. Shaw, unpublished data |
| Aleiodes circumscriptus (Branconidae) | 1–2 | Multiple | M. R. Shaw, unpublished data |
| Aleiodes albitibia (Braconidae) | 1–2 | Multiple | M. R. Shaw, unpublished data |
| Aleiodes rugulosus (Braconidae) | 1–2 | Multiple | M. R. Shaw, unpublished data |
| Bathyplectes anurus (Braconidae) | ≥1 | Single | Dowell (1978) |
| Bathyplectes curculionis (Braconidae) | ≥1 | Single | Dowell (1978) |
| Bathyplectes stenostigma (Braconidae) | ≥1 | Single | Dowell (1978) |
| Venturia canescens (Ichneumonidae) | 1–3 | Single | Beling (1932) |
| Nomada opacella† (Anthophoridae) | 2 | Multiple | Linsley & MacSwain (1955) |
| Nomada edwardsii† (Anthophoridae) | 1–2 | Multiple | Linsley & MacSwain (1955) |

*The existence of multiple probes is inferred from the observation that eggs were glued strongly to the host at very different locations that could not be reached from a single probing site (M. R. Shaw, unpublished data).

†Nomada are cleptoparasites rather than parasitoids in the strict sense. Although Linsley & MacSwain (1955) interpreted their nest dissections as indicating multiple-egg clutches, direct observations of oviposition were not performed; thus further work is needed to demonstrate this rigorously (J. G. Rozen, Jr, personal communication). van Lenteren (1976) cites Wilbert (1964) as having demonstrated that the solitary parasitoid Aphelinus semiflavus deposits multiple-egg clutches; however, neither van Alphen & Nell (1982) nor we have found evidence in this paper for multiple-egg clutches.

production of multiple-egg clutches as a behavioural option. A general model will need to include both host acceptance for parasitism or superparasitism and clutch size. In particular, conditions that have been predicted by existing models to favour self-superparasitism may instead favour multiple-egg clutches (Godfray 1994), thereby reducing the conditions under which self-superparasitism is favoured.

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