



Clutch size decisions of a gregarious parasitoid under laboratory and field conditions

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Under field conditions, insect parasitoids probably experience lower rates of host encounter and life expectancy than under optimal conditions in laboratory studies. We examined the clutch size response of *Mastrus ridibundus*, a gregarious idiobiont parasitoid of codling moth, *Cydia pomonella*, cocoons, to variation in both host encounter rate and life expectancy as possible explanatory variables in a comparison of brood size in the field and laboratory. Under laboratory conditions, mean clutch size (number of eggs laid) declined from 5.8 to 3.4 as host encounter rate increased from one to eight cocoons per day. In contrast, when we reduced life expectancy by withholding honey as a food source, females did not adjust clutch size. Mean brood size (number of progeny surviving to pupation) of females foraging in walnut orchards (3.9) was significantly greater than that under laboratory conditions with excess hosts (3.1). Brood size also increased with host size in the field, but not under laboratory conditions. Brood size fitness curves were derived using both host-finding ability in the field and lifetime fecundity under laboratory conditions as indices of female fitness. Host-finding ability increased exponentially with body size, generating an estimated Lack brood size of 4.3, but lifetime fecundity increased linearly with body size, giving a Lack brood size estimate of 5.5. Under field conditions, female *M. ridibundus* produced brood sizes that closely approximated the Lack brood size estimated from host-finding ability, but that were significantly smaller than that estimated from lifetime fecundity. These observations suggest that, in contrast to lifetime fecundity measures from the laboratory, host-finding ability in the field provides a more accurate estimate of lifetime reproductive success for parasitoids with a low expectation of future reproduction.

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One of the major decisions that a gregarious parasitoid must make is how many, if any, eggs to allocate to an encountered host (Waage 1986; Godfray 1994). Studying birds, Lack (1947) was the first to suggest that females should lay the number of eggs that maximizes the number of progeny surviving from a clutch. This hypothesis has been generalized for parasitoids by stating that a female should lay the number of eggs that maximizes her gain in fitness from a single host (Charnov & Skinner 1985; Godfray 1987; Godfray et al. 1991). In practice, the Lack clutch size of a gregarious parasitoid is estimated from the peak of a fitness curve relating some measure of performance or fitness gain to clutch size.

Fitness curves combine the dependence of fitness on female size with the influence of competition for

resources on the size of progeny produced within a clutch (e.g. Godfray 1994). Among insect parasitoids, there is often a positive relation between fitness and body size (Hardy et al. 1992; Godfray 1994; Vet et al. 1994; Visser 1994; West et al. 1996; Eilers et al. 1998; Rivero & West 2002). For gregarious parasitoids, body size depends upon resource availability during development (Harvey et al. 1998), because siblings within the same brood develop on a single host and compete for a fixed quantity of resources. Thus, to optimize progeny fitness, a parasitoid female is expected to vary the allocation of eggs to a host depending on the host's size or quality. Evidence from numerous parasitoid species suggests that females do indeed allocate more eggs to larger hosts (e.g. Le Masurier 1991; Hardy et al. 1992; Vet et al. 1993; Godfray 1994; Harvey 2000; Zaviezo & Mills 2000; West et al. 2001). Females can precisely measure the size and quality of a host before oviposition to determine how many eggs to lay (Schmidt & Smith 1985; Godfray 1994).

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The majority of studies on clutch size decisions among insect parasitoids have been carried out under laboratory conditions, frequently with isolated individuals given access to excess hosts (but see Le Masurier 1991; West et al. 1999, 2001). Such studies typically observe clutch sizes that are notably lower than the predicted Lack clutch size (Godfray 1994; West et al. 1996). Zaviezo & Mills (2000) provided evidence that egg supply is an important limitation for *Hyssopus pallidus* under laboratory conditions, and that the consequent reduction in clutch size results in a maximization of lifetime fitness rather than the fitness gain from individual hosts (see also Flanagan et al. 1998). Optimal clutch size theory also predicts that when hosts are abundant and the reproductive success of a parasitoid is limited by egg supply (i.e. egg limitation: Rosenheim 1999), females should produce a clutch size that maximizes their fitness gain per egg laid (Rosenheim & Rosen 1991; Godfray 1994).

Despite the need for comparative studies, we are unaware of any that have examined the extent to which parasitoid clutch size differs between laboratory and field conditions. Parasitoids probably encounter hosts at a much lower rate in the field than when provisioned with hosts in laboratory studies, so they are more likely to be time limited under field conditions (Sevenster et al. 1998; Rosenheim 1999; West & Rivero 2000). In this case, optimal clutch size theory predicts that average clutch size in the field should be greater than in the laboratory, but it should never exceed the Lack clutch size (Charnov & Skinner 1984, 1985, 1988; Godfray 1994). In support of this theory, laboratory studies have shown that female parasitoids allocate more eggs to a host when host encounter rates are reduced (Nakamura 1995; West et al. 1999; Zaviezo & Mills 2000). However, in addition to host encounter rates, the life expectancy of parasitoid females is also likely to be reduced under field conditions. For example, female survival can be compromised by predation (Heimpel et al. 1997; Völkl & Kroupa 1997), host defences (Potting et al. 1999), competition between females (Visser 1996) or adverse climatic effects (Weisser et al. 1997). Theory suggests that clutch size should increase inversely with life expectancy (Mangel 1989; Roitberg et al. 1993), but we are not aware of studies with gregarious parasitoids. Life expectancy influenced the tendency of the solitary parasitoids *Leptopilina heterotoma* and *Venturia canescens* to superparasitize hosts (Roitberg et al. 1992; Fletcher et al. 1994), although it did not influence the likelihood of host feeding in *Aphytis melinus* (Heimpel & Rosenheim 1995).

We investigated the clutch size of the gregarious parasitoid *Mastrus ridibundus* (Hymenoptera, Ichneumonidae), an ectoparasitoid of codling moth cocoons, *Cydia pomonella*, under laboratory and field conditions. Codling moth is a key pest of apple, pear and walnut production in California and worldwide (Bezemer & Mills 2001a). As part of a biological control programme, *M. ridibundus* has been imported from the region of origin of the codling moth in Central Asia for culture and release in California and the Pacific Northwest (Mills 1996; Kuhlmann & Mills 1999). In Central Asia, *M. ridibundus* naturally occurs in apple orchards under environmental conditions similar

to those found in California. *Mastrus ridibundus* appears to be a specialist parasitoid of codling moth, and is a non host-feeding idiobiont that attacks mature host larvae once they have spun a cocoon under the bark of the host tree. First we tested whether *M. ridibundus* can adjust its clutch size (defined as the number of eggs laid) in response to variation in host encounter rate and parasitoid life expectancy under laboratory conditions. Then we compared brood size (defined as the number of progeny surviving to pupation) under natural conditions in the field and in the laboratory. Finally, we determined fitness curves for *M. ridibundus*.

METHODS

Insect Cultures

Material for laboratory and field studies was reared from cultures maintained in the Insectary and Quarantine Facility at the University of California, Berkeley, U.S.A. Codling moth eggs were purchased from the Sterile Insect Release Facility in Kootenay, Canada. Hatching larvae developed on apples in cardboard rearing trays (100 × 100 cm) at 15°C and 8 h daylight to induce diapause. On completion of development, the codling moth larvae spun cocoons in strips of corrugated cardboard (1.9 cm wide) that lined the sides of each tray. The diapausing cocoons were stored in a cold room at 2°C until needed. *Mastrus ridibundus* was cultured on diapausing codling moth cocoons under constant conditions of 25°C and 16:8 h light:dark photoperiod. Female parasitoids were maintained in sleeved oviposition cages (45 × 45 × 45 cm) with glass lids and provided with both undiluted honey and water. Cardboard strips containing diapausing codling moth cocoons were added to the oviposition cages at weekly intervals to maintain continuous cultures.

Clutch Size Response to Host Encounter Rate

The response of clutch size (i.e. the number of eggs laid) to variation in host encounter rate was tested in the laboratory under controlled conditions (25°C, 70% RH). Newly emerged females (1–3 h old) were kept individually in plastic vials (7 × 4 × 4 cm) with a male to ensure mating, and with continuous access to undiluted honey. The male was kept in the vial until it perished. To manipulate encounter rates, we gave each female either one, two, four or eight diapausing codling moth cocoons per day for 2 days in corrugated cardboard strips (1.9 cm wide). Under laboratory conditions, *M. ridibundus* usually attacks only one host per day and does not self-superparasitize. We used 30–38 replicate females for each encounter rate. To allow parasitoids to habituate to the encounter rate, we did not monitor host strips from the first day of exposure to hosts and dissected only the cocoons removed at 48 h to record clutch size and host size (head capsule width, see below) for each parasitized cocoon. On the second day of the experiment, 13 females produced clutches on two hosts (two at host

density 2; six at host density 4; five at host density 8), one female produced clutches on three hosts (host density 8), and the remaining females produced either zero or one clutch per day. We averaged clutch size for females that produced multiple clutches per day and for the analysis we used females as replicates. Square-root transformed data were analysed with unbalanced analysis of variance (ANOVA) and Tukey's test for multiple comparisons of means.

Clutch Size Response to Parasitoid Life Expectancy

The response of clutch size to variation in life expectancy was also tested in the laboratory under controlled conditions (25°C, 70% RH). The availability of a carbohydrate food source is perhaps the single most important factor influencing the survivorship of most insect parasitoids (McDougall & Mills 1997; Jacob & Evans 2000). Thus, we monitored life expectancy and the response in clutch size in the presence and absence of honey. To estimate life expectancy, we monitored the longevity of two sets of 65 newly emerged unmated females (1–3 h old) every 4–6 h, one set in the absence and the other in the presence of undiluted honey, replenished daily. We determined the response in clutch size over 48 h using two sets of 36 newly emerged females (1–3 h old), in the presence or absence of honey, with eight diapausing codling moth cocoons replaced daily. Females were kept individually in plastic vials (7 × 4 × 4 cm), and we determined clutch size by dissection of host cocoons. Square-root transformed clutch sizes (averaged per female per day) were compared with two-way ANOVA, with food and parasitoid age (1 or 2 days) as independent variables. Inspection of the probability plot indicated that the errors were normally distributed.

Food deprivation might influence egg availability in *M. ridibundus* and compromise the ability of females to respond to reduced life expectancy, so it was necessary to determine the influence of food on egg maturation and egg load. Unmated females were kept individually from the time of emergence in plastic vials (7 × 4 × 4 cm) in the presence or absence of honey under controlled conditions in the laboratory (25°C, 70% RH). We dissected 20 females immediately to determine the number of mature eggs present in the ovarioles at emergence, and subsequent sets of 15–20 females from each treatment 8, 24, 48, 72, 96, 168, 240 and 336 h after emergence and determined the number of mature eggs. In the absence of honey, the majority of females did not survive for more than 3 days. Errors were normally distributed and egg loads were compared with two-way ANOVA, with age and food as independent variables. To enable comparison, we confined age to data from days 1–3 only.

Brood Size Under Laboratory and Field Conditions

Laboratory

Under controlled conditions (25°C, 70% RH) 28 newly emerged females were kept individually in plastic vials

(7 × 4 × 4 cm) with a male to ensure mating, and were presented daily until death with host strips containing eight codling moth cocoons. Three females produced only male progeny, indicating that they had not mated successfully, and were excluded from the data set. After exposure, the parasitized hosts were incubated individually until progeny emerged. For each parasitized host, we recorded brood size (i.e. the number of progeny surviving to pupation), host size (head capsule width), sex ratio (proportion of females) and average female progeny size (from hindtibia length, HTL). We also measured the size (HTL) of each of the 25 successfully mated parent females and, for each female, calculated lifetime fecundity (total number of progeny produced). We measured brood size rather than clutch size to facilitate comparisons with field data where only brood sizes were available. Head capsule width of the hosts used in the experiment varied from 1.35 to 1.75 mm. To verify that head capsule width was a reasonable estimator of host size, in a separate experiment we measured the fresh weight and head capsule width of 150 larvae extracted from host cocoons. Although there was considerable variation, there was a highly significant positive relation between head capsule width and larval weight (weight (g) = 0.048 × head width (mm) – 0.030; $r^2 = 0.17$, $F_{1,149} = 31.4$, $P < 0.001$).

Field

We measured brood size in six walnut orchards in the Central Valley of California, U.S.A., in 1999. In each orchard, the trunks of 50 trees were wrapped with corrugated cardboard (7.5 cm deep) in early August to trap diapausing codling moth larvae descending from the nuts to spin a cocoon. *Mastrus ridibundus* is an exotic parasitoid recently released in California from Kazakstan (Kuhlmann & Mills 1999), but it is not yet present in the six orchards used in this study. We released 4000 parasitoids (50% female) that had been exposed to codling moth cocoons in each orchard in late August and recovered the trap bands in November. Host cocoons from the bands were isolated, and after 2 months of cold storage at 2°C they were incubated to allow parasitoid progeny emergence. We recorded brood size (number of progeny surviving to pupation) for all 214 parasitized hosts. Because some head capsules were damaged, host size could be recorded only for 98 parasitized hosts. Many of the broods recovered from the field had either already emerged or produced uneven emergence after cold storage, so we were not able to estimate brood sex ratios. Average female hindtibia length, however, could be measured for 97 broods from which at least one female successfully emerged. Laboratory and field brood sizes were log transformed and analysed with unbalanced one-way ANOVA. Parent females were used as replicates for the laboratory data, but individual broods were considered replicates for the field data, because released females tend to attack only a single host individual in the release orchard (Bezemer & Mills 2001b). To analyse the influence of host size on brood size for the laboratory and field data we used regression analysis.

Estimation of Fitness Curves

Brood competition

The relation between average female progeny size (s) and brood size (c) was estimated from the data set on brood size under laboratory conditions with linear regression analysis:

$$s=bc+d \quad (1)$$

Adult female fitness

Most adult size–fitness studies with parasitoids have been carried out under laboratory conditions and have used longevity or fecundity as measures of fitness. For parasitoids such as *M. ridibundus* that encounter only a limited number of hosts in the field (Bezemer & Mills 2001b), it is doubtful whether longevity or fecundity directly determines fitness. We compared two estimates of fitness based on host-finding ability in the field and on lifetime fecundity in the laboratory.

Host-finding ability in the field. To estimate the relation between fitness and female size for *M. ridibundus* under field conditions, we carried out a release–recapture experiment in a walnut orchard selected for the absence of a natural codling moth population and in which no parasitoids had been previously released. As noted previously, there was no natural population of *M. ridibundus* present in the release orchard. We mapped out an orchard plot of 120 × 165 m (18 rows with 640 trees). Every other tree in each row was marked and an index card (12 × 8 cm) with a corrugated cardboard strip containing 5–10 codling moth cocoons was fixed to the trunk of the tree. In the centre of the orchard plot, we released 7000 female *M. ridibundus* and, for 6 h, commencing 2 h after release, checked all the marked trees for parasitoids that were either searching on the card or ovipositing on the cocoons in the cardboard strip. The parasitoids were collected and brought back to the laboratory to verify their identity and to record the size (HTL) of each recaptured female. Females were then classified into a series of 20 size classes, ranging from 0.55 to 1.50 mm in class widths of 0.05 mm. To estimate the size distribution of the 7000 released parasitoids, we also recorded the hindtibia length of a random subsample of 565 females from the release population and classified the data into the same size classes. The percentage of released females of size class s that were recaptured, an index of size-dependent fitness, was estimated with the formula:

$$f_{(s)}=100[RC_s]/[RLN_s/N] \quad (2)$$

where $f_{(s)}$ = the fitness of a female of size class s , RC_s = number of recaptured females of size class s , RL = total females released (7000 in this case), N_s = number of females of size class s in the subsample of the release population, and N = total females in the subsample (565 in this case). The number of released females differed between size classes. Large and small females were released in lower frequencies, but the sizes of females of

the released population did not follow a normal distribution (goodness of fit Kolmogorov-Smirnov test: $D=0.11$, $N=565$, $P<0.01$). To estimate the relation between size and fitness, we adjusted the approach described in West et al. (1996) and used a weighted nonlinear regression fit in which the relative fitness of a parasitoid of size s is of the form:

$$F_{s(\text{field})}=\exp(a_1+a_2s+a_3s^2) \quad (3)$$

In this method, the release number of a size class determines the importance during the fitting process. The exponential linear model provided a better fit to the parasitoid recapture data than a null model excluding the effect of female size ($\chi^2_1=34.04$, $P<0.001$), but the inclusion of a quadratic term did not improve the fit of the model ($\chi^2_1=0.80$, $P=0.63$).

A possible source of bias in the recapture of field-released *M. ridibundus* is that, if larger females spend longer than smaller females in handling hosts, they may consequently have a greater probability of recapture. We therefore tested the dependence of host handling time on female size in the laboratory. We offered an experienced mated female a single codling moth host (1.5 mm head capsule width) in a small strip of corrugated cardboard placed centrally in a petri dish (8 cm diameter), and used a video camera to record the oviposition behaviour and time spent handling the host. The size (HTL) of each female was measured after she had left the host. We repeated this for 15 females of variable size and used regression analysis to examine the relation between female size and host handling time.

Lifetime fecundity in the laboratory. The relation between the lifetime number of female progeny produced and female size (s) was estimated from the data set on brood size under laboratory conditions with linear regression analysis:

$$F_{s(\text{laboratory})}=\alpha s+\beta \quad (4)$$

where $F_{s(\text{laboratory})}$ is the lifetime fecundity, or fitness index, of a female of size s .

Estimation of the Lack brood size. We estimated the gain in fitness $W(c)$ from a brood of size c by combining the brood competition relation (equation 1) with the size–fitness relation derived from equations (3) or (4):

$$W(c)_{\text{field}}=F_{s(\text{field})}cf=cf[\exp(a_1)\exp(a_2(bc+d))] \quad (5)$$

$$W(c)_{\text{laboratory}}=F_{s(\text{laboratory})}cf=cfa[bc+d]+\beta \quad (6)$$

where f is the proportion of female progeny in a brood. The Lack brood size can then be estimated as the brood size c that maximizes the gain in fitness $W(c)$ from a single host. Brood sizes obtained from laboratory and field studies followed a normal distribution and were therefore compared to the Lack brood size based on laboratory and field estimates using a one-sample t test.

Table 1. Clutch size of *Mastrus ridibundus* in relation to host encounter rate and mean host size in the laboratory at 25°C, 70% RH

Encounter rate	No. of reproducing females	No. of clutches	Parasitoid clutch size	Host head capsule width (mm)
1 host/day (35)	13	13	5.77±0.70 ^a	1.58±0.025 ^a
2 hosts/day (38)	18	20	5.08±0.60 ^a	1.60±0.016 ^a
4 hosts/day (36)	27	33	4.51±0.43 ^{ab}	1.58±0.015 ^a
8 hosts/day (30)	19	26	3.39±0.35 ^b	1.54±0.014 ^a

Means are shown ±SE. Level of initial replication (females) is given in parentheses. Number of reproducing females and actual egg clutches produced are also given. Within-column means followed by different letters are significantly different at $P<0.05$, based on a Tukey comparison test.

RESULTS

Clutch Size Response to Host Encounter Rate

On the second day of the experiment, 55% of the female parasitoids laid at least one clutch (Table 1). The mean clutch size of individual females declined with increasing host availability (ANOVA: $F_{3,73}=3.70$, $P=0.015$; Table 1). Average clutch size with eight hosts per day was significantly lower than with one or two hosts per day. Host size remained constant between encounter rates ($F_{3,73}=2.56$, NS; Table 1).

Clutch Size Response to Parasitoid Life Expectancy

In the presence of honey, females survived up to 40 days, but in the absence of honey, no females survived more than 4 days (Fig. 1). Over the 2 days of the clutch size response experiment, the number of hosts a female attacked per day was not affected by the presence or absence of honey ($\bar{X} \pm \text{SE}$; honey present: 0.94 ± 0.09 ; honey absent: 0.98 ± 0.09 ; $F_{1,140}=0.087$, NS). There was also no difference in the average clutch size of the two sets of females that experienced very different life expectancy ($\bar{X} \pm \text{SE}$; day 1: honey present: 4.3 ± 0.4 ; honey absent: 4.1 ± 0.3 ; day 2: honey present: 3.5 ± 0.3 ; honey absent: 3.1 ± 0.2 ; ANOVA: Food: $F_{1,96}=0.329$, NS).

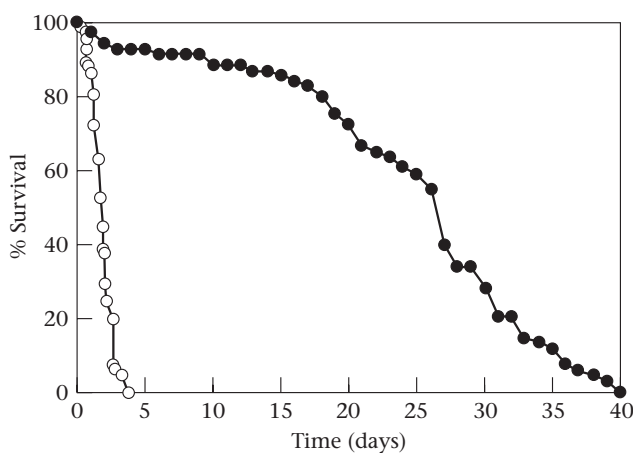


Figure 1. Survivorship curves for *Mastrus ridibundus* in the presence (●) or absence (○) of honey replenished daily ($N=65$ for both sets).

Food*Day: $F_{1,96}=0.326$, NS). Independent of food availability, clutch sizes produced on day 1 were significantly larger than those produced on day 2 ($F_{1,96}=6.505$, $P<0.05$).

In the absence of a significant interaction term, the availability of honey did not influence the number of mature eggs present over the first 3 days of adult life ($F_{1,146}=2.55$, NS; Fig. 2). In contrast, there was a clear effect of time on egg maturation over this same period ($F_{3,146}=21.34$, $P<0.001$). The average number of mature eggs present at day 3 and beyond was 16.8, and females emerged with an average of 5.1 eggs.

Brood Size Under Laboratory and Field Conditions

Average rates of parasitism under both laboratory and field conditions were relatively low: $\bar{X} \pm \text{SE}=13.4 \pm 0.7\%$ for isolated females in the laboratory and $16.5 \pm 1.6\%$ for females released in the field. In the laboratory, average brood size was greatest on the first day after emergence, remained constant over the subsequent 9 days, and declined over the last 5 days of adult female life (Fig. 3a). The average proportion of female progeny in a brood remained constant over the first 11 days after emergence,

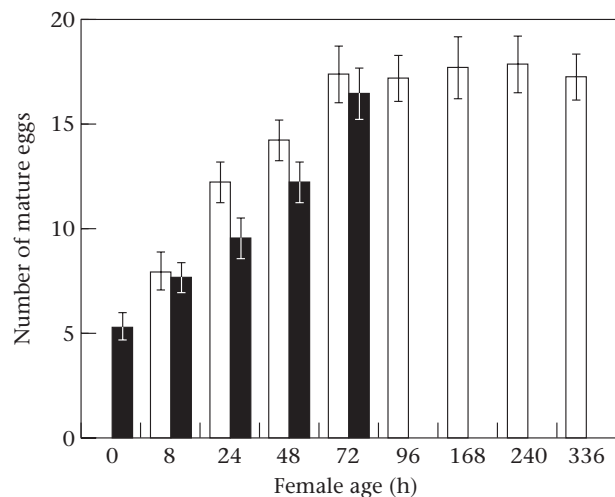


Figure 2. Mean±SE number of mature eggs present in ovarioles of *Mastrus ridibundus* in the presence (□) or absence (■) of honey replenished daily. Females were not provided with hosts.

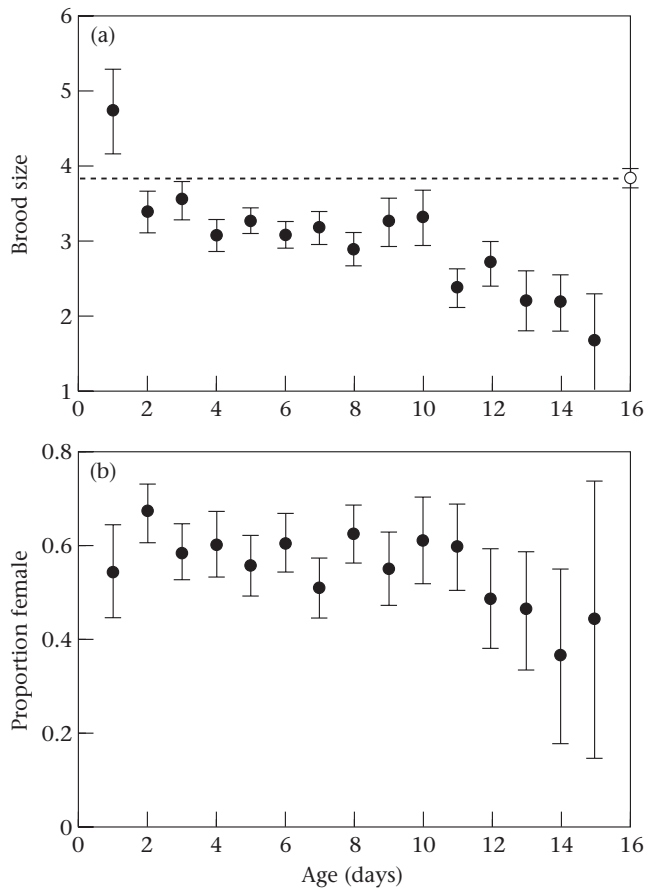


Figure 3. Mean \pm SE (a) brood size and (b) brood sex ratio of *Mastrus ridibundus* in relation to age under laboratory conditions with access to excess hosts. ---, \circ : Corresponding mean \pm SE estimate of brood size from the field.

but subsequently declined during the last 4 days (Fig. 3b). There was no relation between brood size and the proportion of female progeny in a brood ($F_{1,335}=2.58$, $P=0.11$). The mean \pm SE brood size was 3.00 ± 0.13 , the brood sex ratio (proportion female) 0.61 ± 0.04 , and the average female progeny size 1.33 ± 0.01 over the lifetime of the parent females. Lifetime estimates of brood characteristics under laboratory conditions may not be directly comparable with field-collected broods, however, because parent female age in the field is unknown. Field-released females were older than 1 day, but may not have survived for more than 10 days under field conditions. Laboratory brood characteristics remained constant over this age range (Fig. 3a, b), so they provide a more suitable comparison with broods collected from the field. Over an age range of 2–10 days, mean \pm SE laboratory brood size was 3.06 ± 0.13 and significantly lower than the average brood size of 3.85 ± 0.12 observed in the field ($F_{1,237}=4.26$, $P<0.05$). The average proportion of female progeny from the laboratory broods was 0.63 ± 0.05 , but could not be adequately assessed from the field broods. Consequently, average female progeny size was significantly larger in the laboratory (1.33 ± 0.01) than in the field (1.23 ± 0.02 ; $F_{1,120}=8.95$, $P<0.01$), because average host size under both conditions was not significantly

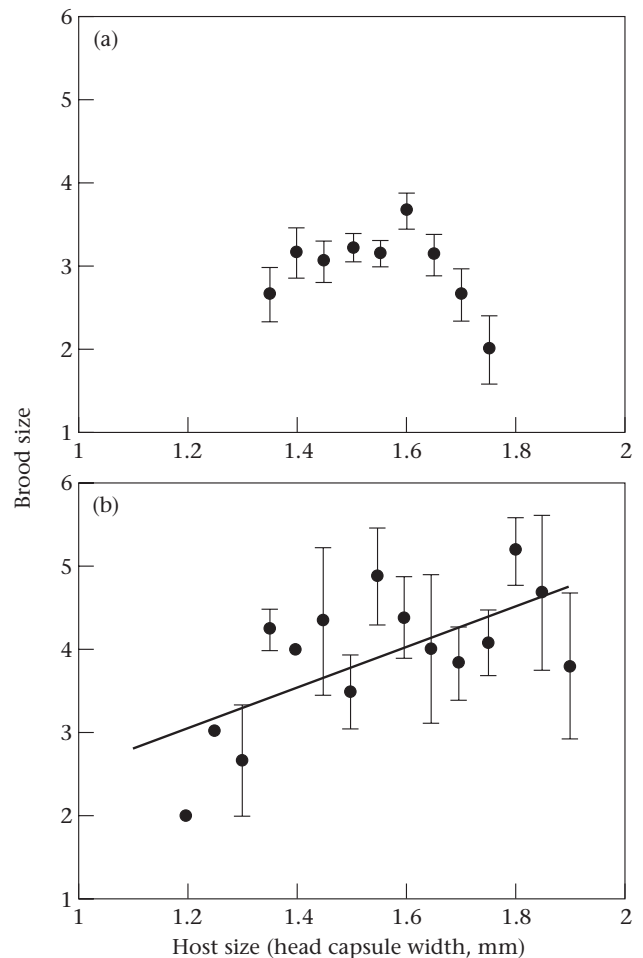


Figure 4. Relation between brood size of *Mastrus ridibundus* and host size under (a) laboratory conditions with access to excess hosts ($N=259$) and (b) in the field ($N=98$). For clarity, only means \pm SE are shown. Regression (laboratory): $F_{1,257}=0.06$, NS; regression (field): brood size = $0.10 \times$ host size + 0.94 , $r^2=0.04$, $F_{1,96}=4.05$, $P<0.05$.

different ($F_{1,121}=0.013$, NS). Brood size in the laboratory was unaffected by host size (Fig. 4a), but in the field, broods developing on large hosts were significantly larger than broods developing on small hosts (Fig. 4b).

Estimation of Fitness Curves

Data from the experiment on brood size under laboratory conditions were used to determine the influence of brood competition on the size of female progeny. For a mean \pm SE size host of 1.54 ± 0.01 mm, the average hindtibia length of female progeny declined from 1.44 mm for a brood size of one to 1.02 mm for a brood size of eight parasitoids (Fig. 5). This reduction in size from competition within the brood was highly significant ($F_{1,268}=262.3$, $P<0.0001$).

Host-finding ability in the field

We recaptured 162 released females in the host-finding ability experiment in the field. The mean \pm SE size of the individuals from the release population (1.17 ± 0.01 mm)

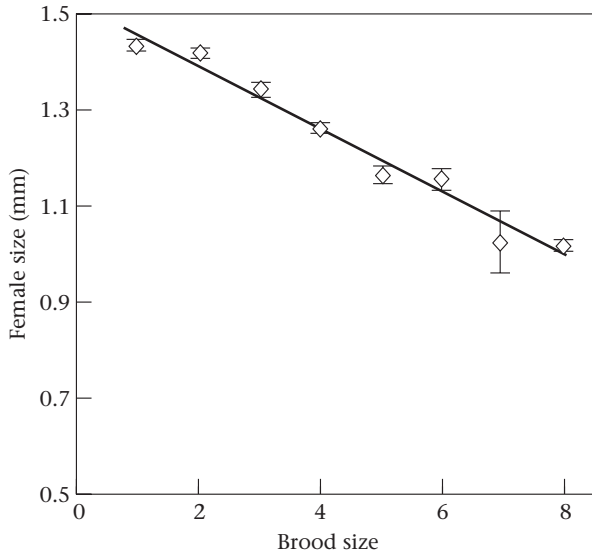


Figure 5. Relation between female offspring size (s , hindtibia length) and brood size (c) for *Mastrus ridibundus* under laboratory conditions, based on an average size host of 1.54 mm. For clarity only the means \pm SE are shown. Regression: $s = -0.067c + 1.54$, $r^2 = 0.53$, $F_{1,268} = 305.7$, $P < 0.001$.

was significantly smaller than that of the recaptured individuals (1.24 ± 0.01 mm; Kolmogorov–Smirnov test for two populations: $D = 0.2$, $N_1 = 565$, $N_2 = 162$, $P < 0.0001$). Percentage recapture as an index of size-dependent fitness (estimated from equation 3) varied from 0 to 7. Released females belonging to size classes smaller than 0.85 mm were not recaptured, and recapture success increased exponentially with size ($F_{2,14} = 70.5$, $P < 0.0001$; Fig. 6a). Video analysis of the oviposition behaviour of *M. ridibundus* showed that host handling time was not affected by the size of the ovipositing female ($F_{1,13} = 1.55$, NS; Fig. 6b).

The fitness curve for *M. ridibundus*, estimated from the combination of brood competition (based on a host with an average head capsule width of 1.54 mm), female host-finding ability in the field, and an estimated brood sex ratio of 0.63 (Fig. 3) increased for brood sizes from one to four and subsequently declined (Fig. 6c). The optimal or Lack brood size of 4.3 was not significantly different from the average brood size found in the field (3.85; $t_{213} = 1.57$, $P = 0.12$), but was significantly larger than the average brood size found in the laboratory (3.06; $t_{24} = 7.60$, $P < 0.001$).

Lifetime fecundity in the laboratory

Lifetime fecundity varied considerably from 4 to 82. None the less, there was a significant positive relation between female size and lifetime fecundity ($F_{1,31} = 17.6$, $P < 0.001$; Fig. 7a). Using lifetime fecundity as an index of fitness, the estimated Lack brood size (based on a host with an average head capsule width of 1.54 mm) is 5.5 (Fig. 7b) and is significantly larger than average brood sizes found in the field ($t_{213} = 13.94$, $P < 0.0001$) and in the laboratory ($t_{24} = 22.79$, $P < 0.0001$).

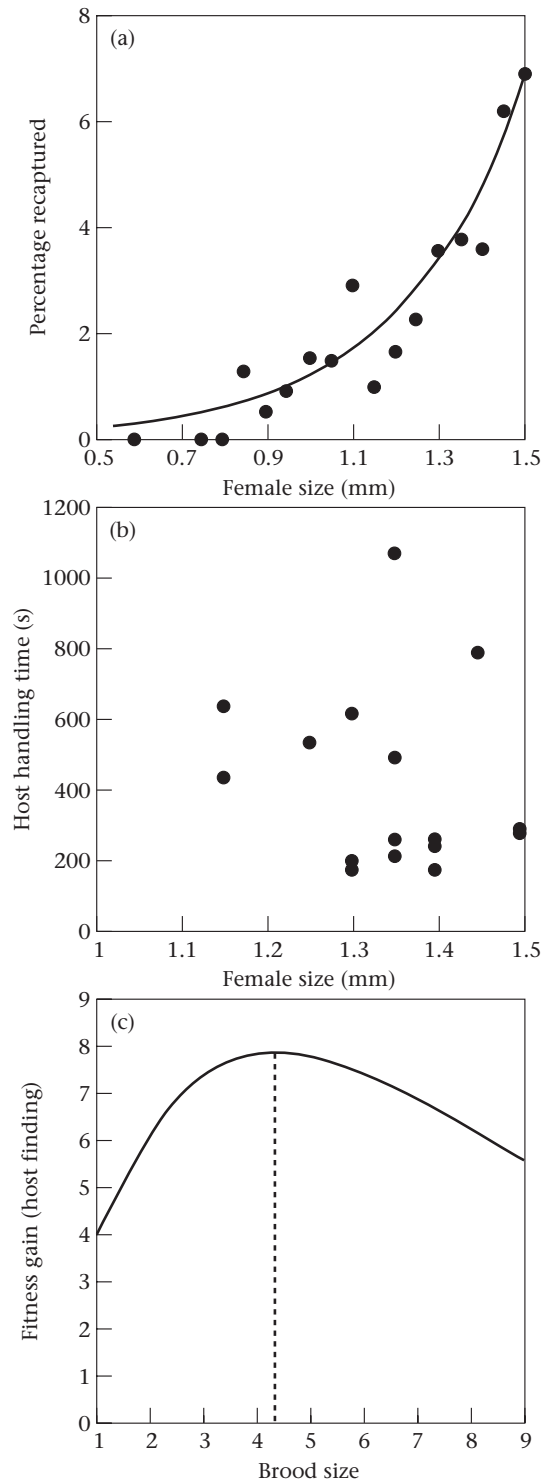


Figure 6. Field fitness estimate. (a) Relation between host-finding ability ($F_{s(\text{field})}$) and female size (s , hindtibia length) for *Mastrus ridibundus* under field conditions. (b) Relation between host handling time and female size. (c) Fitness curve for *M. ridibundus* showing the relation between fitness gain ($W_{s(\text{field})}$) and brood size. In (a), fitness was estimated from the percentage of recaptured females for each size class. Regression: $F_{s(\text{field})} = \exp(-3.24) \exp(3.45s)$, $r^2 = 0.85$, $F_{2,14} = 70.5$, $P < 0.001$. In (c), the vertical line indicates the Lack brood size of 4.3.

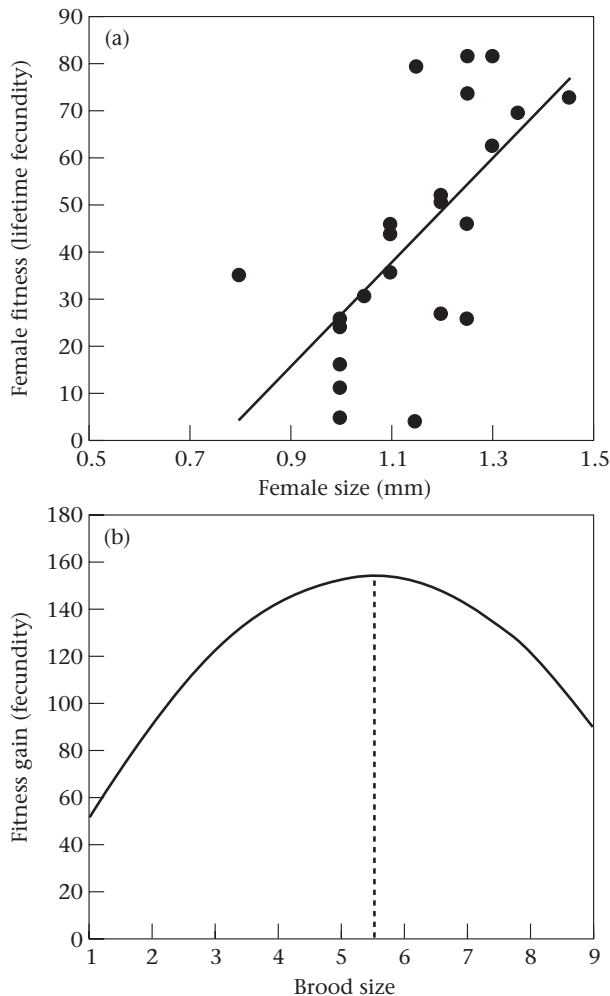


Figure 7. Laboratory fitness estimate. (a) Relation between fitness ($F_{s(\text{laboratory})}$) based on lifetime fecundity and female size (s), measured in the laboratory for parasitoids that had access to honey and excess hosts. (b) Fitness curve showing the relation between female fitness ($W_{s(\text{laboratory})}$) and brood size. In (a), regression: $F_{s(\text{laboratory})} = 112.3s - 85.4$; $r^2 = 0.44$, $F_{1,31} = 17.6$, $P < 0.001$. In (b), the vertical line indicates the Lack brood size of 5.5.

DISCUSSION

Empirical observations have shown that oviposition decisions vary according to the physiological status (egg load and age) of a parasitoid (Rosenheim & Rosen 1991; Flanagan et al. 1998; Papaj 2000), and the abiotic (wind and rain) and biotic quality (host abundance and quality) of its environment (Fink & Völkl 1995; Nakamura 1995; West et al. 1999, 2001; Zaviezo & Mills 2000, 2001). The dynamic nature of parasitoid oviposition has also been effectively captured through use of dynamic optimization models to explore the trade-offs between current and future reproduction (e.g. Mangel 1989; Visser 1994; Mangel & Heimpel 1998; Rosenheim 1999; Ellers et al. 2000).

In the case of gregarious parasitoids, such as *M. ridibundus*, the outcome of oviposition decisions is mediated through adjustment of clutch size. We have shown that, under laboratory conditions, *M. ridibundus* responds to a

reduction in host encounter rate by increasing the average clutch size allocated to hosts, as has been shown for other gregarious parasitoid species (Le Masurier 1991; Vet et al. 1993; Zaviezo & Mills 2000).

Contrary to expectations, however, *M. ridibundus* made no adjustment of clutch size under conditions of reduced life expectancy (absence of honey). It could be argued that adult food deprivation often leads to egg resorption for the support of somatic maintenance, particularly among host-feeding parasitoids (Godfray 1994), which could pose an important constraint on the adjustment of clutch size. However, egg maturation and egg load of *M. ridibundus*, a nonhost-feeding species, were not compromised under conditions of food deprivation (Fig. 2) and thus would not have prohibited an increase in clutch size. It could also be argued that eight hosts per day might induce egg limitation in *M. ridibundus* and thus limit the ability of females to respond to a reduction in life expectancy. However, on average, females attacked just less than one host cocoon per day and laid clutches of three to four eggs, providing little opportunity for egg limitation in a parasitoid that has a mean \pm SE egg load of 10.46 ± 0.40 ($N = 117$) in the presence of excess hosts (N. J. Mills, unpublished data). Clutch size responses to reduced life expectancy have not been tested for any other gregarious parasitoids, so the generality of the response of *M. ridibundus* is unknown. This result is similar, though, to the observation by Heimpel & Rosenheim (1995) that life expectancy does not influence investment in current (oviposition) versus future (host feeding) reproduction in *Aphytis melinus*. In contrast, life expectancy is considered an important determinant of clutch size in birds (Lima 1987; Ghalambor & Martin 2001).

The lifetime reproductive success of parasitoids is likely to be limited by longevity and host encounter rates in the field, suggesting that most parasitoids probably experience time limitation (Sevenster et al. 1998), although synovigenic species, which continue to mature eggs after emergence, may also experience temporary egg limitation when hosts are abundant (Driessen & Hemerik 1992; Heimpel & Rosenheim 1998; Heimpel et al. 1998). As a result, expected lifetime reproductive success in the field is likely to be lower than under laboratory conditions. Heimpel et al. (1998), for example, estimated the lifetime reproductive success of the scale parasitoid *Aphytis aonidiae* to range from 3 to 37 eggs in the field, with a central estimate of only 6.25 eggs, compared to a lifetime fecundity of 35–102 eggs in the laboratory. Thus, under field conditions, gregarious parasitoids should maximize their fitness gain from individual broods. In contrast, under laboratory conditions, with greater expectation of future reproduction, gregarious parasitoids should maximize their fitness gain per egg by reducing brood size (Charnov & Skinner 1985; Godfray 1994).

The average brood size of individual *M. ridibundus* provided with excess hosts in the laboratory was significantly smaller than that under field conditions. *Mastrus ridibundus* responded to host encounter rate, but not to life expectancy, under laboratory conditions, so we assume that the larger brood sizes in the field were caused by limited host availability and a low expectation of future

reproduction. Studies of *Cotesia glomerata*, a gregarious endoparasitoid, have also shown a greater brood size in the field than in the laboratory (cf. Tables 1 and 2, Le Masurier 1991; Table 1, Brodeur et al. 1998; Table 1, Geervliet et al. 2000). Brood size of *M. ridibundus* on large, high-quality hosts was also significantly greater than on small, low-quality hosts under field conditions, but no such response was found in the laboratory. These results provide further evidence that when host availability limits the reproductive success of *M. ridibundus*, a female more precisely assesses the quality of an encountered host to maximize the fitness gain from individual broods. In contrast, when hosts were not limiting under laboratory conditions, females produced an average of three individuals per brood regardless of host size or quality, and thereby increased their lifetime fitness gain through reduced brood competition and the production of larger female progeny.

Our study has also highlighted the importance of using field estimates of female fitness in estimating fitness curves and the Lack brood size of gregarious parasitoids. The majority of previous estimates of Lack brood size for parasitoids have been based on laboratory estimates of longevity or lifetime fecundity (but see West et al. 1999, 2001). Under field conditions, when the expectation of future reproduction may frequently be low, host-finding ability is likely to provide a more accurate representation of lifetime reproductive success. Our results show that, at least for *M. ridibundus*, the estimated Lack brood size based on a fitness measure obtained under laboratory conditions (lifetime fecundity) was much higher than that based on a field estimate of host-finding ability. Thus, the laboratory-based measure of fitness clearly underestimated the disadvantage of being small. How general this phenomenon is remains unknown, but it provides a possible explanation for why laboratory studies of brood size in insect parasitoids have consistently found observed brood size to be well below the predicted Lack brood size (Godfray 1994; West et al. 1996; Zaviezo & Mills 2000).

Females emerging from broods in the field were significantly smaller than those emerging from broods in the laboratory. Based on Fig. 5, we would expect females with an average HTL of 1.23 mm to emerge from broods of more than four, but the average brood size observed in the field was 3.9. We believe that this is because Fig. 5 is based on hosts with a head capsule width of 1.54 mm, but in the field, parasitized hosts were frequently smaller (Fig. 4b). For smaller hosts, the slope in Fig. 5 increases (T. M. Bezemer, unpublished data). The difference in size of female progeny could also have been influenced either by the earlier emergence of, or the mortality that occurred within, the field-collected broods, but there is no obvious reason why either of these effects would have differentially affected progeny size. As noted by Rivero & West (2002), the fitness costs of being small are generally greater under field than laboratory conditions, and thus the consequences of clutch size decisions on progeny fitness are particularly acute in this case. Most models of the evolution of clutch size in parasitoids also assume that broods share resources equally. However, recent

studies on optimal progeny size and the variance in body size within parasitoid broods (Charnov & Downhower 1995; Mayhew & Glaizot 2001; West et al. 2001) suggest a need for more accurate estimation of this aspect of clutch size–fitness relations in future field studies.

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