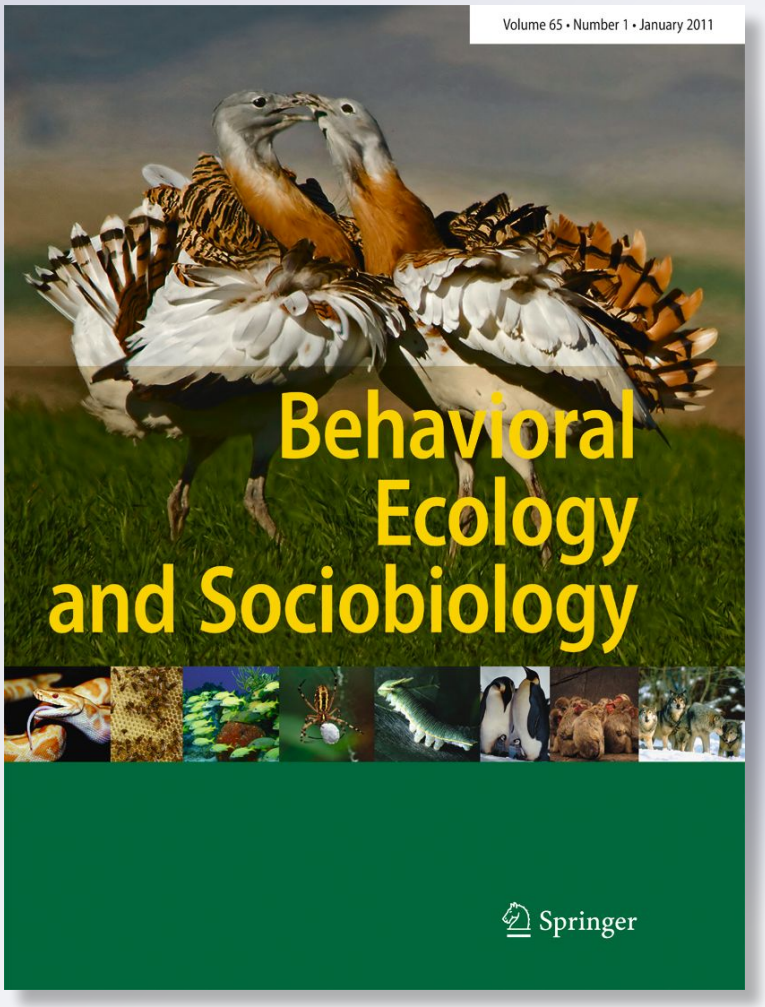
Low maternal host-encounter rate enhances offspring proliferation in a polyembryonic parasitoid

Na'ama Morag, Ally R. Harari, Amos Bouskila & Tamar Keasar

Behavioral Ecology and Sociobiology

ISSN 0340-5443
Volume 65
Number 12

Behav Ecol Sociobiol (2011)
65:2287-2296
DOI 10.1007/s00265-011-1239-6



Springer

Behav Ecol Sociobiol (2011) 65:2287–2296
DOI 10.1007/s00265-011-1239-6

ORIGINAL PAPER

Low maternal host-encounter rate enhances offspring proliferation in a polyembryonic parasitoid

Na'ama Morag · Ally R. Harari · Amos Bouskila · Tamar Keasar

Received: 1 June 2011 / Revised: 20 July 2011 / Accepted: 2 August 2011 / Published online: 14 August 2011

Abstract Mothers can epigenetically influence their progeny's characteristics in response to environmental conditions they experience, thereby increasing offspring adaptation to anticipated future conditions. When resource shortage is anticipated, females are expected to produce larger offspring, as large body size often confers competitive and dispersal advantages. We tested this hypothesis using the polyembryonic parasitoid, *Copidosoma koehleri*. In this wasp, each egg proliferates into a clone of genetically identical individuals within its moth host, and body size correlates negatively with clone size. We expected females anticipating resource limitation to produce fewer and larger offspring per clone than females that anticipate abundant resources. Encounter rates of parasitoid females with hosts were manipulated to simulate varying levels of resource availability. High-encounter-rate females were introduced to ten hosts successively, while low-encounter-rate females encountered each of ten hosts at 8-h intervals. To control for female age at oviposition, we also introduced females at different ages to a single host. Contrary to our predictions, low-encounter-rate females produced larger offspring clones than high-encounter-rate females, and offspring body size did not differ between treatments. Low-encounter-rate females were shorter-lived than females that encountered hosts successively. Single-oviposition females resembled the high-encounter-rate females in longevity but produced as many offspring per clone as in the low-encounter-rate treatment. Female age, and number of previous host encounters, did not affect offspring clone size. These results suggest that offspring proliferation bears a cost to mothers, thus mothers that repeatedly induce high proliferation in their offspring pay an increased price.

Keywords Body size · Host density · Epigenetic effect · Parasitoid · Polyembryony

Communicated by R. Moritz

N. Morag · A. R. Harari · A. Bouskila
Department of Life Sciences, Ben Gurion University,
P.O. Box 653, Beersheba 81405, Israel

A. R. Harari
Department of Entomology, Agricultural Research Organization,
the Volcani Center,
P.O. Box 6, Bet Dagan 50250, Israel

T. Keasar (✉)
Department of Biology, University of Haifa–Oranim,
Tivon 36006, Israel
e-mail: tkeasar@research.haifa.ac.il

Introduction

Mothers can react to environmental stimuli they experience by modifying their offspring's traits, in ways that will increase the progeny's adaptation to anticipated environmental conditions. These non-genetic influences, also called maternal effects, are often mediated via the transfer of nutritional supplements to the laid egg, such as yolk and hormones, or through pre- and post-natal provisions of food. Maternal effects can also be manifested through other means: mate choice, selection of oviposition sites, transmission of pathogens and antibodies to the offspring (either through the blood system pre-birth, or by active feeding), and imitative behavior (reviewed by Bernardo 1996; Mousseau and Fox 1998a, b). The molecular mechanisms underlying maternal effects may involve cytoplasmic inheritance and direct covalent modifications of nucleotides, often expressed by their methylation (Richards 2006).

Adaptive maternal effects are expected to influence important life history characteristics in the offspring. One such trait is offspring body size, which is often positively correlated with fitness measures such as life span, reproductive success, and dispersal abilities (Honek 1993; Kazmer and Luck 1995; Solbreck 1995; Ellers et al. 1998). Progeny body size in arthropods was shown to be affected by multiple cues in the maternal environment, such as feeding status, host plant, density, and rearing temperature (Fox et al. 1995, 1996; Rosenheim and Hongkham 1996; Jann and Ward 1999; Lardies et al. 2004; Creighton 2005). Offspring body size can be regulated through maternal control over clutch size, because clutch- and body-size are traded off under resource limitation (Mayhew and Glaizot 2001). Body size may also be controlled by adjustment of embryonic development rate, as a prolonged embryonic phase often results in larger-sized offspring (Santos et al. 1994).

Offspring body size is often increased in response to resource limitation experienced by their mothers. Examples include crustaceans, which produce larger offspring under mild food shortage (reviewed by Fox and Czesak 2000, but see also for examples of smaller eggs produced by starved females). Similarly, *Drosophila* females that experience poor environmental conditions, such as high density leading to limited food resources, produce offspring with extended development time. This allows them to feed on a larger proportion of resources and reach a larger body size as adults, compared with progeny of low-density females (Santos et al. 1994).

The encounter rate of parasitoid females with hosts is an environmental cue that could indicate future resource availability for the offspring. Low host-encounter rates may indicate to searching females that host density in the patch is low, or may correlate with other biotic or abiotic stress conditions. Such conditions are predicted to favor the production of larger offspring through epigenetic regulation, in species with scramble competition such as gregarious parasitoids (Ives 1989). This is because producing fewer offspring increases the amount of food available to each of them and their final body size and hence may improve their competitive abilities under conditions of superparasitism (Honek 1993). However, this prediction is complicated if low host-encounter rates result in a higher egg load for females, leading to increased clutch sizes (Bezemer and Mills 2003). This may come at the expense of body size (Godfray 1994). In agreement with this reasoning, female gregarious parasitoids allocate more eggs to each host under low host-encounter rates (West et al. 1999; Zaviezo and Mills 2000). Therefore, the effect of host-encounter rates on offspring sizes may be difficult to disentangle from their effect on clutch sizes. Here, we deal with this problem by studying a polyembryonic parasitoid. In polyembryonic development, the number of offspring arising from each oviposition is determined through the repeated proliferation of a single egg into a clone of individuals. Thus, their development is extremely gregarious (Godfray 1994; Strand 2003). This developmental feature means that maternal egg load and clutch size are not directly correlated. An additional advantage of polyembryonic species as experimental models lies in the genetic identity of siblings, which eliminates the conflict regarding clutch- and body-size among clone-mates. Thus, the parent–offspring conflict too is reduced in single-clone situations, because fitness is maximized at the same body- and clutch-size for both parent and offspring (Godfray 1994). Finally, a previous study on a polyembryonic parasitoid indicates that clone size (defined as the number of individuals per clone), and hence, offspring body size, may be influenced by maternal effects (Morag et al. 2011).

We tested two predictions: (1) Low encounter rates with hosts would result in the production of fewer, although larger, offspring from each egg. (2) Larger offspring body sizes would be attained through prolonged larval development when host-encounter rates are low than when host encounters are frequent (Santos et al. 1994).

Materials and methods

The study species and rearing conditions

Copidosoma koehleri (Blanchard) (Encyrtidae: Hymenoptera) is a polyembryonic egg-larval parasitoid wasp that parasitizes the potato tuber moth, *Phthorimaea operculella* (Zeller) (Gelechiidae: Lepidoptera) (Kfir 1981). It is fully pro-ovigenic, i.e., the females' full egg complement (ca. 100 eggs (Kfir 1981)) is already mature at adult emergence (Jervis et al. 2001). The egg proliferates within 8 days of oviposition to form a clone of approximately 40 genetically identical embryos (Segoli et al. 2009a, b). Female clones contain a soldier larva, which attacks competitors in the host and dies before reaching maturity. Male clones do not form soldiers. Sex determination follows haplo-diploid genetics, i.e., virgin females produce only haploid sons, whereas mated females can produce both haploid sons and diploid daughters (Doutt 1947). Egg-to-pupa development requires ca. 16 days at 27 C. All individuals in a clone emerge on the same day.

We used a laboratory stock of *C. koehleri* originating from South Africa and raised it according to Berlinger and Lebiush-Mordechi's protocol (1997). The culture was initiated in 1991, was supplemented with field-collected wasps in 2005, and contained tens of thousands of *C. koehleri* adults. A stock of hosts originating from local field populations was raised at 27°C, 40% RH, and a 12:12 L/D

schedule. Hosts were reared on potatoes during their four larval instars and were provided with honey and water throughout adulthood. Adult females oviposited on non-woven polypropylene sheets that covered their cages. These sheets were replaced every few hours and were used as a source of host eggs of uniform age. The size of the host egg is not related to final host size and to the number of parasitoids it can support (unpublished data). Therefore, host eggs were selected haphazardly for experiments, regardless of their size.

Host presentation

We used fresh *P. operculella* eggs (0–5 h of oviposition) as hosts in all experiments. We placed each host singly in a Petri dish, directed the ovipositing female to the host by rotation of the vertically held dish and observed it during oviposition (Segoli et al. 2009b). A female that did not oviposit within 5 min of first contact with the host was introduced to a second host. If parasitism did not take place within five additional minutes, we scored the female as a rejector and excluded it from the experiment. In the high- and low-encounter-rate treatments (see below), we housed the females individually in Petri dishes and supplied them with honey between successive ovipositions and after completing ten ovipositions. The females were then housed in individual 10-ml test tubes at room temperature and supplied with honey until they died. We reared the parasitized hosts individually on a slice of potato at 27°C until the emergence of the parasitoid brood.

Experimental design

We collected 15 females from each of 26 *C. koehleri* all-female broods within 24 h of emergence and supplied them with honey as a food source. We kept the females virgin to reduce the genetic variance of their offspring and to limit them to the production of sons only. Since male clones do not form soldiers, this precluded any possible complication through brood size manipulation by soldier larvae. We haphazardly designated three wasps from each clone to the high- and low-encounter-rate treatments, respectively, and the other nine to the age-control treatment.

High-encounter-rate treatment We exposed each female within this treatment to ten hosts consecutively within 8 h of emergence and allowed it to oviposit once inside a host. As soon as the oviposition was completed, we introduced the female to a subsequent host. We confirmed oviposition through direct observation. The mean time between ovipositions was 3:00±0:10 min (range, 1–30 min). Five females allocated to this treatment escaped before their first oviposition, reducing sample size from 78 to 73.

Low-encounter-rate treatment We introduced each of the females within this treatment (n=78) to ten hosts at time intervals of approximately 8 h (mean±SD, 8:03±00:41 h; range, 6–10 h), thus they encountered hosts over a period of 72 h. Each female was allowed one oviposition per host, as above.

Age-control treatment This treatment aimed to test whether differences between the low- and high-encounter-rate treatments are due to the females' age at oviposition rather than to differences in host-encounter rates. To this end, we allowed females of different ages to each oviposit once in a host. The females' age groups were 8, 16, 24, 32, 40, 48, 56, 64, and 72 h post-emergence. Thus, these age groups corresponded to the ages at oviposition of females from the low host-encounter-rate treatment. Each age group comprised 15–24 females, each of a different brood, totaling 192 individuals. Ovipositions were confirmed through direct observation.

A scheme of the experimental treatments is provided in Fig. 1.

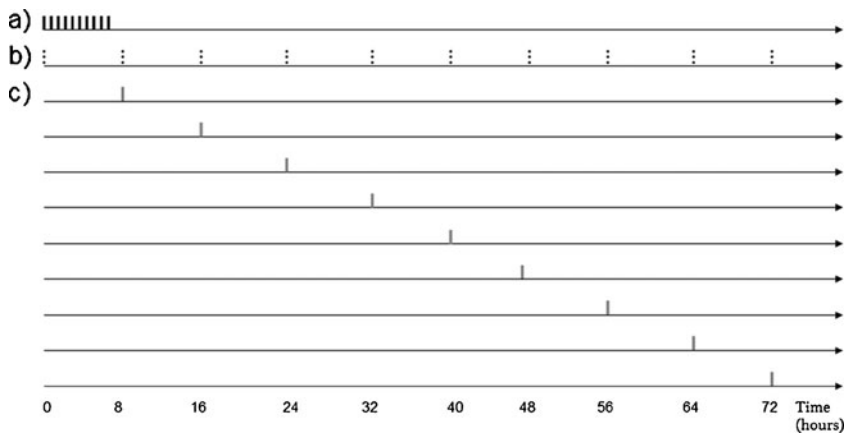


Fig. 1 A scheme of host encounter sequences. The three treatment groups are depicted as follows: **a** The high encounter rate: each female oviposited ten times consecutively; **b** the low encounter rate: each female oviposited ten times every 8 h, and **c** the age-control group: each female oviposited once at each time point. We presented females from this treatment with a host 8–72 h after the initiation of the experiment

The feeding-control experiment In the previously described treatments, we allowed females to feed throughout their encounter period with hosts. Since this period was much longer in the low-encounter treatment (72 h) than in the high-encounter treatment (<2 h), maternal nutritional condition per se, rather than host-encounter rate, could affect offspring traits. To test for this possibility, we supplied half of the wasps from each of 20 female clones with honey (a source of both energy and water) and deprived the rest of food for 24 h (n=270 wasps). We then allowed each female to oviposit once in a host. These females originated from different broods than those used for the high-encounter, low-encounter, and age-control treatments. We recorded female longevity and offspring traits as in the host-encounter experiment (see below).

Dependent variables measured

We assessed pupation of the developing parasitoids at daily intervals to determine the egg-to-pupa development time. We determined the mass of the parasitized hosts upon pupation of the wasps within their hosts' cuticle. At this stage, host tissues had already been completely consumed by the parasitoids, therefore, the mass of the host mummy provides an indication of the parasitoids' mass. Each mummy was placed in a separate test tube. Test tubes were kept at −20°C for 1 h or more after the emergence of the adult wasps, and the dead wasps were subsequently counted. We measured the head width of five randomly selected dead individuals per clone to the nearest 1 μm, using the software QCAPTURE PRO as an indicator of body size.

To test for direct effects of host-encounter rate on ovipositing females, we documented two behavioral parameters. First, we measured the time between the first contact with each host and the completion of ovipositor insertion (starting time). Second, we measured the duration of oviposition (oviposition duration), from the end of the ovipositor insertion until its removal from the host. Additionally, we documented the number of days from the mothers' emergence from the natal host to death, as a measure of maternal physiological condition.

Host dissections

C. koehleri females usually release a single egg per ovipositor insertion, but two eggs are occasionally found in hosts after a single oviposition (Keasar et al. 2006). In the congener, *Copidosoma floridanum*, low host-encounter rates increase the frequency of ovipositions of two eggs per ovipositor insertion, which could increase brood sizes (Ode and Stand 1995). To test for this possibility, we allowed virgin wasps to oviposit once into each of two hosts, either consecutively or at a 24-h interval. We used a maximum of five wasps from each of several all-female broods for this experiment. The wasps were exposed to the first host within 24 h of emergence and had no previous host encounters. We dissected the hosts immediately after the oviposition and recorded the number of wasp eggs in them. Dissections were performed in insect Ringer's solution under a phase-contrast microscope. Hosts that received an ovipositor insertion of less than 10 s were excluded from the sample because they often contained no wasp egg at dissection (unpublished data). Only wasps that released one egg in their first host were presented with a second one. One hundred seventeen hosts, each parasitized by a different naive wasp, were dissected, and a single wasp egg was recorded in 55 of them. Eighteen of the wasps that parasitized these hosts were immediately presented with a second host, and 34 wasps were provided with a second host 24 h later. The remaining three wasps died or escaped before ovipositing in their second host.

Data analysis

We used a χ^2 test to compare the frequency of parasitoid emergence from hosts across experimental treatments. We separated the data regarding the first oviposition from data on ovipositions 2–10, because both low- and high-encounter-rate females experienced identical environments until their second host encounter. To overcome difficulties in data analysis due to low sample sizes, we combined data from ovipositions 3–4, 5–6, 7–8, and 9–10 in the low- and high-encounter-rate treatments, and from ages 16–24, 32–40, 48–56, and 64–72 h in the low-encounter-rate and in the age-control treatments. Data obtained from females' second oviposition and from ovipositions of 8-h-old females were not united with other age/oviposition groups. This combination was chosen because older females and females that have reached late ovipositions tended to escape, die, or reject further hosts. Thus, sample sizes were smaller for older females than for young ones, increasing the need to combine data from later ovipositions. We used these combined data for the statistical analyses of all dependent variables.

Analysis of covariance (ANCOVA) was used to test for the effects of treatment on clone size, body size, development time, and host mass. In the low- vs. high-encounter-rate comparison, we used the number of previous ovipositions as a covariate within the ANCOVA. In the comparison between the low-encounter-rate and the age-control treatment, the female's age was used as a covariate. In both analyses, the treatment was defined as a factor. The high-encounter-rate and the age-control females were not compared directly at this stage, since they differed both in

age and in the number of ovipositions: age-control females oviposited only once but at different ages, whereas high-encounter females oviposited ten times but at the same age. Repeated-measures ANOVA was not appropriate for the analysis of this data set because a large proportion of the parasitized hosts did not complete their development, and this resulted in too many missing values. Due to logistical constraints, the experiment could not be repeated to overcome this problem.

We found that maternal age and oviposition experience did not affect clone size, body size, developmental rates of offspring, and host mass in any treatment, as detailed in the "Results" section. Next, we selected one oviposition by each female (excluding first ovipositions) for further analysis by drawing a random number between 2 and 10. This was done to avoid pseudoreplication when comparing treatments. This reduced data set met the assumptions for parametric statistics. We therefore used one-way ANOVAs, followed by post hoc procedures, to compare clone size, body size, development time, and host mass at pupation across the three treatments.

We compared the life spans of all mothers from the three treatment groups using a Kaplan–Meier survival analysis with a log-rank test and used Mann–Whitney *U* tests as a post hoc procedure. We analyzed the differences in clone size, body size, and development time between offspring of fed and unfed females using Mann–Whitney *U* tests. To test for the effect of treatment on host handling durations (starting time and oviposition duration), we employed *t* tests. We compared the regression slopes describing the tradeoff of clone- and body-size between the two treatments using Chow test (Belsley et al. 2004).

Results

Some of the females died or escaped before they completed ten ovipositions, or rejected their hosts. This led to a reduced sample size in both high- (n=59 females that completed ten ovipositions) and low-encounter-rate (n=35 females) treatments. Overall, high-encounter females oviposited 9.135±0.238 times (mean±SE), and low-encounter females oviposited 7.433±0.318 times. The proportion of parasitoid clones that completed their development was rather low: Out of 1,447 ovipositions in all treatments combined, only 217 resulted in successful emergence of wasps. Part of the hosts developed into adult moths, although the wasps inserted their ovipositor into them. Some of these hosts may have been rejected by the females after probing (hence received no parasitoid egg), while others may have encapsulated the parasitoid egg and managed to develop normally. Other hosts died, mostly due to fungal infestations. The fraction of hosts that did not yield adult parasitoids did not differ among treatments (χ^2= 1.134, df=2, P=0.567), but further reduced sample sizes.

Clone sizes

Females from the low-encounter and from the age-control treatments produced offspring clones of similar sizes (n=80 and 31 females, respectively). Offspring clone size was also unaffected by maternal age (ANCOVA, $F_{1,110}$=0.153, P= 0.696 for the effect of treatment; $F_{1,110}$=0.393, P=0.532 for the effect of age). We therefore pooled the data obtained from all females in the age-control treatment, since these females differed in age but not in oviposition experience.

The mean clone sizes of offspring that developed in the first host encountered by a female were similar across treatments (Fig. 2, left-most bars; Kruskal–Wallis, χ^2= 0.223, df=2, P=0.895). This finding is not surprising because females from all treatments had identical host encounter experience until their second oviposition. A different pattern emerges when ovipositions 2–10 are compared: Sons of females from the low-encounter treatment (n=80 clones) formed significantly larger clones than sons of females from the high-encounter treatment (n=84 clones; Fig. 2; ANCOVA, $F_{1,164}$=8.191, P=0.005 for the effect of treatment; ANCOVA, $F_{1,164}$=0.076, P=0.783 for the effect of oviposition number). Since oviposition experience did not significantly affect offspring clone size, we next randomly selected one of ovipositions 2–10 from each female and compared offspring clone sizes across treatments. The number of sons per clone varied significantly among treatments (Table 1; ANOVA, $F_{2,114}$=5.25, P=0.007).

Head width

The head width of sons of age-control (n=32) and low-encounter females (n=78) did not differ significantly (ANCOVA, $F_{1,109}$=0.633, P=0.428 for treatment; ANCOVA, $F_{1,109}$=0.017, P=0.898 for female age). Thus, we pooled the data obtained from all females in the age-control treatment, as described previously.

No differences were found in the average head width of sons of females that oviposited for the first time among the three treatment groups (Kruskal–Wallis, χ^2=0.18, df=2, P= 0.914). The average head width of sons of high- and low-encounter-rate females was also not affected by the treatment (ANCOVA, $F_{1,160}$=0.648, P=0.422) nor by the previous number of ovipositions (ANCOVA, $F_{1,160}$=0.045, P=0.833) when ovipositions 2–10 were considered. We compared the same randomly selected oviposition per female as for clone size across treatments. In accordance

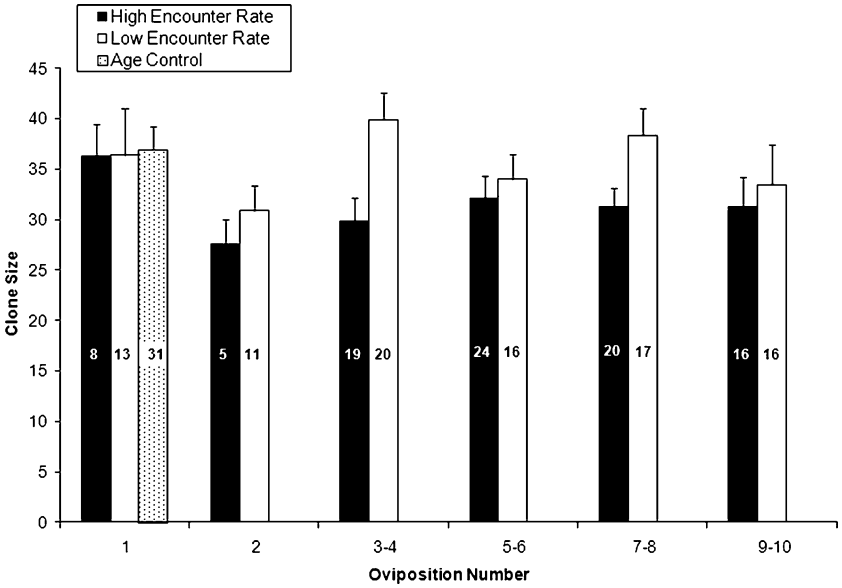


Fig. 2 The effect of host-encounter rates and the number of previous ovipositions on the number of offspring per clone (mean+SE)

with the previous results, offspring body size was not significantly affected by the maternal host-encounter rate (Table 1, $F_{2,111}=0.51$, $P=0.60$). However, clone size and head width were negatively correlated within each treatment (Pearson correlation test, $n=82$, $r=-0.52$, $P<0.001$ for sons of females that encountered hosts at a high rate; $n=78$, $r=-0.56$, $P<0.001$ for sons of females that encountered hosts at a low rate; and $n=31$, $r=-0.78$, $P<0.001$ for sons of age-control females). The correlation remained highly significant also after we pooled the data from all three treatments (Pearson correlation test, $n=191$, $r=-0.58$, $P<0.001$). The coefficients of the correlations between clone size and body size did not significantly differ among treatments (Chow test, $F=0.379$, $P=0.539$).

Development time

Offspring development time was measured from oviposition to pupation. A comparison between the low-encounter ($n=58$ clones) and the age-control ($n=25$ clones) treatments revealed that neither treatment nor maternal age affected the developmental period (ANCOVA, $F_{1,82}=0.283$, $P=0.596$ for treatment; ANCOVA, $F_{1,82}=1.276$, $P=0.262$ for age). Since the age of the female during oviposition did not affect the development time, the data from the age-control treatment were pooled, as was done with the clone size and head width data.

Clones in the low-encounter-rate treatment ($n=58$) had shorter egg-to-pupa development times than in the high-encounter-rate treatment ($n=52$), but not significantly (ANCOVA, $F_{1,109}=3.356$, $P=0.07$). Oviposition number did not significantly affect offspring development time (ANCOVA, $F_{1,109}=0.968$, $P=0.327$). When one oviposition per female (out of ovipositions 2–10) was compared among treatments, no significant differences were found (Table 1, $F_{2,89}=1.98$, $P=0.14$).

Host mass

Hosts mass at pupation did not differ between hosts that were parasitized by low-encounter ($n=35$) and by age-control ($n=13$) females (ANCOVA, $F_{1,47}=0.095$, $P=0.759$

Table 1 The influence of maternal host-encounter rate on offspring developmental parameters

	Experimental treatment		
	High encounter	Low encounter	Age-control
Number of wasps/clone	29.39±1.51 (41)a	36.73±1.93 (45)b	36.97±2.30 (31)b
Offspring head width (mm)	0.457±0.004 (38)	0.457±0.005 (44)	0.463±0.004 (32)
Egg-to-pupation duration (days)	21.9±0.47 (30)	22.29±0.36 (38)	23.21±0.52 (24)
Mass of host mummy (g)	0.0114±0.0004 (21)a	0.0129±0.0005 (19)ab	0.0144±0.0010 (12)b

The data include one randomly selected oviposition, out of ovipositions 2–10, for each mother in the high- and low-encounter-rate treatments. Means with their standard errors are reported. Sample sizes are indicated in parentheses. Different letters indicate significant differences in post hoc tests

for treatment, $F_{1,47}$=2.018, P=0.162 for maternal age). Neither the treatment (ANCOVA, $F_{1,71}$=3.566, P=0.63) nor the oviposition number (ANCOVA, $F_{1,71}$=0.608, P=0.438) affected the pupal mass of hosts parasitized by high- (n=37) and low-encounter-rate (n=35) females. However, the comparison of one oviposition per female across the three treatments revealed significant differences (Table 1, $F_{2,49}$=5.60, P=0.006). These reflected the fact that host mummies in the age-control were significantly heavier than in the high-encounter treatment (P=0.02). The differences in host mass between the high- and low-encounter treatments were marginally significant (P=0.07).

Starting time and oviposition duration

Mean starting time of high-encounter females (n=538) was significantly shorter than that of low-encounter females (n=479; t test, t_{1015}=−3.244, P=0.001). Mean oviposition duration was also lower for high-encounter females (n=540) than for low-encounter individuals (n=477; t test, t_{1015}=−2.038, P=0.042).

Longevity

Longevity of females differed among treatments (Fig. 3, Kaplan–Meier survival analysis: χ_2^2=15.03, P=0.001). Low-encounter-rate females had significantly lower mean longevity than high-encounter-rate females (Mann–Whitney, U=205.5, P=0.04) and age-control females (Mann–Whitney, U=430, P=0.001). High-encounter-rate females did not differ in average life span from age-control females (Mann–Whitney, U=843.5, P=0.472).

Feeding control experiment

Female longevity was reduced in females that were deprived of food (3.5±0.09 days for starved females (n=70) compared with 30±2.08 days for fed females (n=31); t test, t_{99}=−19.221, P<0.001). However, starved and fed females produced clones of similar sizes (32; 26.5–37, n=20 and 36; 27–45.5, n=34 (median and interquartile distance) for sons of starved and fed females, respectively. Mann–Whitney, U=284.5, P=0.319). Moreover, their sons did not differ in head width (0.462; 0.446–0.483 mm, n=20 for sons of starved females; 0.463; 0.445–0.474 mm, n=34 for sons of fed females; Mann–Whitney, U=318.5, P=0.7) and in development time (21; 20–21.5 days, n=21 for sons of starved females; 21; 20–24 days, n=43 for sons of fed females; Mann–Whitney U test, U=408, P=0.742).

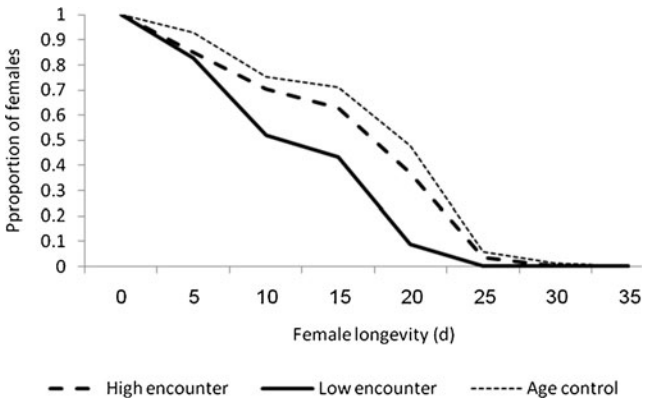


Fig. 3 The effect of mothers' encounter rate with hosts on their longevity

Host dissections

The numbers of hosts with 0, 1, and 2 wasp eggs are reported in Table 2. The number of eggs per host in the second oviposition was independent of the time interval (immediate or 24 h) from the first oviposition (χ_2^2=0.327, P=0.85). The distribution of egg numbers per hosts differed significantly between the first and the second oviposition (data for all second ovipositions were combined, χ_2^2=6.49, P=0.04). This was due to the higher proportions of 0 and 2 eggs per host in the first oviposition compared with the second one.

Discussion

The phenotype of offspring can be highly influenced by the environmental and physiological conditions experienced by their mothers before or during oviposition (Bernardo 1996; Mousseau and Fox 1998a, b). In the present experiment, maternal encounter rates with hosts clearly affected offspring clone size, while their age, oviposition experience, and feeding status had no effect. Interestingly, the epigenetic effects of maternal host-encounter rates on offspring traits differed from our a priori predictions.

Females that encountered hosts every approximately 8 h were predicted to produce larger but fewer offspring than females that encountered hosts consecutively. Development time was predicted to be longer for these offspring, compared with offspring of high-encounter-rate females, as a possible mechanism for attaining a larger adult size. The rationale for this prediction was that large body size can confer competitive and dispersal benefits under resource-limited conditions. For example, large *C. koehleri* males mate sooner than small ones (Morag et al. 2011). Contrary to these predictions, host-encounter rate did not significantly affect offspring body size, and low-encounter females produced more sons per clone than high-encounter females. Furthermore, host-encounter rates did not affect offspring development time. The host dissections exclude the interpretation that the larger broods in the low-

Table 2 The numbers and proportions (in parentheses) of eggs per host recorded in a sample of hosts that were dissected immediately after oviposition. None of the hosts contained more than two parasitoid eggs

Type of oviposition	Number of dissected hosts containing		
	no egg	1 egg	2 eggs
First	45 (0.38)	55 (0.47)	17 (0.15)
Second, immediately after first	4 (0.22)	13 (0.72)	1 (0.06)
Second, 24 h later	10 (0.29)	22 (0.65)	2 (0.06)
Second, both intervals	14 (0.27)	35 (0.67)	3 (0.06)

encounter treatment are due to a higher proportion of two-egg ovipositions than in the high-encounter treatment. The dissections also indicate that two eggs were released more frequently in the females' first oviposition than in the second one, possibly reflecting the wasps' learning of the oviposition technique (Segoli et al. 2009c). The higher frequency of two-egg releases by naive wasps may also account for the somewhat larger broods observed in the first oviposition of the high-encounter wasps compared with later ovipositions (Fig. 2).

Offspring clone sizes were clearly negatively correlated with individual body sizes, but additional factors could influence the offspring's body size as well. Indeed, hosts parasitized by low-encounter-rate females tended to be heavier than hosts parasitized by high-encounter-rate females (Table 1), suggesting that host mass was also somewhat affected by maternal host-encounter rates. Similarly, parasitized larvae of *C. koehleri* and its congener *C. floridanum* attain a higher larval body mass than non-parasitized individuals (Strand 1989; Segoli et al. 2010). Females from the low-encounter-rate treatment may have manipulated the hosts' feeding rates, causing them to reach slightly larger body sizes. Alternatively and perhaps more likely, this manipulation may have been carried out by offspring of low-encounter-rate females (Segoli et al. 2010). The increased host mass may have allowed parasitoids in the low-encounter treatment to grow larger and avoid paying a cost in biomass for their increased proliferation. Similar trends were found in a previous study on *C. koehleri*, where female clones were larger than some of the male clones, with no associated decrease in individual body size (Morag et al. 2011).

Our data suggest that the reproductive output per oviposition of low-encounter females exceeds that of high-encounter females. This raises the question why high-encounter females did not induce a higher proliferation rate in their offspring as well. We speculate that this reflects differences between treatments in maternal resource allocation patterns. *Copidosoma* females do not allocate resources in the form of yolk but may adjust offspring developmental patterns using accessory secretions deposited with the egg or maternal proteins/RNAs. We suggest that females may be limited in these resources, which are required to induce proliferation in their developing offspring. This possibility is supported by the effect of environmental factors, e.g., host age (Corley et al. 2005) and endocrine state (Strand et al. 1991), on embryonic proliferation in the related species *C. floridanum*. Although low-encounter-rate offspring did not pay a price for their large number in the form of reduced body size, their mothers' lifespan was significantly reduced compared with high-encounter-rate mothers (Fig. 3). It could be further speculated that the increased resource allocation to offspring proliferation reduced the longevity of the low-encounter mothers. Thus, increased proliferation perhaps results in a cost, but contrary to our expectation, it may affect mothers rather than offspring. This interpretation is compatible with the life span of age-control females, which was similar to that of high-encounter females. Possibly, those females invested fewer resources in offspring proliferation than low-encounter females, because they only oviposited once. Thus, the creation of large offspring clones, similar in size to those of sons of low-encounter-rate females, did not result in a decrease in their longevity.

This resource allocation interpretation, proposed for a polyembryonic parasitoid, is analogous to the allocation of more eggs to each host (i.e., increased clutch size) under host limitation in monoembryonic gregarious species (Mangel 1987; West et al. 1999). For example, Zaviezo and Mills (2000) found that females of the gregarious endoparasitoid *Hyssopus pallidus* increased their clutches by up to 83% when they experienced a decrease in the encounter rate of hosts. A further example involves the polyembryonic parasitoid wasp *C. floridanum*. Females of this species laid mostly two-egg clutches when they encountered hosts rarely, whereas the frequency of single-egg clutches increased at high host-encounter rates (Ode and Stand 1995). Our resource allocation interpretation is also analogous to foraging theories predicting increased patch exploitation when environmental conditions are poor (Charnov 1976; Stephens and Krebs 1986). Although the number of eggs deposited in each host was fixed in our experiment, maternal resource allocation may have varied under different environmental conditions, such that they invested more resources per host in poor environments. Possibly, the production of larger clones despite the

accompanied cost is due to the low-encounter females' uncertainty regarding future encounters with hosts. High-encounter mothers, on the other hand, may optimize their lifetime fecundity by producing fewer offspring upon each host encounter, since their expectation of future encounters is higher. Thus, they can produce many clutches during their lives and avoid paying a survival price for increased proliferation. According to this interpretation, maternal longevity and offspring proliferation are traded off. The fitness gained by those two strategies (higher proliferation and shorter life span vs. lower proliferation and longer reproductive opportunities) can be compared by measuring the lifetime reproductive output of females that experience different host-encounter rates. This would require an experimental design that does not limit the number of hosts provided to the females.

The differences in the mean starting time and oviposition duration between high- and low-encounter-rate females provide a behavioral indicator that the two treatments were perceived as different by the wasps. Furthermore, perhaps learning is time-limited in this species, meaning that females can better specialize in oviposition and reduce the host handling time if they encounter hosts at a high rate. The reduced handling time by high-encounter females may reflect their better learning of the host-handling technique compared with low-encounter ones, as their memory may rely mainly on short-term experience.

Female age did not influence offspring traits in the present experiment, as opposed to previous studies in insects that have shown that offspring of older mothers had lower egg-to-adult viability (Fox and Dingle 1994; Hercus and Hoffman 2000), higher mortality at infancy (Fox and Dingle 1994; Fox et al. 2003), and a shorter life span (reviewed by Priest et al. 2002; Fox et al. 2003). Possibly because low-encounter and age-control females were no older than 72 h at their last oviposition but lived for a mean of another 9 days after completing it, adverse epigenetic effects due to the females' age were not yet observed.

To summarize, in *C. koehleri*, low host-encounter rate does not induce the production of larger male offspring, but rather the production of more of them. The manipulation of offspring number may allow mothers to overcome the uncertainty regarding future encounters with hosts and simultaneously create offspring that would be able to avoid the harsh conditions. Although our experiment controlled for the number of eggs laid per host, it did not control for additional components of maternal investment. We suggest that the allocation of maternal non-genetic investment may be affected by host-encounter rates and may account for differences in offspring phenotypes and maternal longevity. The mechanism by which mothers affect offspring traits is yet to be found.

Acknowledgments We are grateful to Z. Abramsky, S. Azriel, Y. Keynan, Y. Lubin, I. Malka, M. Kishinevsky, O. Ovadia, M. Segoli, I. Scharf, S. Yehuda, and Y. Zilka for their comments, suggestions, and technical help. This work was supported by The Israel Science Foundation (Grants no. 184/06 and 414/10).

All research reported here was performed in compliance with the Israeli law. The authors declare that they have no conflicts of interest.

References

Berlinger MJ, Lebiush-Mordechi S (1997) The potato tuber moth in Israel: a review of its phenology, behavior, methodology, parasites and control. Trends Entomol 1:137–155

Belsley DA, Kuh E, Welsch RE (2004) Regression diagnostics: identifying influential data and sources of collinearity. Wiley-Interscience, New Jersey, USA

Bernardo J (1996) Maternal effects in animal ecology. Am Zool 36:83–105

Bezemer MT, Mills NJ (2003) Clutch size decisions of a gregarious parasitoid under laboratory and field conditions. Anim Behav 66:1119–1128

Charnov EL (1976) Optimal foraging, the marginal value theorem. Theor Popul Biol 9:129–136

Corley LS, White MA, Strand MR (2005) Both endogenous and environmental factors affect embryo proliferation in the polyembryonic wasp *Copidosoma floridanum*. Evol Devel 7:115–121

Creighton JC (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. Behav Ecol 16:1031–1036

Doutt RL (1947) Polyembryony in *Copidosoma koehleri* Blanchard. Am Nat 81:435–453

Ellers J, van Alphen JJM, Sevenster JG (1998) A field study of size-fitness relationships in the parasitoid *Asobara tabida*. J Anim Ecol 67:318–324

Fox CW, Dingle H (1994) Dietary mediation of maternal age effects on offspring performance in a seed beetle (Coleoptera: Brucidae). Funct Ecol 8:600–606

Fox CW, Waddell KJ, Mousseau TA (1995) Parental host plant affects offspring life histories in a seed beetle. Ecology 76:402–411

Fox CW, Martin JD, Thakar MS, Mousseau TA (1996) Clutch size manipulations in two seed beetles: consequences for progeny fitness. Oecologia 108:88–94

Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. Ann Rev Entomol 45:341–369

Fox CW, Bush ML, Wallin WG (2003) Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*. Funct Ecol 17:811–820

Godfray HCJ (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton (NJ)

Hercus MJ, Hoffman AA (2000) Maternal and grandmaternal age influence offspring fitness in *Drosophila*. Proc Roy Soc Lond B 267:2105–2110

Honek A (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66:483–492

Ives AR (1989) The optimal clutch size of insects when many female oviposit per patch. Am Nat 133:671–687

Jann P, Ward PI (1999) Maternal effects and their consequences for offspring fitness in the Yellow Dung Fly. Funct Ecol 13:51–58

Jervis MA, Heimpel GE, Ferns PN, Harvey JA, Kidd NAC (2001) Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. J Anim Ecol 70:442–458

Kazmer DJ, Luck RF (1995) Field tests of the size-fitness hypothesis in the egg parasitoid *Trichogramma pretiosum*. Ecology 76:412–425

Keasar T, Segoli M, Barak R, Steinberg S, Giron D, Strand MR, Bouskila A, Harari AR (2006) Cost and consequences of superparasitism in the polyembryonic parasitoid *Copidosoma koehleri* (Hymenoptera: Encyrtidae). Ecol Entomol 31:277–283

Kfir R (1981) Fertility of the polyembyonic parasite *Copidosoma koehleri*, effect of humidities on life length and relative abundance as compared with that of *Apanteles subandinus* in potato tuber moth. Ann Appl Biol 99:225–230

Lardies MA, Carter MJ, Bozinovic F (2004) Dietary effects on life history traits in a terrestrial isopod: the importance of evaluating maternal effects and trade-offs. Oecologia 138:387–395

Mangel M (1987) Oviposition site selection and clutch size in insects. J Math Biol 25:1–22

Mayhew PJ, Glaizot O (2001) Integrating theory of clutch size and body size evolution for parasitoids. Oikos 92:372–376

Morag N, Bouskila A, Segoli M, Rapp O, Keasar T, Harari AR (2011) The mating status of mothers, and offspring sex, affect clutch size in a polyembryonic parasitoid wasp. Anim Behav 81:865–870

Mousseau TA, Fox CW (1998a) The adaptive significance of maternal effects. Trends Ecol Evol 13:403–407

Mousseau TA, Fox CW (1998b) Maternal effects as adaptations. Oxford University, Oxford (NY)

Ode PJ, Stand MR (1995) Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. J Anim Ecol 64:213–224

Priest NK, Mackowiak B, Promislow DEL (2002) The role of parental age effects on the evolution of aging. Evolution 56:927–935

Richards EJ (2006) Inherited epigenetic variation—revisiting soft inheritance. Nature Rev Genetics 7:395–401

Rosenheim JA, Hongkham D (1996) Clutch size in an obligately siblicidal parasitoid wasp. Anim Behav 51:841–852

Santos M, Fowler K, Partridge L (1994) Gene–environment interaction for body size and larval density in *Drosophila melanogaster*: an investigation of effects on development time, thorax length and adult sex ratio. Heredity 72:515–521

Segoli M, Bouskila A, Harari AR, Keasar T (2009a) Developmental patterns in the polyembryonic parasitoid wasp *Copidosoma koehleri*. Arth Struct Devel 38:84–90

Segoli M, Harari AR, Bouskila A, Keasar T (2009b) Brood size in a polyembryonic parasitoid wasp is affected by relatedness among competing larvae. Behav Ecol 20:716–767

Segoli M, Harari AR, Bouskila A, Keasar T (2009c) Host handling time in a polyembryonic wasp is affected both by previous experience and by host state (parasitized or not). J Insect Behav 22:501–510

Segoli M, Bouskila A, Harari A, Keasar T (2010) The effect of host starvation on parasitoid brood size in a polyembryonic wasp. Evol Ecol Res 12:1–9

Solbreck C (1995) Variable fortunes in a patchy landscape—the habitat template of an insect migrant. Res Popul Ecol 37:129–134

Strand MR (1989) Development of the polyembryonic parasitoid *Copidosoma floridanum* in *Trichoplusia ni*. Entomol Exp Appl 50:37–46

Strand MR (2003) Polyembryony. In: Carde R, Resch V (eds) Encyclopedia of insects. Academic, San Diego, pp 928–932

Strand MR, Baehrecke EH, Wong EA (1991) The role of host endocrine factors in the development of polyembryonic parasitoids. Biol Cont 1:144–152

Stephens DW, Krebs JR (1986) Foraging theory. Princeton University, Princeton (NJ)

West SA, Flanagan KE, Godfray HCJ (1999) Sex allocation and clutch size in parasitoid wasps that produce single-sex broods. Anim Behav 57:265–275

Zaviezo T, Mills N (2000) Factors influencing the evolution of clutch size in a gregarious insect parasitoid. J Anim Ecol 69:1047–1057