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# The effect of host starvation on parasitoid brood size in a polyembryonic wasp

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## ABSTRACT

**Hypothesis:** Offspring of polyembryonic parasitoid wasps (in which each egg divides clonally to produce several individuals inside a host body) adjust their numbers according to the host carrying capacity.

**Organism:** The polyembryonic parasitoid wasp, *Copidosoma koehleri*, parasitizes the potato tuber moth, *Phthorimaea operculella*.

**Methods:** We starved parasitized host larvae during the wasp embryonic division phase. We recorded host mass and the number of wasps in sub-samples of dissected hosts throughout development and upon pupation and emergence.

**Results:** Starvation significantly reduced larval host mass but this was largely compensated at the pupal stage, probably through delayed pupation. Starved hosts tended to harbour fewer wasps but this effect was marginally non-significant.

**Conclusions:** Wasp offspring do seem to adjust their numbers in response to host starvation, but not strongly.

*Keywords*: brood size, *Copidosoma koehleri*, host quality, host starvation.

## INTRODUCTION

Brood size has important implications for the fitness of both parents and offspring. Since resources for the production and care of offspring are often limited, offspring number is traded-off with quality (Lack, 1947). Thus, parents often adjust the number of offspring that they produce to the carrying capacity of their immediate or anticipated environment. Examples of this phenomenon include, among others, butterflies that adjust clutch size to the quality of the host plant (Bergstrom *et al.*, 2006; Kagata and Ohgushi, 2002), burying beetles that adjust clutch size to the size of the carcass (Nagano and Suzuki, 2007), and Siberian jays that reduce clutch size in an environment perceived as risky (Eggers *et al.*, 2006).

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The trade-off between the number of offspring and their quality may be especially pronounced in parasitoids, where the offspring develop on a limited amount of resources, i.e. the body of the host (Godfray, 1994). Parasitoids show a very high variation in brood size, both within and among species. In solitary parasitoids, only one individual emerges from each host, while in gregarious parasitoids, all larvae within a host can complete development and emerge (Mayhew and Glaizot, 2001). As expected, females of gregarious species often adjust the number of eggs they lay to the quality of the host [e.g. host species (Godin and Boivin, 2000), size (Reitz and Adler, 1995; Wang *et al.*, 2008), developmental stage (Sato *et al.*, 1986; Chong and Oetting, 2006), and the risk of superparasitism (Pexton and Mayhew, 2005)], laying larger clutches in higher quality hosts (reviewed by Godfray, 1994).

In some cases, however, female parasitoids may be limited in their ability to assess the quality of the host. For example, in koinobiont parasitoids, where the host continues to develop after parasitism, the amount of resources that will be available to the developing offspring largely depends on the amount of food that will be consumed by the host after parasitism (Harvey, 2005; Strand and Casas, 2008). In addition, offspring may have to compete for resources with other larvae inside the host in the case of later superparasitism (for example, parasitism by additional females). As a consequence, a female may face difficulties in predicting the host's final carrying capacity, and adjust her clutch size accordingly. One possible adaptation for this limitation is to pass part of the control over brood size to the offspring themselves (Craig *et al.*, 1997).

An example for possible sharing of control over brood size may come from polyembryonic parasitoid wasps, in which many genetically identical embryos develop from each egg. In the process of polyembryonic development, a primary cell mass (morula) proliferates to create a mass of many developing embryos (polymorula). Proliferation continues until wasp embryos differentiate into active larvae at the final stages of host development. These larvae consume the host tissues, pupate, and emerge as adult wasps (Grbic *et al.*, 1998; Strand, 2003; Segoli *et al.*, 2009a). While the mother wasp may control the number of eggs to be laid, offspring may have the potential to further adjust brood size by controlling the degree of proliferation during embryonic stages. Low levels of proliferation, producing broods that are too small, may be disadvantageous due to the inability of the developing larvae to consume all of the host tissue and to emerge from the host. On the other hand, large brood sizes may have a negative effect on individual body size and consequently on its fitness (Ode and Strand, 1995). Thus, the adjustment of brood size to the host carrying capacity is very important for the fitness of individuals in the brood. Since all wasps that develop from one egg are genetically identical, rivalry between them is expected to be minimal [kin-selection theory (Hamilton, 1963, 1964)]. High cooperation between offspring may enhance host utilization and may result in a brood size that is optimal for both the mother and her offspring (Godfray, 1994).

In this study, we tested the hypothesis that embryos adjust the level of clonal-proliferation according to the body condition of the host, in the polyembryonic wasp, *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidea). We manipulated body condition by starving hosts during the wasp proliferative phase. Host starvation was previously shown to affect parasitoid survival, development, and body size (Beckage and Riddiford, 1983; Harvey *et al.*, 1995). In a study on the polyembryonic wasp *C. floridanum*, host starvation for 48 h substantially decreased host mass and the number of wasps emerging from the host (Giron *et al.*, 2004). It is unclear, however, whether this decrease was due to increased embryonic mortality or reduced proliferation. To address this, we dissected samples of hosts at fixed time intervals

following starvation and counted the number of developing wasps inside the hosts. Similar data were recorded for non-starved parasitized hosts that served as controls. We predicted that if embryos adjust proliferation according to host condition, starvation would reduce the number of embryos, larvae, and adults produced.

## METHODS

### Study organism

*Copidosoma koehleri* Blanchard is a polyembryonic parasitoid wasp that parasitizes the potato tuber moth (*Phthorimaea operculella* Zeller, Gelechiidae, Lepidoptera), and is used as a biological control agent of this pest (Horne, 1990; Kfir, 2003). The adult female lays her eggs into the moth egg. Females usually lay one egg per oviposition event, but superparasitism by the same female or by different females is common (Doutt, 1947; Keasar *et al.*, 2006). The moth larva hatches and develops, while the wasp egg divides clonally to produce many embryos. Proliferation occurs mainly during the first and second larval instars of the host, and normally ceases within 10 days of parasitism (when reared at 29°C), as the host enters the third instar. Wasp embryos develop into larvae, which consume the entire host tissues before pupation. Female clones produce more embryos than male clones (40–50 vs. 30–40 individuals, respectively). The wasp life cycle takes about a month and is highly synchronized with that of the host (Segoli *et al.*, 2009a). In several polyembryonic species, a proportion of embryos develop into specialized soldier larvae that attack competitors inside the host (Cruz, 1981, 1986; Giron *et al.*, 2004). In *C. koehleri*, dissections of developing hosts indicate that each female clone contains one female soldier. Male clones, on the other hand, do not produce soldiers (Doutt, 1952; Keasar *et al.*, 2006; Segoli *et al.*, 2009a).

A laboratory stock of *C. koehleri* was used in the experiment. The stock originated from field-collected individuals from South Africa (courtesy of Dr. R. Kfir, Plant Protection Institute, Pretoria). Parasitoids were housed at 27°C, under natural daylight, and fed with honey. A laboratory stock of potato tuber moth (PTM) was housed at 27°C, under natural daylight, and fed with honey and water. The PTM eggs were collected daily and were used within 24 h, since the age of the eggs is known to influence the parasitoids' oviposition decisions (Ode and Strand, 1995). Wasps were used within 3 days of emergence.

### Starvation experiment

To avoid bias in the number of developing wasps due to the sex of the clone or the presence of a soldier larva, we used virgin females that produce male clones only. To reduce genetic variability among embryos within a brood, hosts were singly parasitized. We placed one PTM egg at the centre of a Petri dish. We then introduced a female to the plate and directed her towards the host. This was done by rotating the Petri dish while holding it vertically, using the tendency of wasps to walk upwards on vertical surfaces. Females that touched the host with their antennae normally started ovipositing. The female was removed immediately after oviposition. We added a slice of potato to each Petri dish and incubated it at 29°C.

Parasitized hosts were randomly assigned to two treatments: control and starvation. Hosts of the starvation treatment were starved on the eighth day after parasitism, during the proliferative phase of the parasitoid larvae (Segoli *et al.*, 2009a). Each moth larva from this

sample was removed from the potato on the eighth day and kept in a Petri dish with a wet cotton ball. Approximately 24 h later, the moth larvae were returned to individual Petri dishes and were provided with a slice of potato. A group of parasitized moth larvae that were not starved served as a control.

Samples of hosts from each treatment were dissected at 2-day intervals following the timing of starvation (days 10, 12, and 14 post-parasitism). At each dissection we recorded host mass and counted the number of developing wasps inside the host. The remaining host larvae were kept until wasp pupation and emergence. Hosts were checked daily and the timing of wasp pupation, together with the mass of mummy at wasp pupation (including all the wasps that pupated inside it), were recorded. Mummy mass at wasp pupation may represent the carrying capacity of the host, since at this stage all of the resources accumulated by the host during its growth are allocated to the wasp progeny. Following emergence, we counted the number of wasps per brood, and measured the head width of five wasps per host using the integrated Soft Imaging Software (SIS) image analysis package (Soft Imaging Software, GmbH, Münster, Germany). The experimental design is shown in Fig. 1.

Some of the hosts ($N = 36$) that developed on potatoes were apparently non-parasitized (no developing wasps were found inside the host at dissection, or the larva developed into a moth). These hosts were removed from the data set. Thus, we were able to obtain data from 57 hosts of the control treatment ($n = 6$ for day 10, $n = 14$ for day 12, $n = 18$ for day 14, and $n = 19$ that pupated), and 52 hosts of the starvation treatment ($n = 12$ for day 10, $n = 11$ for day 12, $n = 13$ for day 14, and $n = 16$ that pupated). In several cases, we failed to obtain measures of variables such as host mass, wasp number or time to pupation; thus, accordingly, samples were further reduced.

## Statistical analyses

We used two-way analysis of variance (ANOVA) to test the effects of the starvation treatment and the developmental stage on host mass and on the number of wasps per host. We used one-way ANOVA to test the effect of the starvation treatment on the time until pupation, and on the size of emerging wasps. We used linear regression to test the relationships between number of wasps per host and host mass at different developmental stages for control and starved hosts combined. Similarly, we used linear regression to test the relationships between the number of emerging wasps and wasp size, for control and starved hosts combined.

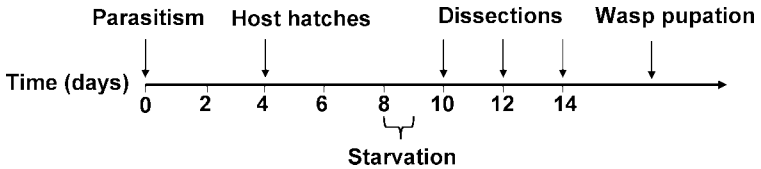


**Fig. 1.** Experimental design: the timing of host starvation and dissections. Starvation was conducted over 24 h starting at day 8 after parasitism and a sample of host larvae from this treatment was dissected on days 10, 12, and 14. A sample of control hosts was dissected at days 10, 12, and 14. In addition, a sample from each treatment was kept until pupation and emergence.

## RESULTS

### The effect of starvation on host mass

The mass of parasitized hosts was significantly affected both by starvation and by timing after parasitism (including days 10, 12, 14, and upon pupation) (Treatment: $F_{1,94} = 11.4$, $P = 0.001$; Day: $F_{3,94} = 40.1$, $P < 0.001$; Treatment × Day: $F_{3,94} = 2.6$, $P = 0.06$; two-way ANOVA) (Fig. 2). Mass of hosts increased throughout development and decreased again for mummified hosts, probably due to the loss of water at this stage. The negative effect of starvation on host mass was consistent through all developmental stages, but its magnitude decreased towards the final stage.

### The effect of starvation on the number of wasps

There was a trend for starved hosts to contain fewer wasps than control hosts. In addition, the number of wasps was significantly affected by the day after parasitism (Treatment: $F_{1,92} = 3.1$, $P = 0.08$; Day: $F_{3,92} = 10.2$, $P < 0.001$; Treatment × Day: $F_{3,92} = 0.4$, $P = 0.74$; two-way ANOVA) (Fig. 3). The day effect was probably due to the larger number of wasps upon emergence than found at dissections.

### The effect of starvation on the timing of pupation

Wasps developing in starved hosts pupated later than those developing in control hosts (one-way ANOVA: $F_{1,29} = 4.8$, $P = 0.04$; 17.9 ± 0.6 days for control hosts and 18.5 ± 0.8 days for starved hosts; mean ± S.D.).

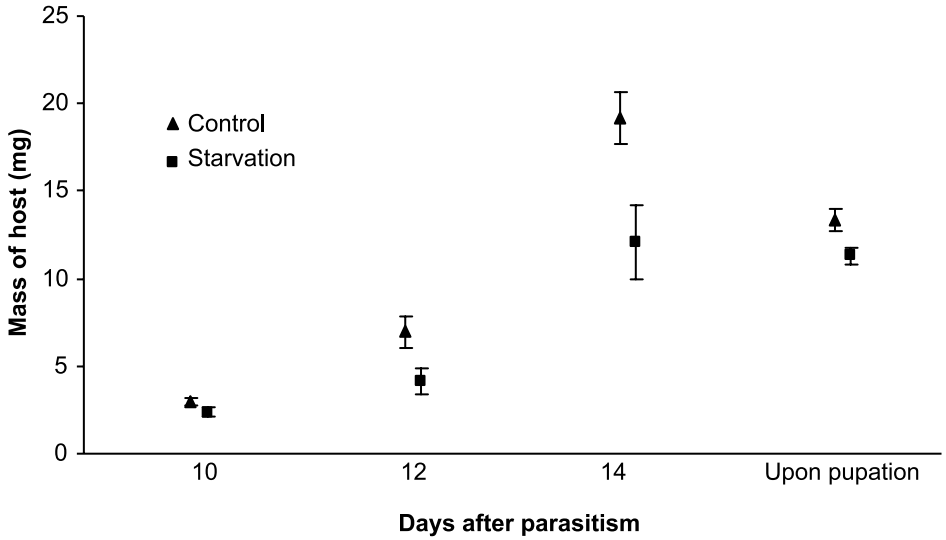


**Fig. 2.** Mass of control (triangles) and starved (squares) hosts (mean ± S.E.). Starvation was conducted over 24 h starting at day 8 after parasitism. Measurements were taken at 2-day intervals following host starvation (days 10, 12, and 14) and upon wasp pupation.

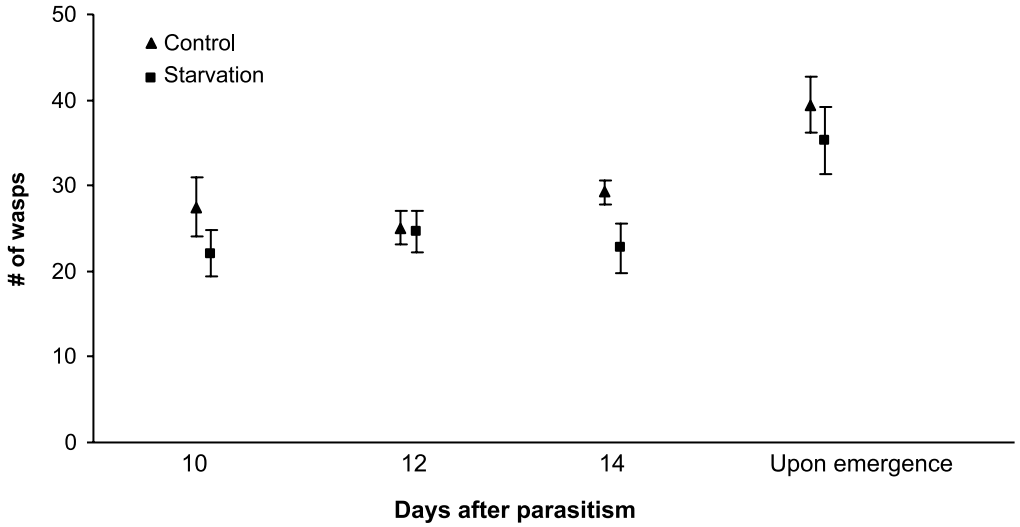


**Fig. 3.** Wasp brood size inside control (triangles) and starved (squares) hosts (mean ± S.E.). Starvation was conducted over 24 h starting at day 8 after parasitism. Measurements were taken by dissecting hosts at 2-day intervals following host starvation (days 10, 12, and 14) and upon wasp emergence.

### The effect of starvation on the size of emerging wasps

Starvation had no significant effect on the head width of emerging wasps (one-way ANOVA: $F_{1,29} = 0.57$, $P = 0.46$; 459 ± 26 $\mu$m for control hosts and 450 ± 40 $\mu$m for starved hosts; mean ± S.D.).

### The effect of host mass on the number of wasps

Pooling control and starved hosts, we found no significant relationship between host mass and the number of developing wasps per host at any timing of dissection (linear regression, Day 10: $n = 11$, $R^2 = 0.06$, $P = 0.33$; Day 12: $n = 23$, $R^2 = 0.05$, $P = 0.96$; Day 14: $n = 30$, $R^2 = 0.04$, $P = 0.15$). We found a marginally non-significant positive relationship between mass of mummy and the number of emerging wasps (linear regression, number vs. mass: $n = 31$, $R^2 = 0.09$, $P = 0.06$). Furthermore, we found a significant negative relationship between the number of emerging wasps and wasp head width (linear regression, $n = 31$, $R^2 = 0.42$, $P < 0.001$).

## DISCUSSION

Polyembryonic development was suggested to represent a counter-adaptation for parents' inability to predict the future carrying capacity of their offspring's environment (Craig *et al.*, 1997). One prediction that emerges from this hypothesis is that the offspring are able to adjust their numbers according to environmental cues they experience during development. In this study, we tested whether offspring of the polyembryonic parasitoid wasp *C. koehleri* adjust their numbers to the body condition of the host. The results did not strongly support our predictions: although starvation had a significant effect on host mass throughout development, it only had a minor effect on the number of wasps per host.

Starved hosts tended to contain fewer wasps than control hosts, but this effect was not significant and was only apparent at some of the developmental stages (e.g. days 10 and 14). This may in part be explained by the relatively small sample size resulting in a low power of the test (power = 0.42 for the effect of treatment), but nevertheless suggests that the effect of starvation on the number of wasps is less strong than its effect on host mass. In addition, there was no significant relationship between host mass and the number of wasps per host during development. This may suggest that embryos of *C. koehleri* cannot fully assess, or do not strongly respond to, the host's body condition.

Although there was no relationship between brood size and host mass during development, we found a marginally non-significant positive relationship between the final carrying capacity of the host (mummy mass) and the number of emerging wasps. This positive relationship, although weak, may indicate that wasps affect the carrying capacity of the host according to their numbers, rather than adjusting their numbers to the host. Indeed, gregarious parasitoids are known to increase consumption by their hosts, resulting in a larger mass for parasitized versus non-parasitized hosts (Slansky, 1986). There are several examples for increased body mass by hosts parasitized by *Copisodoma* species [e.g. *C. floridanum* (Strand, 1989); *C. bakeri* (Byers *et al.*, 1993); *C. koehleri* (Segoli *et al.*, 2009a)]. Moreover, in *C. koehleri* there is evidence that host mass increases according to the number of developing wasps in their body, as hosts parasitized twice (containing two wasp clones) are heavier than hosts parasitized once (Segoli *et al.*, 2009b). This effect may reduce the adaptive value of brood size adjustment at earlier stages.

The combined results suggest that hosts largely compensated for the loss of mass due to starvation: at days 12 and 14 starved hosts weighed ~40% less than control hosts compared with only ~15% less upon pupation. In accordance, starvation had no effect on the size of the emerging wasps. Delayed pupation might have allowed starved hosts to compensate for the loss of mass by increased consumption. It is possible that a stronger treatment [such as longer starvation, as applied by Giron *et al.* (2004)] might have had a larger impact on host carrying capacity than observed in our study. However, the proliferation phase of *C. koehleri* occurs mostly during the host second instar (Segoli *et al.*, 2009a). At this stage, host larvae rarely survive starvation for more than 24 h (M. Segoli, personal observation). Thus, it seems that longer starvation is not relevant at the proliferation phase of this wasp. Other manipulations might elicit a stronger response through proliferation levels. For example, the quality of the host diet, rather than starvation, could perhaps provide reliable information on the future carrying capacity of the host (Ode, 2006). Finally, in species with larger broods, in which the proliferation phase is longer [e.g. *C. floridanum* (Strand, 2003)], offspring may better adjust their numbers to the conditions inside the host.

One puzzling result was that the number of wasps per host at emergence was larger than their numbers at day 14. Such a result was unexpected and was not observed in a previous study (Segoli *et al.*, 2009a), although the trend was similar. There are two possible explanations for this result: first, we might have overlooked some of the larvae during dissections, but this is unlikely because at day 14 larvae are relatively large and conspicuous; second, smaller broods were perhaps less probable to survive to emergence (Ode and Strand, 1995). Unfortunately, we did not keep a record of hosts that died before the final stage, as it was often impossible to find their remains in the potato to determine the stage at which they perished.

In summary, our results do not strongly support the hypothesis that offspring of the polyembryonic wasp *C. koehleri* adjust their number according to the body condition of their host. However, the results suggest that the applied starvation manipulation was not

a strong indicator of the host carrying capacity. The observed trend for smaller broods in starved hosts suggests that offspring have some ability to adjust their numbers to their immediate environment. This ability should be further investigated using additional manipulations of host quality and other polyembryonic species.

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