

SHORT COMMUNICATION

Trans-generational effects of maternal rearing density on offspring development time in a parasitoid wasp

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Abstract. Maternal pre-reproductive experience can impose phenotypic changes on offspring traits. These modifications may result from physiological constraints, although they can also increase the adaptation of offspring to their anticipated environment. Distinguishing between the two interpretations is often difficult. The effects of virgin female rearing density on their longevity and the characteristics of their male offspring are explored in the polyembryonic parasitoid wasp *Copidosoma koehleri* (Blanchard) (Encyrtidae: Hymenoptera). High rearing density may adversely affect maternal physiology or, alternatively, act as a cue for anticipated competition during the lives of the mothers and their offspring. Male offspring of group-reared females reach pupation significantly sooner than male offspring of females reared alone. This accelerated development may provide an advantage when competition from superparasitising individuals is expected. The lifespan of high-density females is longer than that of singly-reared females, and their male offspring survive longer, suggesting that crowded rearing does not reduce the fitness of females or offspring. The shortened development time of male offspring may reflect an adaptive epigenetic response to predicted competitive conditions.

Key words. Competition, *Copidosoma koehleri*, development time, maternal effects, polyembryony.

Introduction

The environment experienced by mothers can influence many of the characteristics of their offspring, such as survival, growth rates, competitive abilities and evasion of predators. Such maternal effects can be mediated by adjustment of the amount, composition and timing of nutrients or hormones provided to the developing offspring (Bernardo, 1996; Mousseau & Fox, 1998). At the molecular level, epigenetic trans-generational effects can be brought about by direct covalent modification of the offspring genome, often expressed by methylation of the nucleotides in the gametes (Richards, 2006).

Maternal effects are often viewed as evolutionary optimisation processes that increase the adaptation of offspring to future environments (Bernardo, 1996; Mousseau & Fox, 1998; Rotem *et al.*, 2003). These effects may also be interpreted as responses to constraints imposed by the mother's physiological condition

(Mousseau & Fox, 1998; Moore & Harris, 2003). The effects of population density experienced by the mother on offspring phenotype illustrate the difficulty of disentangling adaptive interpretations from explanations involving constraints. The adaptive interpretation may consider high population density as a cue for potential future intraspecific competition among offspring over resources, such as food, mates or oviposition sites (Visser, 1996; Pexton & Mayhew, 2005; Allen *et al.*, 2008). A game-theory model predicts that clutch size should be adjusted to the type of competition expected during the progeny's development (Ives, 1989). In species with scramble competition, where resources are divided among several competitors (whether siblings or non-kin), small clutches are predicted (Pexton & Mayhew, 2005). This is because the production of fewer offspring increases the amount of food available to each of them, their final body size and hence their individual fitness (Honek, 1993). Thus, the production of fewer, although larger, offspring can increase clutch fitness. A test of this prediction is supported in several species of Hymenoptera and Lepidoptera, as well as in vertebrates (Visser, 1996; Pexton & Mayhew, 2005; Goubault *et al.*, 2007;

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Leips *et al.*, 2009). Under contest competition, where only one individual monopolises the resources, a female should be selected to produce large clutches. This improves the chances for one of her offspring to win the competition (Ives, 1989; Visser, 1996; MacKauer & Chau, 2001). In agreement with this prediction, the parasitoid wasp *Comperiella bifasciata* produces multiple egg-clutches in the presence of conspecifics, although only one individual eventually survives within each host (Rosenheim & Hongkham, 1996).

Alternatively, high population density may be viewed as a factor that reduces the reproductive potential of females. This can cause them to produce fewer, smaller and/or lower-quality offspring, or to suffer increased mortality, compared with mothers reared at low density. Examples for adverse, non-adaptive, effects of adult crowding include reduced longevity and fertility in the moth *Chilo partellus* (Hari *et al.*, 2008), and decreased fecundity and egg viability, accompanied by elevated egg cannibalism, in ladybird beetles (Mishra & Omkar, 2006).

The present study tests how crowding of adult virgin female parasitoids affects their life history, as well as that of their male progeny, in light of the above interpretations. Accordingly, phenotypic differences between sons of females that experience different densities pre-oviposition are studied in the wasp *Copidosoma koehleri* (Blanchard). In this polyembryonic parasitoid, several genetically identical embryos develop clonally from a single egg (clone mates). Clone members (all individuals that arise from a single egg) share the host's tissues during their larval development. Superparasitism in *C. koehleri* becomes more frequent as parasitoid density increases (Keasar *et al.*, 2006). Male embryos of different clones probably experience scramble competition when superparasitising a host because they lack morphological and behavioural adaptations to eliminate each other, and eventually share host resources (Segoli *et al.*, 2009).

If the adaptive interpretation described above is correct, high population density during pre-oviposition indicates a high risk of superparasitism (scramble competition conditions) for developing offspring. Females under these conditions are predicted to produce smaller clones, whose body size is larger than that of singly-raised females (Ives, 1989). If the constraint interpretation is correct, lower survival in high-density mothers compared with low-density mothers is anticipated, as well as lower egg-to-adult survival of offspring of high-density mothers. Because both interpretations may be applicable, their predictions are not mutually exclusive.

Copidosoma koehleri is a suitable model for studying maternal effects after crowding as a result of several life-history features. First, maternal care in this species, as in other parasitoids, is limited to host selection. It is therefore easy to manipulate the maternal pre-oviposition environment, whereas developmental conditions for offspring are kept constant. Second, by using a polyembryonic species, parent-offspring conflict regarding optimal clutch size is minimised as a result of the genetic identity between the siblings (Segoli *et al.*, 2009). Thus, the observed clutch sizes are expected to maximise both maternal and offspring fitness, rather than presenting a compromise between the fitness interests of the mother and her

offspring. Finally, adult females emerge synchronously but later disperse to search for hosts. Thus, they experience both high and low densities of conspecifics under natural conditions.

Materials and methods

Study species and rearing conditions

Copidosoma koehleri is an egg-larval pro-ovigenic parasitoid of the potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). An insectary stock of *C. koehleri* was initiated from a field collection from South Africa in 2003. The *P. operculella* stock originated from repeated field collections in Israel during 2002 and 2003. Parasitoids and their hosts were reared using modifications on established protocols (Berlinger & Lebiush-Mordechi, 1997). Throughout the experiment, *C. koehleri* and moth larvae were maintained under an LD 12 : 12 h photocycle at 27 °C and 40% relative humidity.

Experimental design

Virgin females that originated from 46 all-female unrelated clones were allocated to two treatment groups within 24 h of emergence. For the first, high-density treatment, 25 females from each clone were randomly selected and housed together in a single test tube (13 × 100 mm). Five other females from each clone were randomly allocated to the low-density treatment group, and each was housed alone in a test tube of the same size. Females of both treatments were supplied with food (honey) without restriction. After 48 h, the five low-density females and five of the high-density females were each introduced to a different host egg, and were allowed a single ovipositor insertion assessed by direct observation. Because *C. koehleri* females release a single egg per oviposition (Keasar *et al.*, 2006), this corresponded to one egg, at most, laid per host. The experimental design is shown schematically in Fig. 1.

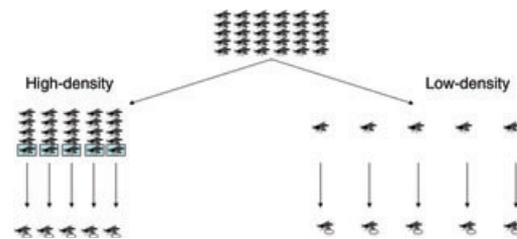


Fig. 1. Experimental design. Female *Copidosoma koehleri* from single clones were assigned to the high-density treatments (25 wasps housed together for 48 h) or to the low-density treatment (five wasps, each housed individually for 48 h). Five individuals from the high-density treatment, and each of the wasps from the low-density treatment, were allowed a single ovipositor insertion into a host egg. Maternal and offspring life-history traits were recorded. The experiment was replicated with females from 46 clones.

The parasitised hosts were reared until emergence of adult insects. The emergence of parasitoids was scored as successful parasitism. The emergence of an adult host indicated that the host was rejected after it was probed by the wasp or that oviposition did occur but offspring died during either the egg, larval or pupal stage. Parasitised hosts were weighed at parasitoid pupation using a Sartorius (Germany) CP224S balance (accuracy ± 0.1 mg). Wasps pupate within the host's cuticle, after fully exploiting the host's internal tissues as the only food source; hence, host mass at this time point provides a measure of the consumed biomass. The aim of weighing was to test whether wasp larvae from the two treatments induced differential growth in their hosts. Both parasitoid pupation and adult emergence dates were documented. Head widths of five individuals from each wasp clone were measured using the software QCAPTURE PRO as an indicator of parasitoid body size (Segoli *et al.*, 2009).

Maternal survival

To test for the effect of maternal rearing density on adult survival, the lifespan of females reared either alone or in a group was documented. Lifespan was defined as the number of days from adult emergence to death. One hundred and thirty-two females that were reared alone, and nine groups of 25 individuals each, were supplied with honey. The wasps were not exposed to hosts, aiming to eliminate any possible effect of oviposition on longevity. The number of dead individuals was recorded daily.

Statistical analysis

The recorded data did not meet the assumptions of parametric tests. Accordingly, medians and interquartile ranges are reported as descriptive statistics, and nonparametric tests were used to compare between treatments. Differences in offspring development durations were tested using Fisher's exact test. Mann-Whitney *U*-tests were used to examine the effects of density treatment on host mass at offspring pupation, time until offspring pupation, the number of individuals per clone and their size. A Cox proportional hazard model

was used to compare the longevity of mothers belonging to both treatment groups, employing the S-PLUS software (<http://spotfire.tibco.com/Products/S-Plus-Overview.aspx>). The calculation of median head width per density treatment (with the associated interquartile range) is based on the mean head width of each of the clones.

Results and Discussion

Survival of offspring and mothers

In 41.1% of the 129 hosts, parasitoids reached adulthood and emerged through the host cuticle. The rest (58.9%) of the hosts died as eggs, larvae or pupae, or developed into adult moths. Hosts that developed into moths may have encapsulated the parasitoids or were possibly rejected by the wasps after ovipositor insertion (i.e. may have not been parasitised initially). This relatively low proportion of parasitoid development is compatible with the low egg-to-pupa survival rates of unparasitised hosts (<50%) observed in a previous study (Keasar & Steinberg, 2008). The egg-to-adult survival of high-density offspring (born to females raised in groups) (51.5%) was significantly higher than that of low-density offspring (30.2%) (Fisher's exact test: $P = 0.02$). The longevity of mothers reared at high density (LT₅₀: 5 days, median and interquartile range: 5 ± 1 days, $n = 245$) was also significantly higher than that of females reared under low-density conditions (LT₅₀: 4 days, median and interquartile range: 4 ± 1 days, $n = 132$) (Cox proportional hazard model: likelihood ratio test = 2.78, d.f. = 1, $P = 0.048$).

Offspring traits

Table 1 compares offspring traits between the two rearing density treatments. Offspring clones of low-density mothers were similar in number to clones from high-density females. Offspring mean head width was not affected by maternal rearing density. Moreover, no significant differences were found in the mass of hosts parasitised by low-density and high-density females after the parasitoids had pupated within the host cuticle. Clones of sons of high-density females pupated

Table 1. Characteristics of male offspring produced by high- and low-density reared female *Copidosoma koehleri* and effect on host biomass after parasitoid pupation.

Parameter	High-density treatment			Low-density treatment			Significance of difference (Mann-Whitney)
	Median	Interquartile range	<i>n</i>	Median	Interquartile range	<i>n</i>	
Number wasps/clone	27	24.5–32.5	27	33	28.5–38	20	$U = 185.5, P = 0.069$
Head width (mm)	0.4843	0.4737–0.4979	27	0.4705	0.4546–0.4947	20	$U = 376.5, P = 0.84$
Host mass (g) after parasitoid pupation	0.0137	0.0116–0.0168	28	0.016	0.0122–0.0176	18	$U = 202.5, P = 0.265$
Egg-to-pupa development time (days)	19	18–19.5	24	20	19–21	11	$U = 67.5, P = 0.02$
Pupa-to-adult development time (days)	13	12.5–13	30	13	12–13	13	$U = 182, P = 0.744$

significantly sooner than sons of low-density females. There were no differences in the time from pupation to emergence of wasp offspring from the two maternal rearing density treatments.

High population density could indicate to parasitoid mothers that their offspring may experience competition during embryonic development as a result of superparasitism (Visser, 1996). Theoretical considerations predict a decrease in offspring clutch size, and an associated increase in body size, as an adaptive maternal response to anticipated scramble competition (Ives, 1989). This response is expected even if the anticipated competition between clones of offspring does not occur (as in the present study). By contrast to this prediction, maternal rearing density does not affect the number of wasps per offspring clone, or their body size. However, the duration of larval development is shortened after maternal high-density rearing. Unexpectedly, although faster development usually results in smaller body size (Honek, 1993), sons of high-density females are not smaller than sons of low-density females.

Accelerated larval development can be regarded as a strategy to avoid expected competition, instead of confronting it. Fast-growing embryos may be able to obtain a larger share of the host resources during development compared with slower-growing competitors (Blanckenhorn, 1998; Metcalfe & Monaghan, 2001). Faster development can also reduce the time window available for other individuals to superparasitise the host. Depending on whether the wasp egg or larval stages are accelerated, the risk of intraspecific or interspecific competition may be reduced.

Although sons of high-density females attain a similar body size to sons of low-density females, the faster development might result in other costs. Previously described consequences of accelerated development include decreased maintenance efficiency, reduced ability to repair cellular damage, developmental instability, higher susceptibility to diseases and a shorter life span (Metcalfe & Monaghan, 2001). These possible costs raise the question of whether the accelerated development strategy is profitable. Indeed, other species confront competition by extending their development, rather than shortening it (Wall & Begon, 1987; Santos *et al.*, 1994; Fox *et al.*, 1999; McKintyre & Gooding, 2000; Sousa & Spence, 2000). However, elongated development may bear a cost as well because individuals are more vulnerable during their developmental stages and expanded development delays reproduction (Metcalfe & Monaghan, 2001). The fitness implications of modified larval development rates should be measured directly, in the presence and absence of competitors, to address these competing hypotheses.

The proportion of hosts from which wasps emerged was higher in the high-density (51.5%) than in the low-density treatment (30.2%). This finding may reflect maternally-induced phenotypic differences between the two types of offspring. Alternatively, it may arise from differences in host selectivity between the high- and low-density mothers. Low-density females may reject more hosts after ovipositor insertion, which could lead to lower wasp emergence rates from probed hosts. This possibility can be tested by dissecting host eggs immediately after observing ovipositor insertion by low- and

high-density females and looking directly for the parasitoid egg.

By contrast to the interpretation of maternal crowding inducing maladaptive physiological effects, maternal longevity is increased after high-density rearing. Furthermore, high-density females produce faster-developing offspring, which do not suffer from reduced body size, clutch size or embryonic survival compared with offspring of low-density mothers. These results thus suggest that the effects induced in *C. koehleri* by high rearing density do not reflect physiological constraints imposed by crowding. Rather, they may provide an adaptive response to anticipated competition but by mechanisms different from those previously suggested.

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