



Evidence for *trans*-generational effects on egg maturation schedules in a *syn*-ovigenic parasitoid

Yael Keinan^a, Tamar Keasar^{b,*}

^a Evolutionary and Environmental Biology, University of Haifa, Israel

^b Biology and the Environment, University of Haifa – Oranim, Israel

ABSTRACT

The lifetime reproductive success of a female parasitoid is limited by (1) host (or time) limitation – the number of hosts available for oviposition during its lifetime; and (2) egg limitation – its egg supply. Host limitation is expected to select for increased longevity and/or foraging efficiency, while increased fecundity is predicted to evolve under egg limitation. If the limiting factor varies, phenotypic plasticity in egg maturation schedules may be advantageous, i.e. adjusting investment in egg production to host availability. In the polyembryonic parasitoid *Copidosoma koehleri*, environmental conditions experienced during development indeed influence resource allocation to egg maturation. However, whether parasitoids' maternal environment also influences their daughters' egg production has hardly been studied. To address this knowledge gap, we tested whether exposure of *C. koehleri* females to previously parasitized hosts (signaling intraspecific exploitation competition and risk of host limitation) reduces their daughters' initial egg loads. We presented newly-emerged females with hosts that were either fresh or parasitized by conspecifics. The following day, we exposed both groups to additional fresh hosts, and reared out the daughters of these previously experienced, 24+ h old, individuals. The daughters' egg loads and body sizes were similar under both experimental conditions. Nevertheless, their egg loads were ~30% higher, and body sizes were ~10% lower, than in daughters of just-emerged parasitoids. We suggest that female experience or age, but not conditions associated with host exploitation, trigger maternal effects on the reproductive and developmental physiology of their daughters.

1. Introduction

In many insects, the two main limitations on lifetime reproductive success are the availability of hosts (or time to find them) and the availability of mature eggs. Theory predicts strong selection for traits that reduce the primary constraints to fitness, especially in the most highly productive members of the population (Rosenheim et al., 2010). Hence, if the population's primary constraint to fitness is host availability, the evolution of increased longevity and/or foraging efficiency is expected. On the other hand, if the primary constraint is egg limitation, then we will expect increased fecundity to evolve. However, these two forces act simultaneously. Hence, if the main limiting factor varies in time and place during the course of an individual's lifetime, there may be a significant advantage to non-genetic phenotypic plasticity. That is, the ability of each individual to invest in increased longevity when hosts are scarce and in increased fecundity when hosts are abundant.

Parasitoids, insects whose larvae consume a single arthropod host during their development, have to face the constant challenge of maximizing the match between egg load and host availability. Flanders (1950, cited in Jervis et al., 2001) distinguished between parasitoid species that have all or nearly all of their eggs mature at adult emergence ('pro-ovigenic') and those that continue to mature eggs

throughout their reproductive life ('*syn*-ovigenic'). Jervis et al. (2001) suggested that ovigeny variation among parasitoid species (as well as in other insects) is a continuum, ranging from strict pro-ovigeny, through partial *syn*-ovigeny, to extreme *syn*-ovigeny, rather than a clear dichotomous distinction. They therefore proposed the 'ovigeny index' (OI) to denote the proportion of the initial mature egg load out of an individual's lifetime potential fecundity. An OI of 1 (strict pro-ovigeny) indicates that the female emerges with all of her oocytes mature and ready for oviposition; whereas an OI of 0 (extreme *syn*-ovigeny) denotes that the female emerges with no mature oocytes.

Phenotypic plasticity in the temporal pattern of egg maturation, rather than in the lifetime potential fecundity, provides an additional mechanism for optimizing reproductive success under variable host densities. While pro-ovigeny (or a high OI, in general) provides the benefit of maximizing the number of eggs available early in life, it reduces the females' reproductive plasticity, i.e. her ability to match her egg supply to variation in host availability experienced during her lifetime. By contrast, a low OI (*syn*-ovigeny) carries the disadvantage of having only a small fraction of the lifetime potential egg complement available for laying at any one time. On the other hand, it confers the advantage of greater reproductive plasticity over the females' life span (Eilers et al., 2000; Jervis et al., 2001; Jervis and Ferns, 2004). *Syn*-ovigenic parasitoids are plastic in their egg maturation schedule and

* Corresponding author at: Department of Biology and Environment, University of Haifa – Oranim, Israel.

E-mail address: tkeasar@research.haifa.ac.il (T. Keasar).

can adjust their egg load in response to host availability. By doing so, *syn*-ovigenic parasitoids can potentially reduce the risk of egg / host limitation (Ellers et al., 2000; Jervis et al., 2001). *Syn*-ovigeny is clearly adaptive in stochastic environments and indeed it is by far the most common egg maturation strategy in parasitoid wasps (Jervis et al., 2001; Wajnberg et al., 2012).

Body size and life span are negatively correlated with the OI in cross-species (Jervis et al., 2003; Ellers and Jervis, 2003; Jervis and Ferns, 2004; Abram et al., 2016) and intra-species (Thorne et al., 2006) comparisons. This is probably due to a tradeoff between resource allocation to egg maturation vs. fat body storage, which is strongly correlated with body size and improves life span and dispersal ability (Ellers et al., 1998; Jervis et al., 2001). Thus, parasitoids that are resource-limited as larvae should benefit from a *syn*-ovigenic lifestyle, which does not require heavy investment in egg production in pre-adult stages, as it provides the ability to compensate by maturing additional eggs after adult eclosion. In pro-ovigenic parasitoids, on the other hand, carried-over resources are allocated mainly to egg maturation, at the expense of somatic maintenance and longevity (Jervis et al., 2001). The amount of carried-over resources to the adult stage in parasitoid wasps (fueling both ovigenesis and somatic functions) is determined by a combination of environmental factors such as host size and quality, the period of time available for larval development and the intensity of larval competition inside the host. These environmental factors affect metabolic rates, lipid synthesis and other physiological processes in the developing parasitoids (Ismail et al., 2012; Moiroux et al., 2018). They are thus expected to be major determinants of intraspecific variation in egg maturation schedules (Jervis et al., 2001). This prediction was tested and supported in a small number of experiments, including our own previous work (see below).

In one case study, pre-adult egg maturation was enhanced by low-temperature stress at the pupal stage in *Aphidius ervi* (Braconidae), an aphid parasitoid (Ismail et al., 2012). In a second experiment, Moiroux et al. (2018) exposed *A. ervi* to different temperatures during their development and adult stage. This resulted in increased OI at the lowest end of the temperature range (12 °C), probably because this temperature was too low for the adult females to mature additional eggs. OIs also increased at the highest temperature tested (28 °C). This increase was proposed to be adaptive: since insect life expectancy decreases with increasing metabolic rate, and thus with temperature, females should allocate more resources to early reproduction at higher temperatures, to maximize reproduction before dying. Another study, which focused on fruit-fly parasitoids, found that reproductive allocation in *Diachasmimorpha longicaudata* (Braconidae) varied with the food supplied to its host and hence with host resources. When reared on low quality host substrate (yielding the lightest and least nutritious larvae) *D. longicaudata* females were relatively smaller, produced more and bigger eggs and showed a higher reproductive investment relative to body size. In three other braconid parasitoids of the same host, however, reproductive investment was not affected by the host's diet (Cicero et al., 2011).

Another possible way to achieve phenotypic plasticity in egg maturation may be through maternal effects on daughters' life history traits, according to environmental conditions experienced by their mothers. Maternal effects were repeatedly suggested to provide a mechanism for adaptive *trans*-generational phenotypic plasticity, where the environment experienced by parents is translated into phenotypic variation in the offspring (Mousseau and Fox, 1998; Donelson et al., 2018). According to this view, beneficial mutations accumulate too slowly in the genome to provide organisms with well-adapted phenotypes, if their environment changes occasionally across generations. Phenotypic plasticity can provide selective advantages by allowing rapid and appropriate phenotypic expression (West-Eberhard, 2003; Jablonka and Lamb, 2014). This plasticity can be induced by the parents to enhance offspring fitness, if offspring environmental conditions are predictable from the maternal environment (Uller, 2008).

Ovipositing parasitoids can potentially transfer physiologically active substances (such as hormones) to the eggs, thereby controlling their size and biochemical composition. Such maternally derived factors may regulate offspring gene expression (Ho and Burggren, 2010). Recent studies have provided examples of heritable epigenetic effects in hymenopteran insects as a means of phenotypic regulation (e.g. Zwier et al., 2012; Voinovich and Reznik, 2017; Gosh and Ballal, 2018).

As far as we know, the effects of maternal environments on daughters' egg loads have not been directly investigated. Segoli and Rosenheim (2013) and Segoli et al. (2017) tested the hypothesis that the fecundity of pro-ovigenic parasitoids is positively correlated with the mean expectation for oviposition opportunities in the environment. Females of the parasitoids *Anagrus daanei* and *Anagrus erythroneruae* Trjapitsyn & Chiapini (Hymenoptera, Mymaridae) from agricultural systems, where hosts are often relatively abundant, indeed emerged with higher initial egg loads than those from natural habitats. These differences can potentially result from epigenetic effects, namely modification of offspring phenotypes by the foraging experience of their mothers. However, Andreatza and Rosenheim (2015) found no evidence for *trans*-generational phenotypic plasticity in fecundity of these parasitoids and suggested that the intraspecific differences recorded by Segoli and Rosenheim (2013) may have a genetic basis. Here, we extend the investigation to a *syn*-ovigenic, polyembryonic parasitoid (*Copidosoma koehleri* (Hymenoptera: Encyrtidae)) with considerable intra-specific variation in females' initial egg loads. In polyembryonic parasitoids, used in this study, several genetically identical embryos (clones) develop from each egg via repeated embryonic divisions. This provides the advantage of paired experimental designs when studying non-genetic effects on phenotypic plasticity: Individuals from a single genotype (clone-members) can be exposed to different environments, and the effects on life history traits can be isolated. Our previous studies have already shown both genetic and environmental contributions to the intraspecific variation in egg maturation schedules in *C. koehleri*. We found that the variation in initial egg loads decreased with increasing relatedness between *C. koehleri* females. The lowest variation, found among genetically identical clone members, may result from environmental influences on egg maturation, while the higher, between-clone variation could be explained by either genetic or epigenetic effects (Keinan et al., 2017). In another study we showed that resource allocation between growth and reproduction in *C. koehleri* is significantly affected by the developmental environment experienced during the larval stage, within the parasitized host. Females that developed in starved hosts were smaller in size than females from non-starved hosts. Though initial egg loads did not differ between the two treatment groups, females from starved hosts invested a relatively larger proportion of their resources to egg maturation (Keinan et al., 2018). In the present study we investigate whether the perception of an environmental factor experienced by mothers is translated into phenotypic variation in their offspring's egg maturation patterns, indicating maternal effects.

Our experiment manipulated the type of hosts (previously parasitized or not) encountered by mothers, as a proxy for the intensity of intra-specific competition. Such competition for hosts between adult parasitoids can be direct, when several females visit the same host patch at the same time. It is considered as indirect exploitation competition when a female visits a patch that has already been exploited by another female, as in our experimental design. Unlike prey items, which are immediately removed from the patch, parasitized hosts remain available and can still be parasitized by conspecific or interspecific competitors (Cusumano et al., 2016). However, parasitized hosts often carry chemical marks that allow competitors to detect and sometimes avoid them (Nufio and Papaj, 2001; Kishinevsky and Keasar, 2014).

Being the first to parasitize a host is considered advantageous because the offspring can start exploiting the host's limited resources before the competitors. In species with aggressive larvae such as *C. koehleri* the temporal advantage is even more significant, as the first

individuals attack their younger competitors and often cannibalize them. The order of oviposition and the time interval between ovipositions therefore play a role in mediating host acceptance and larval competition (Giron et al., 2007; Segoli et al., 2010; Cusumano et al., 2016). Tougeron et al. (2017) report previous evidence for a transgenerational effect induced by a cue of intra-specific competition. High density of conspecific parasitoids, when no hosts are available, enhanced the induction of summer diapause in the progeny of two *Aphidius* parasitoids. This diapause protected the following generation from exposure to low populations of suitable hosts and high mortality from superparasitism. They suggested that the perception of intraspecific competition is a proxy for estimating the risk of both superparasitism and host limitation for the future generation.

We hypothesized that high maternal intraspecific exploitation competition (i.e., an environment with previously parasitized hosts) is a cue for a host-limited environment, which will induce the production of larger and more *syn*-ovigenic daughters. Complementarily, we predicted that low maternal intraspecific exploitation competition provides a cue for a host-rich environment, and a risk for egg limitation that will induce the production of smaller and more pro-ovigenic daughters.

2. Materials and methods

2.1. The study organism

Copidosoma koehleri (Hymenoptera: Encyrtidae) is a koinobiont, polyembryonic egg-larval endoparasitoid that parasitizes the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). The species is *syn*-ovigenic (Keinan et al., 2017). The females emerge with ca. 60 mature eggs in their ovaries and continue developing additional eggs after emergence (Keinan et al., 2017, 2018). Adult body length is ca. 1.5 mm. Adult life span is about 30 days after emergence at 25 °C (Keasar et al., 2006; Keinan et al., 2017). Superparasitism occurs frequently as two or more eggs are often laid in a single host by one or several females (Segoli et al., 2009b). While the host hatches from the egg, and goes through four larval instars, the parasitoid egg proliferates inside its body and forms a clone of approximately 40 genetically identical individuals (Segoli et al., 2009a,b). The parasitoid larvae feed on the host tissues until only its cuticle remains, then pupate in the host mummy and eventually emerge as adults. Egg-to-adult development requires ca. 30 days at 27 °C. Sex determination is haplo-diploid, i.e., virgin females produce only haploid sons, whereas mated females can produce both haploid sons and diploid daughters (Doutt, 1947). Female clone-members exhibit a larval caste system: a single soldier larva develops precociously, attacks members of competing clones that superparasitize the same host, and dies before reaching maturity (Doutt, 1947, 1952; Keasar et al., 2006; Segoli et al., 2009b). The remaining female larvae develop normally and form the reproductive caste. Male clones do not form soldiers.

2.2. General methods

Insect rearing protocols, dissections, egg counting, body size measurements and further general methods are described in detail in Keinan et al. (2017, 2018). Insects were reared under conditions of 27 °C, 60% relative humidity and a 12:12 h L:D schedule. Hind tibia length was used as a measure of body size.

2.3. Obtaining parasitized hosts for Day-1 high intraspecific competition treatment

On the day of emergence, groups of 15–20 F1 females were allowed to parasitize 30–50 fresh *P. operculella* eggs, in a petri dish, for two hours. Under these conditions, all the eggs are parasitized 1–9 times, as confirmed in preliminary tests. These parasitized hosts were used for

the high intraspecific exploitation competition treatment on day 1 of the experiment.

2.4. Experimental design

The experiment comprised ten all-female F1 clones. On the day of emergence, a pair of genetically identical F1 females from each clone were mated and then separately subjected to either one of two Day-1 treatments for 20 h: a. Low maternal intraspecific exploitation competition: a petri dish containing 30–50 fresh, non-parasitized *P. operculella* eggs; or b. High maternal intraspecific exploitation competition: a petri dish containing 30–50 *P. operculella* eggs that had been previously parasitized by non-related females. For details see section “Obtaining parasitized hosts for Day-1 high intraspecific competition treatment” above. 20 h later, on Day 2, each female was given ca. 40 fresh *P. operculella* eggs for 4 h. All females were allowed to freely oviposit and parasitize the hosts’ eggs. All Day-2 host eggs as well as some of the low exploitation treatment hosts from Day 1 were reared on potato tubers, separated by F1 clone, age and Day-1 treatment. After pupation, mummies were collected, placed individually in glass test tubes (13/100 mm) and left to emerge.

We aimed for all F2 females to have experienced similar developmental conditions and to originate from a singly parasitized host, i.e. F2 clones of ~45 females (Segoli et al., 2009b). Therefore, on the day of emergence of the adult parasitoid broods, the wasps were sexed and counted to ensure their suitability (40–50 females). All suitable female clones (mean \pm SD numbers of individuals per clone: 43.73 ± 2.87) were deep-frozen on the day of emergence. Three females from each clone were measured and dissected to determine per-clone average body sizes and initial egg loads. The experimental design is summarized in Fig. 1.

2.5. Data analysis

We treated offspring clone as the experimental unit of replication and calculated the per-clone mean tibia length and initial egg loads ($n = 10$ for each treatment, the data collected from the three females that represented each clone were averaged). We used paired samples *t*-tests to check for differences between the two maternal treatments. We applied a linear mixed-effects model to check whether mean per-clone body size and treatment influence initial egg loads. In a second linear mixed-effects model, we tested for the effect of treatment on the initial egg-loads/body size ratio. We used two further LMMs to compare initial egg loads and body sizes between the daughters of Day-1 and Day-2 females. Maternal ID was included in all models as a repeated measures factor, to account for the paired experimental design.

Correlations between per-clone mean initial egg loads and per-clone mean hind tibia lengths were calculated across all wasps, regardless of maternal origin. Pearson’s coefficient for normally distributed data was calculated for the low exploitation treatment and Spearman’s rho for data that did not meet parametric assumptions was calculated for the high exploitation intraspecific competition treatment.

SPSS version 19.0 and 24.0 was used for most statistical analyses. The packages ‘lme4’ and ‘lmerTest’ in R were used for the LMMs (Bates et al., 2015).

3. Results and discussion

Our experiment tested whether the type of hosts encountered by adult females affects their daughter’s investment in pre-adult egg maturation. We expected maternal encounters with parasitized hosts (a proxy for high maternal intraspecific exploitation competition) to enhance allocation towards body size at the expense of initial egg load in their daughters. This trade-off was hypothesized to increase longevity and dispersal ability, which are advantageous under conditions of high intraspecific competition and host limitation. Contrary to our

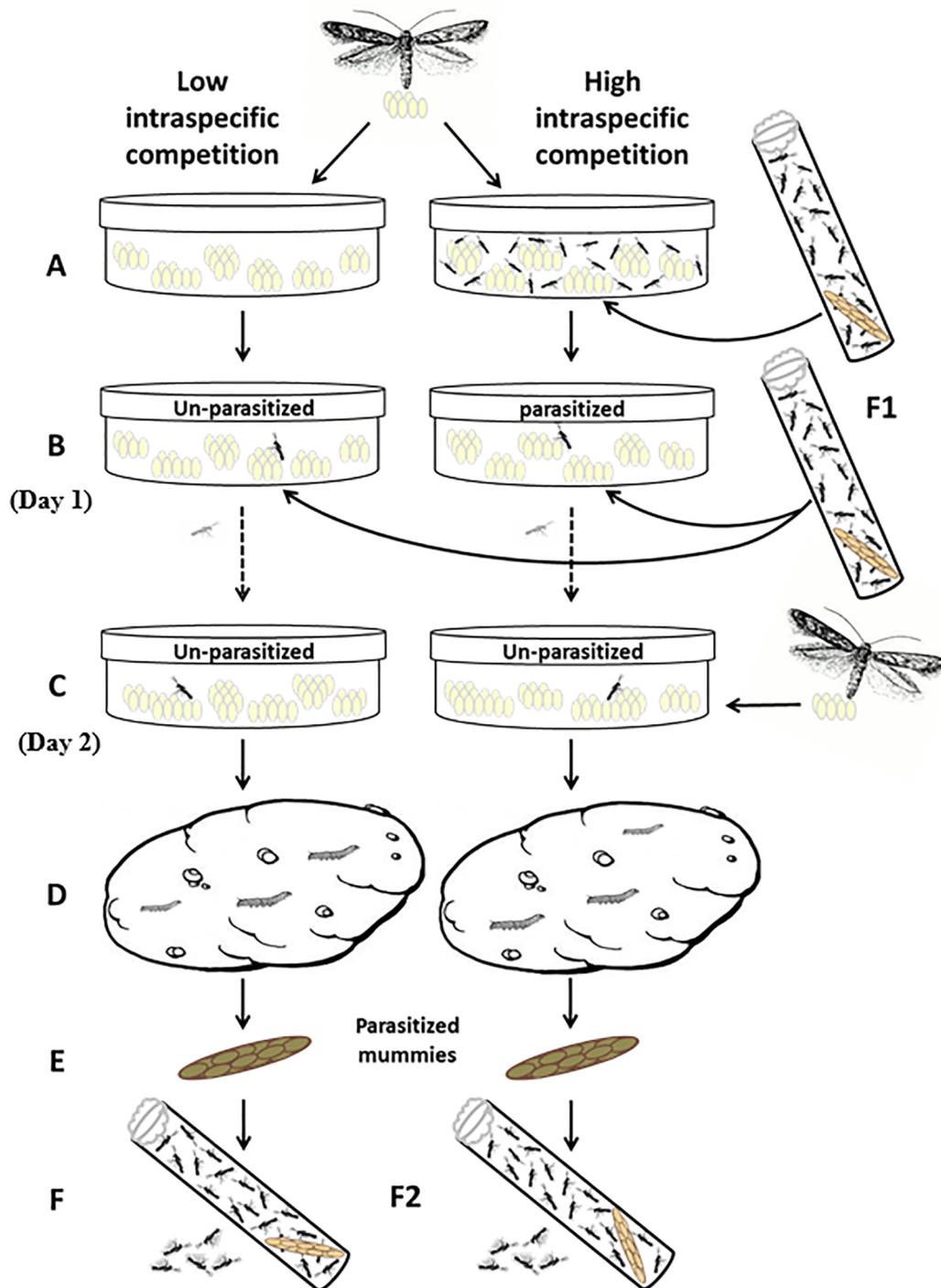


Fig. 1. The experimental design: (A) fresh *P. operculella* eggs are collected and placed in petri dishes. One half of the petri dishes are parasitized by groups of 15–20 *C. koehlerii* females, and the rest are left un-parasitized. (B) On Day 1 of the experiment, two genetically identical females from each of 10 newly emerged F1 clones are mated and placed in the petri dishes that already contain parasitized or un-parasitized host eggs for 20 h. (C) On Day 2 both females are presented with fresh un-parasitized hosts for 4 h. (D) Day-2 hosts (as well as some of the low maternal intraspecific competition hosts from Day 1) are reared out on potato tubers until mummified. (E) Mummies (dead hosts containing parasitoid pupae) are collected and reared individually until F2 adult emergence. (F) Emergence tubes. On the day of emergence all broods are sexed and counted to ensure their suitability for the experiment. Three females from each clone are sacrificed to determine their initial egg loads and hind tibia lengths.

predictions, we found no difference in daughters' initial egg loads and body sizes between the two treatments of high vs. low maternal intraspecific exploitation competition (paired-samples t-tests: $t_9 = 0.169$, $p = 0.870$ and $t_9 = 0.693$, $p = 0.125$ respectively), indicating that maternal effects were not induced by this specific manipulation. As

parasitoids are more likely to encounter hosts that are parasitized by conspecifics rather than heterospecifics, the ability to discriminate between parasitized and un-parasitized hosts is expected to be more common at the intraspecific level (Cusumano et al., 2016). This ability was indeed documented in *C. koehlerii* (Segoli et al., 2010). Hence, the

different host-type treatments were probably detected by the females in our study but failed to elicit the expected response.

The ratio between initial egg loads and body sizes was also unaffected by treatment, reflecting no difference in the allocation of resources to egg production vs. body size (paired-samples *t*-tests: $t_9 = 0.117$, $p = 0.909$). Offspring body size but not treatment had a positive effect on initial egg load within samples paired by their maternal origin (linear mixed model: $F_{1,47} = 8.90$, $p = 0.005$ for body size). The ratio between these two parameters was also unaffected by treatment, reflecting no difference in the allocation of resources to egg production vs. body size (linear mixed model: $F_{1,48} = 0.03$, $p = 0.86$). No correlation was found between initial egg loads and body sizes across mothers in both treatments ($r_{\text{pearson}} = 0.009$, $p = 0.980$ and $r_{\text{spearman}} = 0.389$, $p = 0.266$ for the low exploitation treatments and the high exploitation treatment respectively).

Although counterintuitive and previously considered maladaptive, superparasitism is frequent in several hymenopteran species and has long been recognized as adaptive in some situations (Pereira et al., 2017) such as host scarcity and high levels of competition (Kishinevsky and Keasar, 2014). *C. koehleri* females do not avoid, and sometimes even prefer, hosts parasitized by conspecifics over other host types in two-choice experiments (Segoli et al., 2010). Two possible explanations are that superparasitism increases out-breeding opportunities for the offspring upon emergence (Segoli et al., 2010) and can also inhibit the hosts' defensive response and reduce parasitoid mortality (Keinan et al., 2012; Pereira et al., 2017). These potential advantages of superparasitism may have overpowered the possible risks of high maternal intraspecific competition in our experiment. Had this been the case, we would have expected the response to be opposite to our initial hypothesis: a cue for the presence of "good quality" or preferable hosts that could have possibly induced the production of more pro-ovigenic daughters. Either way, we found no effect of host types on the relative investment to egg maturation.

In a previous study (Keinan et al., 2018) we determined the initial egg loads of wasps from singly-parasitized hosts, which were mothered by just-emerged females with no previous exposure to hosts. In that study, initial egg loads were 37% lower and body sizes were 8% higher than in the present experiment (Table 1). The ratio of egg load to body size in the previous study was lower by 42% than in the present one. The wasps' source population, rearing conditions and the season of the experiment were identical across the two studies. This raised the possibility that the mothers' age or oviposition experience, the only major differences between the two experiments, was the cause of this inconsistency. We explored this hypothesis further by comparing the body sizes and egg loads of females that developed from Day-1 ovipositions of three mothers in the present experiment to those of females that

developed from Day-2 ovipositions. As in the comparison between experiments, daughters of just-emerged (Day 1) females had lower initial egg loads and larger bodies than daughters of Day-2 females (Table 1). These differences were statistically significant (linear mixed models: $F_{1,16.03} = 4.82$, $P = 0.04$ for initial egg load, $F_{1,17.85} = 25.30$, $P < 0.001$ for body size).

This higher relative allocation to egg production vs. body size in daughters of oviposition-experienced females, as compared to just-emerged females, suggests a maternal effect induced by experience or physiological state. It complements evidence for *trans*-generational phenotypic plasticity in other life-history traits in *C. koehleri* (Morag et al., 2011a,b). The oviposition-experienced mothers in our study have already encountered 30–50 fresh or parasitized hosts. Parasitized or not, by the end of this experience the mothers probably perceived their environment as rich in hosts. The high production of eggs by these females' daughters can be interpreted as an adaptive response to the perceived high host density, namely resource investment in early egg-laying at the expense of dispersal ability.

A complementary interpretation of our results involves differences in physiological state, rather than in learning experience, between the host-exposed and the host-naïve females. Host-exposed females were older, both chronologically and physiologically (in terms of previous egg-laying) than the host-naïve wasps. Both of these factors could be involved in maternal effects on their daughters' egg maturation. Consistent with this interpretation, female *Eupelmus vuilleti* (Eupelmidae) parasitoids reduced nutrient provisioning of their eggs as they became older. This led to lower protein and glycogen levels in the adult offspring of older mothers (Muller et al., 2017), and could potentially explain the lower body size in daughters of host-exposed mothers in our study. Nevertheless, differences in maternal age are unlikely to fully explain our results because of the following considerations: (1) the age difference between host-naïve and host-experienced mothers was only one day, out of a life span of > 30 days. (2) Reduced provisioning of nutrients by older mothers does not directly account for the higher initial egg loads of the offspring. (3) Maternal age had only minor effects on offspring body sizes and initial egg loads in the parasitoid *Lysiphlebus fabarum* (Braconidae) (Najafpour et al., 2018).

A few previous studies documented evidence for a genetic influence on the ovigeny index in parasitoids. Laboratory cultures of the *syn*-ovigenic parasitoid wasp *Asobara tabida* Nees (Hymenoptera: Braconidae) that had been collected from different geographic areas (southern vs. northern Europe) showed differences in initial egg loads that persisted over several generations (Ellers and Van Alphen, 1997). These differences were proposed to reflect natural selection, adapting the populations to local conditions, in particular the average availability of hosts (Jervis et al., 2001). Differences among populations in initial egg loads, which are probably genetically based, were also demonstrated in *Anagrus daanei* (Hymenoptera: Mymaridae) (Segoli and Rosenheim, 2013 followed by Andreatza and Rosenheim, 2015). Additional evidence is provided by Wajnberg et al. (2012) who showed a significant genetic component to phenotypic variability in egg maturation patterns in a strain of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) maintained as separate lines under laboratory conditions. We found similar results in *C. koehleri* (Keinan et al., 2017).

The present experiment, combined with our previous work (Keinan et al., 2018), suggests some phenotypic plasticity in *C. koehleri*'s initial egg loads even in the absence of genetic variation (i.e., among clone-mates). Altogether, we find a great deal of intra-specific variation in pre-adult egg maturation schedules, mediated by a combination of genetic, epigenetic and environmental factors. This suggests that the Ovigeny Index (calculated as initial egg load divided by total fecundity) should also be interpreted as a flexible life-history trait rather than a rigid species-specific measure. Learning to control this intra-specific variation in life history traits in general, and pre-adult egg maturation

Table 1

Mean \pm SE initial egg loads and body sizes for daughters of Day-1 and Day-2 females, recorded in the current experiment and in a previous experiment with a similar design (Keinan et al., 2018).

Data source	Number of clones	Initial egg load	Body size
Keinan et al., 2018 (Day-1 females only)	15	53.96 \pm 2.87	0.393 \pm 0.004
Current experiment, Day 1 Low exploitation competition treatment	3	68.22 \pm 0.23	0.439 \pm 0.024
Current experiment, Day 2 Low exploitation competition treatment	10	84.70 \pm 4.36	0.360 \pm 0.004
Current experiment, Day 2 High exploitation competition treatment	10	85.73 \pm 4.95	0.366 \pm 0.006
Current experiment, Day 2 All clones combined	20	85.22 \pm 3.21	0.362 \pm 0.004

in particular, is a promising direction to improve management of parasitoids for pest control programs.

Acknowledgements

The study was supported by the Israel Science Foundation, grant no. 414/10 to TK. We thank Michal Segoli for helpful comments on the manuscript, and Nina Dinov for rearing the insects in the lab.

References

- Abram, P.K., Parent, J.P., Brodeur, J., Boivin, G., 2016. Size-induced phenotypic reaction norms in a parasitoid wasp: an examination of life-history and behavioural traits. *Biol. J. Linn. Soc.* 117, 620–632.
- Andrezza, F., Rosenheim, J.A., 2015. Absence of transgenerational phenotypic plasticity in fecundity in the parasitoid *Anagrus erythronuræ* (Hymenoptera: Mymaridae). *J. Insect Sci.* 15 (1), 138. <https://doi.org/10.1093/jisesa/iev122>.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.
- Cicero, L., Sivinski, J., Rull, J., Aluja, M., 2011. Effect of larval host food substrate on egg load dynamics, egg size and adult female size in four species of braconid fruit fly (Diptera: Tephritidae) parasitoids. *J. Insect Physiol.* 57, 1471–1479.
- Cusumano, A., Peri, E., Colazza, S., 2016. Interspecific competition/facilitation among insect parasitoids. *Curr. Opin. Insect Sci.* 14, 12–16.
- Donelson, J.M., Salinas, S., Munday, P.L., Shama, L.N., 2018. Transgenerational plasticity and climate change experiments: where do we go from here? *Glob. Change Biol.* 24, 13–34.
- Doutt, R.L., 1947. Polyembryony in *Copidosoma koehleri* Blanchard. *Am. Nat.* 81, 435–453.
- Doutt, R.L., 1952. The teratoid larvae of polyembryonic encyrtids (Hymenoptera). *Can. Entomol.* 84, 247–250.
- Ellers, J., van Alphen, J.J.M., 1997. Life history evolution in *Asobara tabida*: plasticity in allocation of fat reserves to survival and reproduction. *J. Evol. Biol.* 10, 771–785.
- Ellers, J., Van Alphen, J.J.M., Sevenster, J.G., 1998. A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *J. Anim. Ecol.* 67, 318–324.
- Ellers, J., Sevenster, J.G., Driessen, G., 2000. Egg load evolution in parasitoids. *Am. Nat.* 156, 650–665.
- Ellers, J., Jervis, M.A., 2003. Body size and the timing of egg production in parasitoid wasps. *Oikos* 102, 164–172.
- Giron, D., Ross, K.G., Strand, M.R., 2007. Presence of soldier larvae determines the outcome of competition in a polyembryonic wasp. *J. Evol. Biol.* 20, 165–172.
- Gosh, E., Ballal, C.R., 2018. Maternal influence on diapause induction: an approach to improve long-term storage of *Trichogramma chilonis*. *Phytoparasitica* 46, 383–389.
- Ho, D.H., Burggren, W.W., 2010. Epigenetics and transgenerational transfer: a physiological perspective. *J. Exp. Biol.* 213, 3–16.
- Ismail, M., Vernon, P., Hance, T., Pierre, J.S., Van Baaren, J., 2012. What are the possible benefits of small size for energy-constrained ectotherms in cold stress conditions? *Oikos* 121, 2072–2080.
- Jablonka, E., Lamb, M.J., 2014. Evolution in Four Dimensions, Revised Edition: Genetic, Behavioral, and Symbolic Variation in the History of Life. MIT Press, Epigenetic.
- Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A., Kidd, N.A.C., 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *J. Anim. Ecol.* 70, 442–458.
- Jervis, M.A., Ferns, P.N., Heimpel, G.E., 2003. Body size and the timing of egg production in parasitoid wasps: a comparative analysis. *Funct. Ecol.* 17, 375–383.
- Jervis, M.A., Ferns, P.N., 2004. The timing of egg maturation in insects: ovigeny index and initial egg load as measures of fitness and of resource allocation. *Oikos* 107, 449–461.
- Keasar, T., Segoli, M., Barak, R., Steinberg, S., Giron, D., Strand, M.R., et al., 2006. Costs and consequences of superparasitism in the polyembryonic parasitoid *Copidosoma koehleri*. *Ecol. Entomol.* 31, 277–283.
- Keinan, Y., Kishinevsky, M., Segoli, M., Keasar, T., 2012. Repeated probing of hosts: an important component of superparasitism. *Behav. Ecol.* 23, 1263–1268.
- Keinan, Y., Kishinevsky, M., Keasar, T., 2017. Intra-specific variability in egg maturation patterns and associated life-history tradeoffs in a parasitoid wasp. *Ecol. Entomol.* 42, 587–594.
- Keinan, Y., Braun, R., Keasar, T., 2018. Phenotypic plasticity of pre-adult egg maturation in a parasitoid: effects of host starvation and brood size. *PLoS ONE* 13, e0195767.
- Kishinevsky, M., Keasar, T., 2014. State-dependent host acceptance in the parasitoid *Copidosoma koehleri*: the effect of intervals between host encounters. *Behav. Ecol. Sociobiol.* 69, 543–549.
- Morag, N., Harari, A.R., Bouskila, A., Keasar, T., 2011a. Low maternal host-encounter rate enhances offspring proliferation in a polyembryonic parasitoid. *Behav. Ecol. Sociobiol.* 65 2296 2287.
- Morag, N., Keasar, T., Harari, A.R., Bouskila, A., 2011b. Trans-generational effects of maternal rearing density on offspring development time in a parasitoid wasp. *Physiol. Entomol.* 36, 294–298.
- Moiroux, J., Boivin, G., Brodeur, J., 2018. Ovigeny index increases with temperature in an aphid parasitoid: is early reproduction better when it is hot? *J. Insect Physiol.* 109, 157–162.
- Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. *Trends Ecol. Evol.* 13, 403–407.
- Muller, D., Giron, D., Desouhant, E., Rey, B., Casas, J., Lefrique, N., Visser, B.J., 2017. Maternal age affects offspring nutrient dynamics. *J. Insect Physiol.* 101, 123–131.
- Najafpour, P., Rasekh, A., Esfandiari, M., 2018. Maternal rearing condition and age affect progeny fitness in the parasitoid wasp *Lysiphlebus fabarum*. *Entomol. Exp. Appl.* 166, 24–31.
- Nufio, C.R., Papaj, D.R., 2001. Host marking behavior in phytophagous insects and parasitoids. *Entomol. Exp. Appl.* 99, 273–293.
- Pereira, K.S., Guedes, N.M.P., Serrão, J.E., Zanuncio, J.C., Guedes, R.N.C., 2017. Superparasitism, immune response and optimum progeny yield in the gregarious parasitoid *Palmistichus elaeis*. *Pest Manag. Sci.* 73, 1101–1109.
- Rosenheim, J.A., Alon, U., Shinar, G., 2010. Evolutionary balancing of fitness-limiting factors. *Am. Nat.* 175, 662–674.
- Segoli, M., Bouskila, A., Harari, A., Keasar, T., 2009a. Brood size in a polyembryonic parasitoid wasp is affected by the relatedness among competing larvae. *Behav. Ecol.* 20, 761–767.
- Segoli, M., Bouskila, A., Harari, A., Keasar, T., 2009b. Developmental patterns in the polyembryonic wasp *Copidosoma koehleri*. *Arthropod Struct. Dev.* 38, 84–90.
- 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.
- Segoli, M., Rosenheim, J.A., 2013. The link between host density and egg production in a parasitoid insect: comparison between agricultural and natural habitats. *Funct. Ecol.* 27, 1224–1232.
- Segoli, M., Sun, S., Nava, D.E., Rosenheim, J.A., 2017. Factors shaping life history traits of two proovigenic parasitoids. *Integr. Zool.* 13, 297–306.
- Thorne, A.D., Pexton, J.J., Dytham, C., Mayhew, P.J., 2006. Small body size in an insect shifts development, prior to adult eclosion, towards early reproduction. *Proc. R. Soc. London B: Biol. Sci.* 273, 1099–1103.
- Tougeron, K., Hraoui, G., Le Lann, C., Van Baaren, J., Brodeur, J., 2017. Intraspecific maternal competition induces summer diapause in insect parasitoids. *Insect Sci. DOI*. <https://doi.org/10.1111/1744-7917.12491>.
- Uller, T., 2008. Developmental plasticity and the evolution of parental effects. *Trends Ecol. Evol.* 23, 432–438.
- Voinovich, N.D., Reznik, S.Y., 2017. On the factors inducing the inhibition of diapause in the progeny of diapause females of *Trichogramma telengai*. *Physiol. Entomol.* 42, 274–281.
- Wajnberg, E., Curty, C., Jervis, M., 2012. Intra-population genetic variation in the temporal pattern of egg maturation in a parasitoid wasp. *PLoS ONE* 7, e45915.
- West-Eberhard, M.J., 2003. Developmental Plasticity and Evolution. Oxford University Press.
- Zwier, M.V., Verhulst, E.C., Zwahlen, R.D., Beukeboom, L.W., Van de Zande, L., 2012. DNA methylation plays a crucial role during early *Nasonia* development. *Insect Mol. Biol.* 21, 129–138.