Intraspecific variability in egg maturation patterns and associated life-history trade-offs in a polyembryonic parasitoid wasp

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Abstract. 1. Life-history theory predicts a trade-off between the resources allocated to reproduction and those allocated to survival. Early maturation of eggs (pro-ovigeny) is correlated with small body size and low adult longevity in interspecific comparisons among parasitoids, demonstrating this trade-off. The handful of studies that have tested for similar correlations within species produced conflicting results.

2. Egg maturation patterns and related life-history traits were studied in the polyembryonic parasitoid wasp, *Copidosoma koehleri* (Hymenoptera: Encyrtidae). Although the genus *Copidosoma* was previously reported to be fully pro-ovigenic, mean egg loads of host-deprived females almost doubled within their first 6 days of adulthood.

3. The initial egg-loads of newly emerged females were determined and age-specific realised fecundity curves were constructed for their clone-mate twins. The females' initial egg loads increased with body size, but neither body size nor initial egg load was correlated with longevity and fecundity.

4. The variation in initial egg loads was lowest among clone-mates, intermediate among non-clone sisters and highest among non-sister females. The within-clone variability indicates environmental influences on egg maturation, while the between-clone variation may be genetically based.

5. Ovaries of host-deprived females contained fewer eggs at death (at ∼29 days) than on day 6. Their egg loads at death were negatively correlated with life span, consistent with reduced egg production and/or egg resorption. Host deprivation prolonged the wasps' life span, suggesting a survival cost to egg maturation and oviposition.

6. It is concluded that adult fecundity and longevity were not traded off with pre-adult egg maturation.

Key words. Body size, *Copidosoma koehleri*, egg resorption, longevity, ovigeny, polyembryony.

Introduction

The temporal pattern of egg maturation is an important component of life-history strategies of insects. The 'ovigeny index' (OI), devised by Jervis *et al.* (2001), is a commonly used indicator of the relative degree to which egg production is concentrated into pre-adult life. It is defined as the number of mature eggs in a female's ovaries at adult emergence, divided by her lifetime egg complement. Fully synovigenic species (OI = 0) mature all of their eggs as adults, while fully pro-ovigenic ones (OI = 1) reach adulthood with a full egg complement and do not produce additional eggs during the adult stage. Many species are partially synovigenic (0 < OI < 1), namely they mature only part of their eggs during adulthood.

Comparative studies, many of which focused on parasitoid wasps, explored how the OI varies among species and with other life-history traits. The OI is negatively correlated with life span (Jervis *et al.*, 2001, 2003) and with body size (Jervis *et al.*, 2003) in parasitoids. These correlations were suggested to reflect a trade-off between resource allocation to early reproduction in

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pro-ovigenic species and higher allocation to energy reserves in synovigenic ones. The relatively larger investment in somatic tissue in synovigenic species is hypothesised to allow their increased size and longevity.

Egg maturation schedules differ within parasitoid species as well. The fraction of the total egg complement that is mature upon adult emergence varies among geographically distinct populations (Ellers & van Alphen, 1997). Even within a single laboratory population, some individuals are more pro-ovigenic than others (Wajnberg *et al.*, 2012; Abram *et al.*, 2016). Theoretically, the trade-off between reproduction and survival is assumed to underlie the intraspecific variation in OIs, similarly to the trade-off suggested to account for the variability among species. Individuals with low OIs are expected to direct fewer resources into early egg maturation, grow larger in size and have a higher survival, while more pro-ovigenic individuals are predicted to be smaller and shorter-lived (Ellers & Jervis, 2003). The rationale offered for this prediction is that larger females are expected to have higher survival and dispersal abilities, to encounter more hosts and hence to run a higher risk of egg limitation than small females (Jervis & Ferns, 2004). This, in turn, selects for continued egg production throughout the adult life (Rosenheim *et al.*, 2000). Thus, some theoretical analyses of ovigeny suggest that large body size selects for synovigeny, while others see it as an outcome of synovigenic development.

The intraspecific trade-offs between the egg maturation schedule, body size and longevity were measured in a few parasitoid species only, and have yielded inconsistent results. As predicted by theory, small individuals were more pro-ovigenic and shorter-lived than large ones in *Aphaereta genevensis* (Braconidae) (Olson & Andow, 1998; Thorne *et al.*, 2006). Similarly, body size was inversely correlated with the OI in *Telenomus podisi* (Platygastridae). However, it was uncorrelated with longevity (Abram *et al.*, 2016). Body size was not correlated with OI in *Ibalia leucospoides* (Ibaliidae), while the correlations of these traits with longevity were not investigated (Fischbein *et al.*, 2013). Increased female size in this species is associated with similar increases in both initial egg load and in total lifetime fecundity, resulting in a constant OI. These scattered and conflicting findings highlight the need for additional case studies.

To address this knowledge gap, we investigated the intraspecific variability in egg maturation patterns and its correlations with life-history traits in a polyembryonic parasitoid. In the polyembryonic parasitoid *Copidosoma koehleri* (Blanchard) (Hymenoptera: Encyrtidae), young embryos undergo repeated cleavage within the body of their host to form a clone of about 40 genetically identical offspring. We took advantage of this unique developmental feature to determine initial egg loads and lifetime realised fecundity in sets of identical twin sisters. We used these parameters to estimate the species' reproductive concentration index (RCI), defined by Wajnberg *et al.* (2012) as the proportion of mature eggs in <24-h-old females out of the total egg complement. Both RCI and OI estimate resource allocation to reproduction at early adulthood. They differ in the

timing of the egg counts: immediately upon adult emergence to determine the OI, or on the day of emergence for the RCI (Wajnberg *et al.*, 2012). We tested for trade-offs between the wasps' initial egg load, RCI, body size and longevity. By comparing the variability in initial egg loads among twins, sisters and non-sister females, we explored the relative contributions of heredity and environment to this trait. The current study, except for one experiment (Experiment 3), relies on natural intraspecific variation in life-history traits and is mostly observational. It is widely acknowledged that in purely observational studies, trade-offs are often masked by differences between individuals (as a result of their different genetic and developmental background). In spite of this inherent limitation, the present work provides baseline data on the natural variation in the RCI, needed for further manipulative experiments.

Our questions were:

- Does *C. koehleri* exhibit intraspecific variation in egg maturation schedules?
- If it does, how is this variation affected by genetic background and environmental conditions?
- What are the relationships between the temporal pattern of egg maturation, body size and adult survival?

Materials and methods

The study organism

Copidosoma koehleri is a koinobiont, polyembryonic egg-larval endoparasitoid that parasitises the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), and is used as a biological control agent of this pest (Kfir, 1981; Keasar & Sadeh, 2007; Keasar & Steinberg, 2008). The species has been reported to be pro-ovigenic, i.e. the females' full egg complement is mature at adult emergence (Baggen & Gurr, 1998, but see our results below). Adult body length is ∼1.5 mm. Adult wasps live for about 30 days after emergence (Keasar *et al.*, 2006) and do not host-feed (Y. Keinan, pers. obs.). Hosts are frequently super-parasitised by two or more eggs laid by one or several females (Segoli *et al.*, 2009b). The host goes through four larval instars after emerging from the egg, while the parasitoid embryos develop inside it. The parasitoid egg proliferates within 8 days of oviposition to form a clone of approximately 40 genetically identical embryos (Segoli *et al.*, 2009a,2009b). Eventually, the parasitoid larvae consume the host tissues until only its cuticle remains, pupate in the host mummy and emerge as adults. Egg-to-adult development requires ∼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 super-parasitise 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.

Insect rearing

A laboratory stock of *C. koehleri* that originated from field-collected individuals from South Africa in 2003 was used in the study. The parasitoids and their hosts were reared using modifications of Berlinger and Lebiush-Mordechi's (1997) and Kfir's (1981) protocols, under conditions of 27 °C, 60% RH and LD 12:12 h. Hosts were fed on potato tubers during larval stages, and on honey and water throughout adulthood. Adult parasitoids were fed on honey without restriction. Hosts used for experiments were 0–12 h old.

Obtaining single-sex genetically identical clones

To obtain all-female genetically identical clones, highly developed parasitised mummies were collected from the laboratory stock, placed in glass test tubes (13/100 mm) with a drop of honey as food source, and left to emerge. Newly emerged virgin females (isolated from all-female broods) were mated with a single male. Mating was observed under a stereomicroscope. The females were then allowed to oviposit individually in fresh *P. operculella* eggs for 3–4 h in a Petri dish. A single oviposition per host is expected under these conditions. Each female's parasitised hosts were placed separately on a potato tuber in a plastic container covered with cloth, and were reared until pupation. F1 mummies were collected, placed individually in glass test tubes and left to emerge. F1 female clones originating from different mothers and fathers were then used in experiments. Some of the emerged all-male F1 clones were used to mate non-sister females in Experiments 2 and 3, as detailed below.

Dissections, egg counting and body size measurements

Female parasitoids were deep-frozen (−20 °C) to arrest their oogenesis and preserve them until dissection. Frozen parasitoids were individually dissected in a droplet of insect Ringer's solution (Laughton *et al.*, 2011). Their ovaries were carefully separated, spread out under a light microscope and photographed using a Dino-lite digital eyepiece camera (Hsinchu, Taiwan). Images were analysed using the IMAGEJ image processing and analysis software (Schneider *et al.*, 2012) to count the number of eggs in each ovary. Each female's hind tibia length was determined, as a measure of body size, using the same equipment.

Experiment 1: Egg loads of 6-day old host-deprived females

The aim of this experiment was to determine short-term egg production in wasps with no access to hosts. Six F1 female clones were obtained as described earlier. Seven to eight females from each clone were sacrificed on the day of emergence and dissected to determine their initial egg loads. The remaining females were kept unmated, each clone in a different test tube, for 6 days. They were supplied with honey but not with hosts. The egg loads of three additional females per clone were assessed on day 6.

Data analysis. We calculated the per-clone mean egg loads on days 0 and 6. The distribution of the mean egg loads on both days did not conform to the assumptions of parametric tests. We used a generalised linear model (GLM) with an inverse-Gaussian error distribution to test the effects of wasp age on the per-clone average egg loads. Age was modelled as a fixed factor, and clone was treated as a random factor.

Experiment 2: Variability in initial egg loads within and among clones

This experiment was designed to explore the sources of variation in initial egg loads among females. Nine all-female F1 clones were obtained following the protocol described earlier. Shortly after emergence, one female per clone was mated, each with a male from a different all-male clone. Each mated female was allowed to oviposit once in each of several hosts, which were reared out to produce the wasps' F2 generation. Three all-female F2 clones from each lineage were randomly selected for determination of initial egg loads. Three females from each of these clones were dissected on the day of emergence. This design resulted in 81 dissected females that originated from the same clone, from sister clones or from non-sister clones.

Data analysis. The initial egg load data met the parametric assumptions. Nested ANOVA was used to compare the variance in initial egg loads among females with different mothers (defined as a random factor), among sister clones (defined as a nested term within each mother) and within each clone (the error term). The procedure 'variance components' was used to estimate the contribution of each of these variables to the total variance in initial egg loads.

Experiment 3: Constructing an age-specific realised fecundity curve

This experiment aimed to determine the realised fecundity of females under optimal conditions (unlimited access to hosts and food) over their whole adult life. One newly emerged virgin female (the 'host-exposed' female) from each of 11 F1 clones was mated with a single male. Each mated female was placed individually in a Petri dish, with unrestricted food and >20 hosts, and allowed to oviposit until she died. Hosts were replaced every 6 h, every day, throughout the parasitoid's entire lifetime and immediately dissected to count the number of parasitoid eggs deposited inside them. Three other individuals per clone were dissected on the day of adult emergence to determine initial egg loads. Five additional females from each clone were also mated and placed in identical Petri dishes with food *ad libitum*. These females were host-deprived and were used as a control group to test for a possible fecundity–longevity trade-off. All parasitoids were deep-frozen after death. They were later measured for body size and dissected to count the eggs left in their ovaries.

Data analysis. Each female's egg complement was calculated as the total number of eggs she laid plus the eggs that remained

in her ovaries after death. The average fraction of parasitoid eggs oviposited each day, out of the total egg complement, was plotted against age to construct an age-specific realised fecundity curve. Average per-clone initial egg loads, tibia lengths and longevity were calculated. The correlations between the average life-history parameters per clone were calculated using Pearson's coefficient for normally distributed data (tibia length, longevity, initial egg load) and Spearman's rho for data that did not meet parametric assumptions (number of eggs laid).

The RCI was estimated for each clone separately. Its numerator was estimated by the average number of mature eggs in the ovaries of the three newly emerged females dissected for initial egg loads. Its denominator was calculated as the total number of eggs laid by the female tested for realised fecundity throughout her entire life time plus the number of eggs left in her ovaries after death. The population's average RCI is the mean of all 11 per-clone RCI values. The SE of this mean provides a measure of the between-clone variability in RCIs.

SPSS version 19.0 was used for most statistical analyses. The package 'lme4' in R was used for the GLM (Bates *et al.*, 2015).

Results

C. koehleri's *ovigeny index (Experiments 1 and 3)*

Females in Experiment 1 emerged with an average of 63.11 ± 6.38 (SE) mature eggs in their ovaries and nearly doubled this number within 6 days, to 104.30 ± 6.34 eggs ($n = 6$ clones; Fig. 1). Experiment 3 yielded similar initial egg loads (59.91 ± 3.31; $n = 11$). The mean lifetime egg complement of these wasps' twin sisters was 214.18 ± 6.23. The population's RCI, which is the ratio of these values, is estimated at 0.28 ± 0.02, which is quite highly synovigenic. The age-specific realised fecundity curve indicates that females that experience high host availability lay most of their eggs during the first 2 weeks of their adult lives (Fig. 2).

Intraspecific variation in egg maturation schedules *(Experiments 1 and 2)*

The females of Experiment 1 exhibited intraspecific variation in initial egg loads [coefficient of variation (CV) = 0.05]. This variation increased with time (CV = 0.18, 6 days later), as individuals matured additional eggs at different rates (Fig. 1). Accordingly, wasp age significantly influenced the variation in egg loads (GLM, $t = 12.00$, $P < 0.001$ for the effect of age, based on 63 individuals from six clones). The variation in initial egg loads that was recorded in Experiment 2 was highest among daughters of different mothers (estimation of variance components: 49% of the total variance). It was lower among non-clone sisters (30% of the total variance) and lowest among clone-mates (21% of the total variance). The variation in initial egg loads due to clone and to the identity of the clone's mother was significant (nested ANOVA, $F_{8,18} = 3.637$, $P = 0.011$ for mother, $F_{28,53} = 4.319$, $P < 0.0001$ for clone). The mean CV in initial egg loads in Experiment 2 was 0.11 within clones and
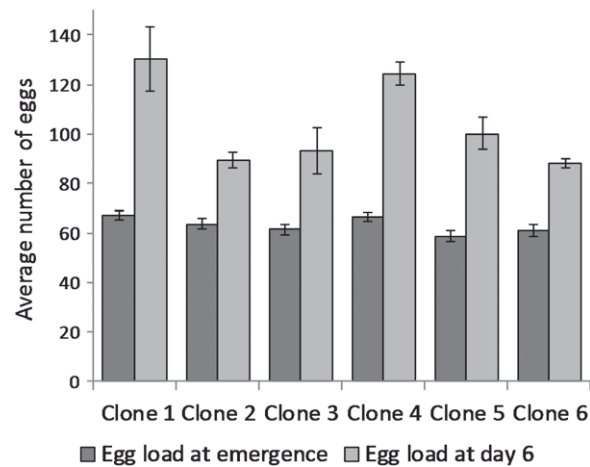

Fig. 1. Average ($\pm$ SE) egg loads at emergence and 6 days after emergence of parasitoids from six different clones, originating from six different mothers.

0.15 between sister clones. The coefficient of variation among non-sister clones was 0.17.

Correlations between the egg maturation schedule, body size and longevity *(Experiment 3)*

Life-history theory predicts a trade-off between resource allocation to somatic and reproductive functions. Thus, individuals with high RCIs are expected to be smaller and have shorter life spans than females with lower RCIs. Contrary to this expectation, the wasps' RCIs were not significantly correlated with their tibia lengths (Pearson's coefficient = 0.326, $P = 0.327$) and with longevity (Pearson's coefficient = -0.446, $P = 0.169$). Body size may confound the relationship between the RCI and life span. To test for this possibility, we calculated a partial correlation between these variables while controlling for tibia length. The correlation remained non-significant (partial correlation coefficient = -0.345, $P = 0.330$).

Average per-clone tibia lengths correlated positively with the average per-clone initial egg loads (Pearson's correlation coefficient = 0.699, $P = 0.017$; Fig. 3). The tibia lengths of the host-exposed females were not significantly correlated with the number of eggs they laid (Spearman's rho = -0.233, $P = 0.491$), a measure of realised fecundity. The longevity of the host-exposed wasps did not correlate with the mean initial egg loads of their twin sisters (Pearson's = 0.195, $P = 0.589$). We also found no correlation between the longevity of the host-exposed females and the number of eggs they laid (Spearman's rho = 0.434, $P = 0.183$), even after correcting for tibia length (partial correlation coefficient = 0.450, $P = 0.192$). However, host-deprived females had a significantly longer life span (mean $\pm$ SE, 28.80 ± 1.25 days) than their host-exposed twin sisters (21.40 ± 2.29 days; paired *t*-test, $t_9 = -3.347$, $P = 0.009$), supporting a survival cost to egg maturation and oviposition. Two lines of evidence suggest that host deprivation reduced egg maturation and/or possibly induced resorption of eggs. First, host-deprived females died with far fewer eggs in their ovaries
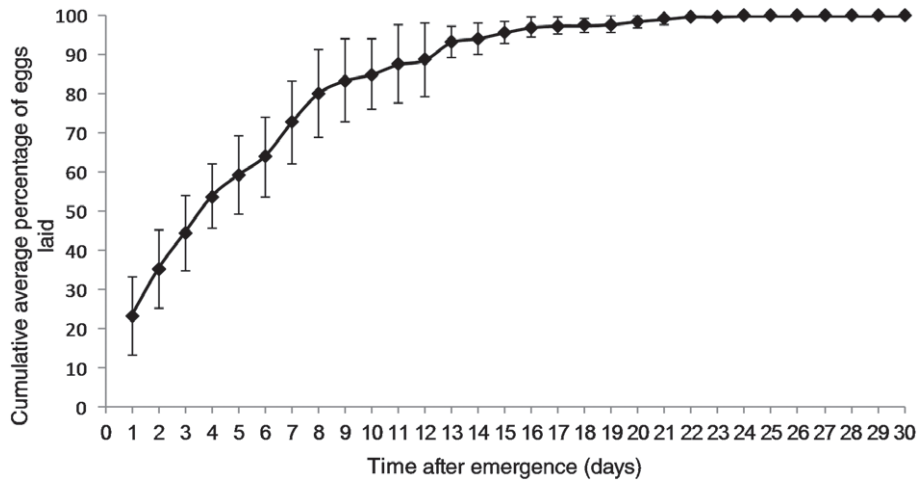
Fig. 2. The cumulative average (± SE) percentage of eggs laid each day by 11 mated females under conditions of high host availability and food *ad libitum*. Females originated from 11 different mothers and were monitored until natural death occurred.
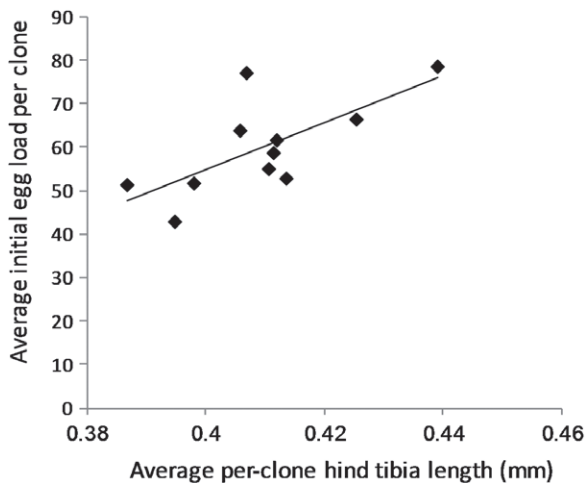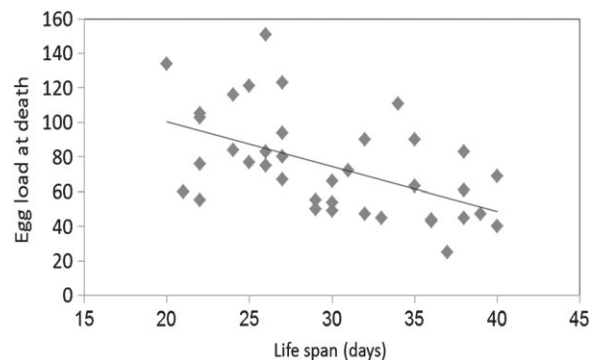

Fig. 3. The correlation between the average egg load at emergence and the average tibia length. Each data point is an average of three clone-mate females. The experiment comprised 11 clones.

Fig. 4. The correlation between the life span of the host-deprived females of Experiment 3 and the number of eggs in their ovaries at the time of their natural death. Each data point represents an individual wasp. We were able to record longevity for 38 females, originating from 10 of the 11 experimental clones.

(75.06 ± 6.61) than they would have produced had they experienced high host availability (number of eggs produced by host-exposed wasps = 214.18 ± 6.23). Second, the egg loads of host-deprived females at the time of death were negatively correlated with their life span (Fig. 4).

Discussion

Our experiments took advantage of the unique clonal development of our study organism. To characterize egg maturation schedules of insects, females need to be dissected shortly after emergence to determine initial egg loads, while fecundity during the adult stage must be measured in other individuals. This necessarily increases the variance in the measured data and reduces its reliability. By using clone-mate females of a

polyembryonic parasitoid species, we aimed to minimise this experimental 'noise' (Experiment 3) and to estimate its magnitude (Experiments 1, 2). Indeed, we found that the variability between clones in initial egg loads was significantly higher than the within-clone variability. This was also reflected in the higher CV of initial egg loads between non-sister clones (0.17) than within clones (0.11). Similar variability levels in initial egg loads were recorded for non-clonal parasitoids in previous studies [e.g. CV = 0.16 in *Ibalia leucospoides* (Fischbein *et al.*, 2013), CV = 0.12 in *Microplitis rufiventris* (Hegazi *et al.*, 2013)]. Nevertheless, some of the egg maturation patterns that were recorded in the present work may be unique to polyembryonic parasitoids. Thus, additional studies of the intraspecific variability in egg maturation, focusing on non-clonal species, are warranted.

The age–specific fecundity curve (Experiment 3) shows that *C. koehleri* females vary in adult egg maturation schedules, in addition to the variation that we documented in their initial egg loads. The species has been previously considered to be

pro-ovigenic (Baggen & Gurr, 1998). Our work updates this view and shows that females mature fewer than one-third of their eggs, on average, before the adult stage. Thus, it is much more synovigenic than the related species *C. floridanum* (OI = 1; Jervis *et al.*, 2001) and *C. bakeri* (OI ∼ 0.8; Saeki & Crowley, 2013). Similar re-examinations, using appropriate protocols, have improved our knowledge of the ovigeny of other parasitoid species as well (Carbone & Rivera, 2003; Riddick, 2005; Irvin & Hoddle, 2009; Boivin & Martel, 2012; Boivin & Ellers, 2016). Our estimate of *C. koehleri*'s RCI is based on dissection of females on their first day as adults. Several hours may have passed between adult emergence and egg-load determination. During this time, the females may have matured some additional eggs. Thus, *C. koehleri*'s mean OI is possibly even lower than 0.28, our estimated mean RCI.

The variability in *C. koehleri*'s egg maturation schedule clearly has a non-genetic component. Initial egg loads varied among clone-mate individuals that are genetically identical (coefficient of relatedness, $r = 1$). This variation must reflect differences in the developmental conditions experienced by individuals within the clone's common host, or differential epigenetic programming. Sister and non-sister clones experienced higher environmental heterogeneity, as each clone developed in a different host. Nevertheless, initial egg loads were significantly more variable among non-sister clones than among sister clones. Sister clones are highly related to one another ($r = 0.75$), suggesting a possible genetic basis for their lower variability in initial egg loads compared with non-sister clones. This possibility is compatible with a previous study, which demonstrated genetically based intraspecific variation in the temporal pattern of egg maturation in the parasitoid *Trichogramma brassicae* (Trichogrammatidae) (Wajnberg *et al.*, 2012). However, an alternative interpretation cannot be ruled out: as all sister clones were reared on the same potato, their developmental environment was possibly more uniform than that of non-sister clones, each of which was reared on a different tuber. The variability in initial egg loads among non-sister clones could also result from maternal effects (Mousseau & Fox, 1998), which have translated the different environments experienced by mothers into phenotypic plasticity in the traits of their offspring. Maternal effects on life-history traits were previously demonstrated in *C. koehleri* (Morag *et al.*, 2011a,2011b), but their role in affecting initial egg loads has not been tested so far. Recent work on the pro-ovigenic parasitoid *Anagrus erythroneurae* (Mymaridae), however, found no evidence for maternal effects on their daughters' fecundity (Andreazza & Rosenheim, 2015).

Our work adds to the scant information on the trade-offs between reproduction and survival at the intraspecific level. Early reproductive investment (manifested by high initial egg loads) was shown to be negatively correlated with body size and longevity in parasitoids in interspecific comparisons (Jervis *et al.*, 2001, 2003). Previous comparisons between populations (Ellers & van Alphen, 1997; Phillips & Baird, 2001) also found a negative relationship between body size and early-life reproductive allocation. However, these studies did not look at individual variation within populations, while our experiment focuses on this variation. Unlike the earlier-cited species- and population-level studies, we found that initial egg loads increased with body size, but that the females' lifetime fecundity and adult longevity did not. Per-clone RCIs, which were determined in Experiment 3, were not correlated with the per-clone average body size. There was also no significant negative correlation between the per-clone RCI and the survival of the host-exposed females, after correcting for body size. Thus, early investment in reproduction does not seem to carry a cost in terms of adult survival or fecundity at the individual wasp level. Nevertheless, host-deprived females survived longer than females that were supplied with unlimited hosts. This suggests that the total lifetime reproductive effort, rather than pre-adult egg maturation, was traded off with life span in our experiment.

Females in our experiment fed on honey *ad libitum*, so as to let them realise their fecundity potential. This unlimited feeding might have masked the hypothesised trade-off between body size and adult survival, and other trade-offs involving vigour-related traits. Honey feeding may have supplied the wasps in our study with sufficient nutrients to sustain both adult reproduction and somatic maintenance, alleviating the need to invest in one of the functions at the expense of the other. Indeed, *C. koehleri* females that fed on honey or floral nectar had higher longevity and a longer period of egg-laying than females that had only access to water (Baggen & Gurr, 1998). Moreover, in the congener *C. bakeri*, adult survival under starvation increased with body size (Saeki & Crowley, 2013). On the other hand, egg loads of sugar-fed *C. bakeri* females, assessed at three time points during adult life, did not correlate with body size. Similarly, the longevity of honey-fed *C. floridanum* adults were unaffected by size (Ode & Strand, 1995). Taken together, these findings indirectly support a role for adult feeding in relaxing the links between size, survival and fecundity. In agreement with this interpretation, feeding on carbohydrate sources was found to promote egg maturation and to prevent egg resorption in some parasitoids (Heimpel *et al.*, 1997; Lee & Heimpel, 2008). The parasitoids in our experiment were also continually supplied with hosts. This probably further reduced the potential advantages of larger females in mobility and host-searching (Segoli & Rosenheim, 2015), which are probably involved in the trade-off between reproduction and survival in the field.

Egg loads in host-deprived females increased from ∼60 to ∼100 over the first 6 days (Fig. 1), but were only ∼75 at the time of death, at around 29 days. This number is much lower than the total number of eggs (>200) matured by host-exposed females throughout their lives. This suggests that host encounters promote egg maturation, while host deprivation arrests egg maturation and possibly even induces egg resorption. To our knowledge, there is no previous evidence of egg resorption in *C. koehleri*. Whether this reduction in egg loads of host-deprived females is a result of oosorption, blocked maturation or both at the same time remains unclear and requires additional testing.

Similar mechanisms to adjust egg loads are known from other synovigenic parasitoids, and were proposed to reduce the risks of host and egg limitation (Papaj, 2000). In spite of these potential mechanisms to regulate egg loads, all of the host-exposed parasitoids died with some (26.64 ± 6.37) mature eggs in their ovaries. In other words, they encountered host limitation. This is consistent with the suggestion that synovigenic parasitoids alternate between periods of host limitation and of egg limitation,

because of the complex dynamics of egg maturation, oviposition and resorption (Casas *et al.*, 2000). It is also compatible with our finding that egg-laying reduced the females' survival, possibly increasing the probability of death while still carrying eggs in their ovaries. *C. koehleri*'s eggs are small in volume and yolk-deficient, and therefore probably do not require high energetic investment. This can reduce the costs of producing more eggs than are eventually oviposited.

The absence of a trade-off between fecundity and adult survival that is indicated by our results, along with *C. koehleri*'s polyembryonic development, theoretically enable it to produce very high numbers of offspring (potentially up to 200 clones of 40 wasps each, i.e ∼8000). This consideration suggests that population growth in this species is limited by egg or larval survival, rather than by adult reproduction or longevity. Jervis *et al.* (2008) suggested that species that parasitise early-instar hosts are more susceptible to mortality by natural enemies or abiotic factors in their early life stages, and are thereby selected for higher fecundity. This idea fits well with *C. koehleri*'s life-history traits – they parasitise their hosts as eggs and go through long development inside the host larvae. In addition, females often super-parasitise eggs. This results in competition within the host and death of some of the competitors (Segoli *et al.*, 2009b). Indeed, a study on the same laboratory population found that <40% of parasitised hosts develop into wasp mummies, even under optimal conditions (Keinan *et al.*, 2012). Under these constraints, *C. koehleri* may be selected mainly for life-history traits that increase larval survival, rather than adult longevity or fecundity. This possibility requires further experimental testing.

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