

Linking reproductive and feeding strategies in the parasitoid *Ibalia leucospoides*: does feeding always imply profit?

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Abstract Resource acquisition and allocation to different biological functions over the course of life have strong implications for animal reproductive success. Animals can experience different environmental conditions during their lifetime, and this may play an important role in shaping their life-history and resource allocation strategies. In this study we investigate larval and adult resource allocation to reproductive and survival functions in the parasitoid wasp *Ibalia leucospoides* (family Ibalidae). The pattern of larval resource allocation was inferred from the relationship between adult body size and ovigeny index (OI; a relative measure of investment in early reproduction determined as the ratio between the initial egg load and the potential lifetime fecundity); and adult resource allocation was explored through the influence of adult feeding on reproduction, maintenance and metabolism, in laboratory experiments. Food acquisition by this parasitoid in the wild was also examined. The relationship between size and OI was constant, suggesting no differential resource allocation to initial egg load and potential lifetime fecundity with size. This finding is in line with that predicted by adaptive models for the proovigenic egg maturation strategy ($OI = 1$). Despite of this, *I. leucospoides* showed a high OI of 0.77, which places this species among the weakly synovigenic ones ($OI < 1$). Adult feeding had no effect on post-emergence egg maturation. However, wasps extended their lifespan through feeding albeit only when food was provided ad libitum. Although the information we obtained on the feeding behaviour of free-foraging wasps is limited, our results suggest that food intake in the wild, while possible, may not be frequent in this parasitoid. We discuss the results relative to the environmental factors, such as reproductive opportunities and food availability, which may have driven the evolution of larval and adult pattern of resource allocation in parasitoids.

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Introduction

A question of great interest to ecologists is how natural selection shapes life history traits and behaviours according to the ecological environment in which an animal lives. Organisms may adopt different energy allocation strategies in relation to the abundance and distribution of resources to enhance reproductive success (Ellers and van Alphen 1997). Consequently, differences in energy allocation towards life-history traits, such as survival and reproduction, result in the observed inter and intraspecific differences in life histories (Jervis et al. 2008).

In parasitoids (insects that lay eggs in or on other arthropods eventually killing them), like in other holometabolous insects, nutritional resource allocation to competing metabolic needs occurs during larval and adult stages. In this way, insects can use both stored larval nutrients (i.e. capital resources) and nutrients acquired through adult feeding (if this actually takes place) to support biological functions such as reproduction, survival and locomotion (Boggs 1981; Boggs and Freeman 2005; Jervis et al. 2008). Both environmental (e.g. resource abundance and distribution) and internal factors (e.g. body size, energetic reserves, egg load) determine the nutrient acquisition and allocation strategies adopted by insects throughout their life cycle (Jervis et al. 2008).

Patterns of larval resource allocation can be inferred from knowledge of the relationship between adult body size and ovigeny index (OI). OI—defined as the ratio between the initial egg load and the potential lifetime fecundity—has been suggested by Jervis and Ferns (2004) as a measure of the relative distribution of insect capital resources to reproduction and other functions. The numerator (the initial egg load) represents the initial energy investment in reproduction and the denominator (potential lifetime fecundity) represents the initial reserves (fat body storage), which can contribute to maintenance and lifetime fecundity. However, contrary to the broadly documented intraspecific positive relationships between size and life-history traits such as initial mature egg load, potential and realized fecundity or longevity, the OI countervaries with body size (Honek 1993; Visser 1994; Ellers et al. 1998, 2001; Jervis et al. 2003; Ellers and Jervis 2003). One explanation is a differential resource allocation; namely, while the two components involved in the OI positively correlate with body size within species, the proportionate increase of initial egg load with body size is smaller than the proportionate increase in potential fecundity, this resulting in a decline of the OI with body size (modeling: Ellers and Jervis 2003; Jervis and Ferns 2004; empirical evidence: Olson and Andow 1998; Thorne et al. 2006).

This explanation is suitable for synovigenic egg maturation strategy; that is, when females emerge with no mature oocytes (extreme synovigeny, $OI = 0$), with only a few (strong synovigeny) or most of them mature (weakly synovigeny, $OI < 1$), while the remaining eggs are matured throughout adult life. In proovigenic species in which females emerge with their entire complement of mature eggs, the initial egg load always equals the potential lifetime fecundity ($OI = 1$) (Jervis et al. 2001). In this case there is no room for a differential resource allocation. Larger individuals can allocate more capital resources to reproduction and, consequently they emerge with a larger mature egg load, however their OI will not alter with body size (Ellers and Jervis 2003). Thus the effects of body size on

the relative allocation of resources to the initial egg load, potential lifetime fecundity and ultimately on variation in the OI should be different from those corresponding to synovigenic strategy.

As adult free-living animals, parasitoids can feed on their hosts (i.e. host-feeders) and/or on non-host foods. The latter, mainly comprises floral and extra-floral nectar and honeydew; and more rarely, food includes pollen or other materials as fungi or fruit exudates (Jervis et al. 1993, 2008; Rivero and Casas 1999). From an array of laboratory studies it is widely known that several species of parasitoids either greatly enhance their longevity, fecundity and flight activity, or change their host searching behavior through feeding [Takasu and Lewis 1995; Stapel et al. 1997; Heimpel et al. 1997; Olson et al. 2000; Siekmann et al. 2001; Giron et al. 2002; Mitsunaga et al. 2004; Wanner et al. 2006; see review by Bernstein and Jervis (2008)]. In metabolic terms, flight is the most costly of all activities for many insects, and generally, in the Hymenoptera, carbohydrates constitute the main fuel burnt during this activity (Chapman 1998). Fecundity can increase by feeding through various mechanisms. Egg load may change as a direct result of feeding because of a reduction of the egg resorption rate (Heimpel et al. 1997) or an increase of egg maturation (Tylianakis et al. 2004; Bezemer et al. 2005). Likewise, access to adult food sources can increase the number of offspring by increasing host attack rates (Schmale et al. 2001). Eventually, feeding can indirectly affect parasitoids' realized fecundity through an increase in longevity which provides more time to mature and lay eggs.

Despite the potential positive effects that feeding may have on key life-history traits, as evidenced by laboratory experiments, knowledge of food foraging in the field is still limited (Sisterson and Averill 2002; Lee and Heimpel 2008a; Desouhant et al. 2010). In nature, the availability of food sources can be highly variable in space and time. Actually, in many instances feeding opportunities may be absent where hosts are found or be so scarce that it is not worth searching for them. Several theoretical models have been developed inquiring how female parasitoids should distribute their foraging time and energy in food and host searching in order to maximize their fitness gain (Sirot and Bernstein 1996; Tenhumberg et al. 2006; Bernstein and Jervis 2008).

Ibalia leucospoides Hochenwarth (Hymenoptera, Ibalidae) is a solitary, koinobiont parasitoid of the primitive xylophagous woodwasp *Sirex noctilio* Boidin (Hymenoptera: Siricidae) (Chrystal 1930; Madden 1968; Spradberry 1974). *S. noctilio* is native to Mediterranean Europe; however, in the last century, it successfully invaded Australia, New Zealand, South Africa, South America, and more recently North America (Hoebeke et al. 2005). In most regions where this woodwasp established, it rapidly became an important pest of pine tree forestations mainly due to its outbreak population dynamics during which tree mortality can be severe (Corley et al. 2007; Corley and Villacide 2011). *I. leucospoides* is one of several biocontrol agents adopted for this pest management (Hurley et al. 2007; Corley and Bruzzone 2009). This parasitoid, also native to Europe, was introduced into Australasia in the early 1960s. Since then, it has become established through the invasion range of its host, *S. noctilio*, mostly via accidental introductions (Madden 1988).

Because of its practical importance, there is a growing research effort into the biology and ecology of this parasitoid species. On the one hand, several studies have focused on its host location and patch use behaviour (Madden 1968; Spradberry 1974; Martínez et al. 2006; Fernández-Arhex and Corley 2005, 2010; Corley et al. 2010; Fischbein et al. 2012). On the other hand, recent work has explored the role of food consumption on different aspects of parasitoid behaviour (Corley et al. 2010; Fischbein et al. 2011; Pietrantuono et al. 2011). Although feeding behaviour of *I. leucospoides* is not known in its native environment, in cultivated pine forests as in many invaded regions such as Patagonia, *I.*

leucospoides potential feeding opportunities are rare. Food sources are scarce where hosts are found, namely, within the pine plantations and their immediate surroundings. If feeding is important for full expression of host searching behaviour in this species, food scarcity may be a handicap for parasitoid performance in the plantations. Hence, the effects of food intake on flight potential, host patch exploitation rules and on the olfactory response to cues associated with food- and host-foraging have been studied in laboratory conditions. Results show that providing adults with food in captivity does not affect either their dispersal capacity or their host patch exploitation (Corley et al. 2010; Fischbein et al. 2011). Moreover, feeding has little influence on female decision-making (Pietrantuono et al. 2011). However, these circumstances do not rule out that *I. leucospoides*, a non-host feeding parasitoid, may still need food to cover other energy demanding functions such as somatic maintenance and egg maturation.

In this study, we investigated how larval and adult resource allocation to reproductive and survival functions is shaped by host and food availability in the parasitoid *I. leucospoides*. We inferred the pattern of larval resource allocation from the shape of the relationship between adult body size and OI. In addition, the consequences of adult food consumption for longevity and reproduction were determined in laboratory experiments. We hypothesized that the higher the initial energy investment in reproduction (high OI), the less dependent females will be on external nutrient acquisition for sustaining egg maturation after adult emergence. However, we expected that, as a non-host feeding parasitoid *I. leucospoides* extends its lifespan through adult feeding. In the first part of this study, we estimated *I. leucospoides* initial egg load, lifetime fecundity and OI and describe their relationship with body size. Secondly, we studied the effects of food provisioning on longevity by examining whether: (a) variations in the timing (duration) during which female wasps are exposed to food (e.g. 1 day or ad libitum) or (b) the age when wasps are provisioned with food (at emergence, middle age or later in their life) affects adult lifespan. Thirdly, we tested the influence of adult feeding on egg maturation. Finally, we examined the influence of food consumption on parasitoid metabolism and attempted to find out whether food acquisition actually occurs in field conditions. This study can shed light on energy allocation strategies and potential explanation concerning the evolution of egg maturation strategies. Lastly, attempts to gain insight into parasitoids use of food in the wild can further our knowledge into parasitoid feeding in agricultural habitats.

Materials and methods

Ibalia leucospoides female wasps used in this study were obtained from pine (*Pinus contorta*) logs collected in the field, at several heavily attacked plantations located in NW Patagonia, Argentina (42°02'S, 71°56'O). Once felled, trees were cut into 1 m-long logs and kept individually in locker-type cages until insect emergence. Each morning, newly emerged parasitoid females were collected from the cages and immediately placed in individual plastic vials in order to be randomly assigned to the different treatments.

Estimation of the initial egg load, lifetime fecundity and ovigeny index

The number of initial mature eggs (initial egg load) was tallied from recently emerged females ($n = 21$) stored in diluted ethanol 60 % V/V until the day of dissection. As Ellers and Jervis (2003) noted, maximum potential fecundity can be estimated as the mean total egg production by females under the most favourable conditions. Under this assumption,

all eggs of 24 day old females (mean lifespan of food-deprived wasps, see below), fed ad libitum and maintained in the absence of hosts were counted ($n = 11$); and then, mean total egg production computed as an estimate of potential lifetime fecundity. Finally, the OI was estimated as the ratio between the mean initial egg load and mean total egg production. Hind tibia length, a standard measurement of size in parasitoids (Jervis et al. 2005), was measured for each female under a binocular lens with a caliper.

Longevity

In order to study the effects of food provisioning on longevity we examined whether (a) variations in the timing (duration) during which female wasps are exposed to food (e.g. 1 day or ad libitum) or (b) the age at which wasps are provisioned with food (at emergence, middle age or later in their life) affects adult lifespan. For this, newly emerged female parasitoids were randomly assigned to each of the following five treatments: (1) access to water only (*unfed wasps*); (2) ad libitum access to diluted honey solution (30 % honey-distilled water solution; *fed ad libitum*); access to diluted honey solution for: (3) 1 day at emergence (*fEd*); (4) 1 day at day 7 (*f7d*) and; (5) 1 day at day 14 (*f14d*). In treatment two, food was replenished every day. All wasps tested were kept individually in plastic vials under semi-controlled temperature, humidity conditions (temperature 21 ± 0.1 °C; RH 54 %) and LD 16:8 h until death.

Egg maturation

To examine the effects of sugar feeding on the egg complement at different ages, newly emerged female parasitoids were randomly assigned to one of two feeding treatments: (1) fed ad libitum with diluted honey solution or (2) with access to water only. In both treatments, wasps were deprived from hosts and males. Females were dissected at each of the following ages: at birth, 3, 14, 24 days since emergence and at death. The experiment was ended 24 days post-emergence because this time span corresponds to the mean lifespan of food-deprived wasps recorded in the longevity experiment (see below, “Results” section). Egg load at death was estimated for unfed wasps only. Since, in the current experiment unfed wasps did not survive as long as in the longevity tests, egg load at death of unfed wasps was estimated at day 19, which was the mean lifespan for this egg maturation assay.

In treatment fed ad libitum, food was replenished every day. All wasps were kept individually in plastic vials under LD 16:8 h and semi-controlled conditions of temperature (21 ± 0.1 °C) and humidity (RH 54 %) up to the looked-for age was reached. Then, females were preserved in alcohol 60 % until dissection. Only mature eggs were counted, because the degree of egg maturation may vary with age and exposure to food whereas the number of immature eggs is considered to be fixed at emergence.

To explore realized ovipositions, mean egg load of field captured wasps was also considered in this assay. Females were caught with traps, baited with α - and β -pinene, placed within and in the vicinity of pine stands, and checked every 15 days. Collected wasps were preserved in alcohol 60 % until dissection.

Feeding status of females foraging in the wild

To gain an insight into *I. leucospoides* feeding under field conditions, the quantification of carbohydrate levels of wasps collected in the field was carried out using a modification of

the colorimetric techniques developed for mosquito analysis (Van Handel 1985; Van Handel and Day 1988). This method has been used efficiently to compare variations in sugar content between different feeding treatments in other studies (Casas et al. 2003; Pelosse et al. 2007; Desouhant et al. 2010). Since extra-floral and floral nectar sugars are predominantly glucose, fructose and sucrose as in a honey meal, we measured levels of total sugar content in field wasps and laboratory females whose feeding regime was known. Then, by comparing the reference values of laboratory-reared individuals with those of captured wasps, we estimated whether or not wasps fed on sugary sources in the field.

Reference values and wasps captured in the field

To record quantitative changes in total sugar content of laboratory individuals whose feeding regime was known and establish reference values, newly emerged females were randomly assigned to each of the following feeding treatments: emergence; one, seven and 14 days unfed (*unfed1*, *unfed7*, *unfed14*); one, seven and 14 days fed ad libitum since emergence (*fed1*, *fed7*, *fed14*); 14 days fed and starved (7 days fed ad libitum and then 7 days unfed; *fed&starved*); 24 days fed ad libitum (*fed24*) and; starved to death. On the one side, the measurements of sugar content allows visualizing quantitative changes in capital reserves through time in starved wasps; and on the other, they are a reliable indicator of feeding occurrence because of their increase with nectar or honey food ingestion. The experiment was ended 24 days post-emergence for fed wasps for the same reason as in egg maturation assay. Finally, since total sugar content of individuals decreases when feeding is followed by food deprivation, “fed then starved” wasps may enable differentiating between parasitoids that had consumed sugars in a relatively distant past and those that have not fed at all. All records of sugar content were expressed relative to wasp weight at time of emergence. The sugar-rich meal consisted of a 30 % honey-distilled water solution. Wasps were conserved at -20°C up to the day of biochemical analysis.

Freely foraging wasps were captured in pine tree plantations located in Patagonia ($42^{\circ}02'S-71^{\circ}56'O$), Argentina, from late January to late March 2010 (wasp flight season). Females were caught while flying using an insect net and immediately placed in cold (camping coolers) to be carried within a few hours to the laboratory where they were then frozen at -20°C .

Biochemical analysis

For the sugar analysis, 200 μl of 2 % sodium sulphate were added to each sample (laboratory or field individuals). After mixed for 1 min at 30 beats/s, 1 ml of methanol was added and samples then vortexed and centrifugated for 2 min at 12,000 rpm. As sugars remain dissolved in the supernatant, 700 μl of this was transferred into an Eppendorf tube and evaporated to nearly 200 μl at ambient temperature. This aliquot was collected and transferred into a volumetric flask in which anthrone reagent was added until 25 ml were completed. Finally, a fraction of this solution was placed at 90°C for 15 min, then cooled and read in a spectrophotometer at 625 nm to estimate the total sugar content for each female. Body sugars are mainly constituted of glucose, however, other sugars such as, fructose, trehalose or melezitose are present. Yet, as non-glucose sugar concentrations are usually low in parasitoids, we considered total sugar content as equivalent of glucose (Desouhant et al. 2010). A calibration curve with a standard solution of glucose (0.1 mg/ml in ethanol 25 %) allowed us to transform absorbance into concentrations.

Statistical analysis

The covariations between initial egg load and total egg production with body size were analyzed by Pearson's correlation test. To study how the OI is related to body size, a Spearman rank correlation coefficient (r_s) was estimated and a bootstrap analysis was carried out. Since both initial egg load and total egg production can only be measured destructively, pairs of similar-sized individuals were identified, for which initial egg load or total egg production were measured. For each pair, the OI and mean tibia length was calculated. By using an a priori size interval, we ensured to constrain the size differences between individuals. We obtained 10 pairs of individuals that met the size interval in which no datum was used more than once. For all pairs, the Spearman rank correlation coefficient between OI and tibia length was estimated and bootstrapped 1,000 times to produce a mean and 95 % bootstrapped confidence interval (Thorne et al. 2006). The null hypothesis was that the OI remains constant with body size ($r_s = 0$) and the alternative, that the OI declines with body size ($r_s < 0$). The proportion of coefficients less than or equal to 0 tests the null hypothesis (the P value). The bootstrapping analysis was repeated with different size intervals to ensure that the result did not depend on this.

The influence of food supplying on longevity of female wasps was analyzed using Cox proportional hazards model and a modified matrix of contrasts to perform a posteriori tests of coefficients. Egg load was analyzed using a linear model and an appropriate matrix of contrasts. To study changes in total sugar content through the time in starved laboratory-reared individuals and the difference in body sugars between recently born and dead wasps, linear models were used. In addition, to examine the effect of feeding treatment (2 levels) and age (3 levels) on body sugars of reference individuals, a generalized linear model assuming Gamma distribution of residuals and an inverse link function was used. A similar model was used to compare the total sugar content of wild-caught female wasps with laboratory reference values. In this case, the data were organized such as, that as a consequence of the contrast matrix used, wild-caught animals constituted the focal group against which all different feeding treatments were compared. The statistical differences between the focal group and laboratory treatments are given by the statistic of the regression coefficients and their significance levels. Finally, in all cases, residuals were examined to confirm that the final models accurately fitted the data. All data analyses were done using the R statistical environment (R Development Core Team 2011).

Results

Initial egg load, lifetime fecundity and ovigeny index

The initial egg load was significantly and positively correlated with body size (mean \pm standard error (SE): 466.76 ± 17.13 eggs; $r^2 = 0.470$, $t = 2.327$, $df = 19$, $P = 0.031$; Fig. 1), as was total egg production (mean \pm SE: 607.91 ± 27.46 eggs; $r^2 = 0.731$; $t = 3.216$, $df = 9$, $P = 0.0105$; Fig. 1). The slope of the size–initial egg load relationship did not significantly differ from the slope of size–total egg production relationship (test for different slopes: $t_{(2,30)} = 0.36$; $P = 0.722$). The OI remained constant with hind tibia length for both size intervals tested: 0.030–0.035 cm (mean $r_s = 0.141$; 95 percentile 0.763; $P = 0.668$) and 0.035–0.040 cm (mean $r_s = 0.271$; 95 percentile 1; $P = 0.719$). The mean OI computed for *I. leucospoides* was 0.77.

Fig. 1 Total egg production (our measure of lifetime potential fecundity) and initial egg load as a function of body size in *Ibalia leucospoides*. The regression equations are: lifetime potential fecundity = $1,545.7 \times$ hind tibia length (cm) – 130.9 and, initial egg load = $1,284.4 \times$ hind tibia length (cm) – 185.6

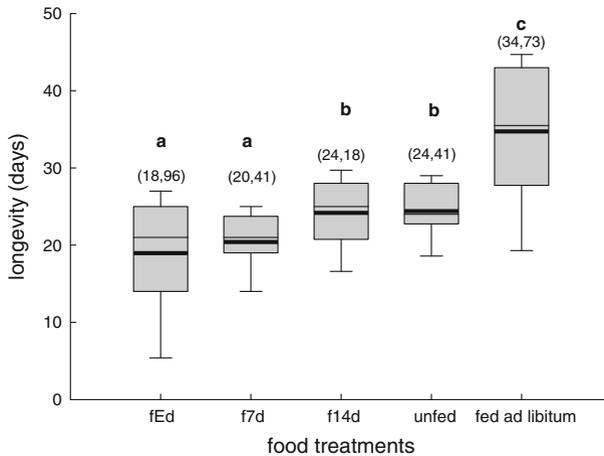
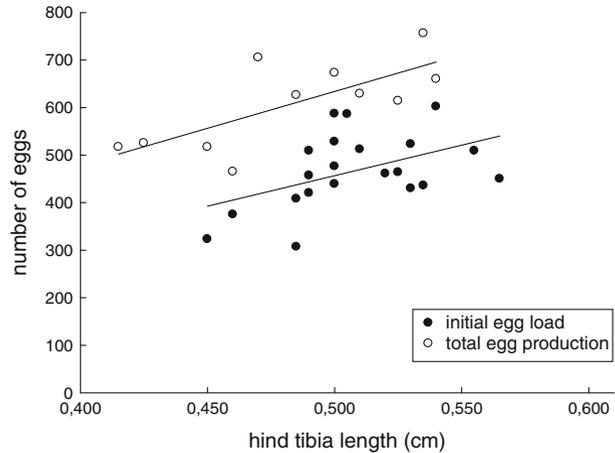


Fig. 2 Effects of the frequency and temporal access to food on longevity in *I. leucospoides* females. The thin horizontal line in each box plot marks the median of the sample and the thick horizontal line marks the sample mean. The mean for each group of wasps is shown above each box. Significantly different treatments are shown with different letters (a, b, c). *fEd*: fed 1 day at emergence (n = 23); *f7d*: fed 1 day at day 7 (n = 24); *f14d*: fed 1 day at day 14 (n = 22); *unfed*: access to water only (n = 22) and *fed ad libitum*: access to diluted honey solution (n = 22)

Longevity

Females with ad libitum access to sugar-rich food gained, in average, 10 days of life compared with starved wasps (*fed ad libitum*, median survival age: 35 days; *unfed*, median survival age: 24 days) (Fig. 2). There was no significant difference in longevity between females fed during one day at emergence or at day 7 ($z = -0.687$; $P = 0.492$). Likewise, females with 1 day honey solution access at day 14 had similar longevity than unfed wasps ($z = -0.313$; $P = 0.755$). However, longevity significantly decreased (4.61 days) when female wasps were fed early in life (*fEd* and *f7d*) compared with unfed and fed wasps at day 14 ($z = -6.546$; $P < 0.0001$).

Egg maturation

The egg load of *I. leucospoides* ranged from 178 to 806 eggs per female (field-caught wasps not included), a 4.5-fold difference between extremes. Sugar-rich food had no significant effect on egg load at any given age (fed vs unfed wasps of 3 days old: $t = 0.857$, $P = 0.394$; fed vs unfed wasps of 14 days old: $t = -0.234$, $P = 0.815$; and 24 days fed vs unfed wasps at death: $t = -0.715$, $P = 0.476$; Fig. 3). However, age had a significant positive effect on the egg complement (emergence vs 3 days old: $t = -7.119$, $P < 0.0001$; 3 vs 14 days old: $t = -5.280$, $P < 0.0001$; Fig. 3) until 14 days old when there were no significant differences between wasps 14 days old and those 24 days and dead wasps ($t = 0.640$, $P = 0.523$; $t = -0.089$, $P = 0.929$, respectively; mean lifespan for unfed wasps in this assay \pm SE: 18.71 ± 1.37 days). Finally, field captured females had a lower number of mature eggs than newly emerged females ($t = -6.174$, $P < 0.0001$).

Ovipositions in the field previously to capture were estimated as the difference between the mean total egg production of reference females and the average egg load of females captured in the wild. Total egg production of reference females was obtained as the egg load average of 14 and 24 days old females, given that, egg load remained constant after 14 days in the absence of hosts (mean \pm SE: 603.74 ± 16 eggs, $n = 43$). Field and laboratory reference wasps were of equal in size (Mann–Whitney U Test: $W = 165$, $P = 0.49$). As a result, wild caught females would have discharged, in average, 276.19 eggs.

Changes in total sugar content values for laboratory-reared individuals

To examine the influence of sugary food consumption on parasitoid metabolism and establish reference values, quantitative changes in total sugar content of laboratory individuals whose feeding regime was known were studied.

The interaction between feeding treatment and age had a significant effect on total sugar content for females of 1, 7 and 14 days old ($df = 2$; $\chi^2 = 4.854$; $P < 0.001$). As expected, the access to food ad libitum produced an increase in total sugar content. One day old fed wasps experienced an increase in body sugars of 29 % compared to one day old unfed

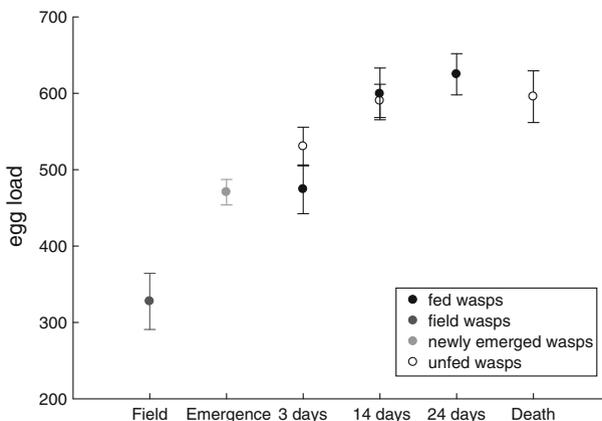


Fig. 3 Egg load (mean \pm SE) of *I. leucospoides* females as a function of age, in the absence or presence of food ($n_{\text{field}} = 9$, $n_{\text{emergence}} = 22$, $n_{3 \text{ days unfed}} = 15$, $n_{3 \text{ days fed}} = 15$, $n_{14 \text{ days unfed}} = 15$, $n_{14 \text{ days fed}} = 15$, $n_{\text{at death/unfed}} = 14$, $n_{24 \text{ days fed}} = 13$)

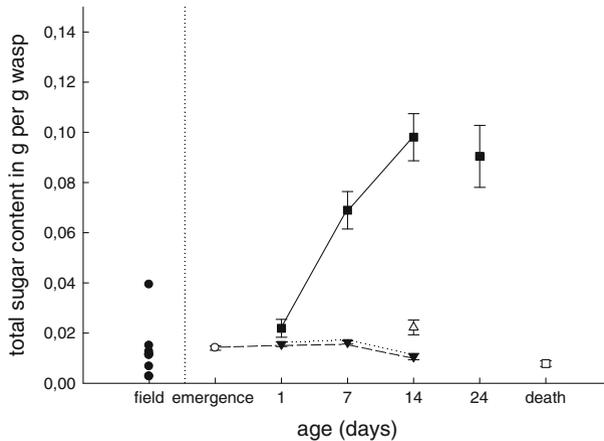


Fig. 4 Total sugar content (mean \pm SE) as a function of age in *I. leucospoides* females. *Open circle* emergence females ($n = 16$); *filled triangles* 1 ($n = 14$), 7 ($n = 15$), 14 ($n = 17$) days old unfed females; *Open triangle* 14 days old fed and starved females ($n = 15$); *full squares* 1 ($n = 16$), 7 ($n = 14$), 14 ($n = 15$) and 24 ($n = 17$) days old wasps fed ad libitum; *open squares* females at time of death ($n = 20$). Field captured wasps ($n = 9$) are represented as individual data with *full circles*

wasps. Likewise, the total sugar content of 7 days old fed females was four times larger than the quantity recorded for 7 days old unfed wasps. Finally, 14 days old fed females had nine times more total sugar content than unfed ones of the same age (Fig. 4).

Age had a significant effect on total sugar content for starved females ($F_{(3,58)} = 6.929$, $P < 0.001$). Fourteen days unfed females had a significantly lower total sugar content than recently emerged females, 1 day and 7 days old unfed females ($t = 2.572$, $P = 0.0127$; $t = 3.544$, $P < 0.001$; $t = 4.176$, $P < 0.001$; respectively). However, similar quantities of body sugars were observed in newly emerged, 1 day old and 7 days old unfed females (emergence vs unfed1, $t = 1.047$, $P = 0.299$; emergence vs unfed7, $t = 1.624$, $P = 0.109$; unfed1 vs unfed7, $t = -0.539$, $P = 0.591$) (Fig. 4). The decline of total sugar content between birth and death was of 44.68 % ($F_{(1,34)} = 17.820$; $P < 0.001$).

Total sugar content of wild-caught female wasps

To find out whether females acquire food when freely foraging in field conditions, we compared the sugar content of wild-caught individuals with laboratory reared reference females. Capital resources of laboratory-reared individuals should be equivalent to those of wild wasps. Note that laboratory parasitoids were obtained from infested logs collected in the field and then stored under natural conditions until insect emergence.

The total sugar content of females captured in the wild (mean \pm SE: 0.0126 ± 0.0036 g body sugars/g wasps) did not significantly differ from the laboratory reference values of starved females, regardless of age ($t = -0.548$, $P_{\text{emergence}} = 0.584$; $t = -0.982$, $P_{\text{unfed1}} = 0.327$; $t = -1.202$, $P_{\text{unfed7}} = 0.231$; $t = 0.824$, $P_{\text{unfed14}} = 0.411$) (Fig. 4). Still, field wasps had significantly higher total sugar content than wasps at death ($t = 2.472$, $P_{\text{death}} = 0.0145$). Finally, field captured wasps showed a total sugar content significantly lower than that recorded for females fed under laboratory conditions, irrespective of age ($t = -2.374$, $P_{\text{fed1}} = 0.018$; $t = -4.889$, $P_{\text{fed7}} < 0.001$; $t = -2.415$, $P_{\text{fed\&starved}} = 0.0169$; $t = -5.241$, $P_{\text{fed14}} < 0.0001$; $t = -5.175$, $P_{\text{fed24}} < 0.0001$).

Only one out of the nine field captured wasps showed a high sugar content (0.0393 g body sugars/g wasps). This was twice the observed value for the individual with the highest sugar content at emergence (teneral level) suggesting that this wasp had probably fed on a sugar-rich source. Thus, the preceding results would be affected by this single data. By removing this individual from the analysis the results were different. Contrary to the former results, the total sugar content of females captured in the wild did significantly differ from the laboratory reference values of young unfed females of 1 day old and 7 days old ($t = -2.247$, $P_{\text{unfed1}} = 0.0260$; $t = -2.432$, $P_{\text{unfed7}} = 0.0161$) and, marginally differed from the recently emerged wasps ($t = -1.896$, $P_{\text{emergence}} = 0.059$). That is, field caught females average sugars were 1.5, 1.69 and 1.77 lower than reference values at emergence, 1 day old and 7 days old starved females, respectively. In addition, body sugars of wild females did not significantly differ from reference values of fourteen day old unfed wasps and wasps at death ($t = -0.700$, $P_{\text{unfed14}} = 0.485$; $t = 0.838$, $P_{\text{death}} = 0.403$). Freely foraging wasps still showed a total sugar content significantly lower than the content of females fed under laboratory conditions, including to those females fed and then starved ($t = -3.382$; $P_{\text{fed\&starved}} < 0.001$).

Discussion

Our main results show that *I. leucospoides* females emerge with a considerable number of already mature eggs and do not depend on external nutrient acquisition to sustain post-emergence egg maturation. However, wasps do extend their lifespan through feeding. Currently, there is limited information on the feeding behaviour of free foraging wasps. The results here presented suggest that food intake in the wild, although possible, may not be a frequent event for this parasitoid. In this discussion we take into account the environmental factors that may influence the larval and adult patterns of resource allocation.

Ibalia leucospoides, like other parasitoids, exhibits positive relationships between body size and initial egg load and total egg production (Visser 1994; Rivero and West 2002). Consistently, *I. leucospoides* females showed a large initial investment in reproduction, with a high OI of 0.77, which places this species among the weakly synovigenic parasitoid species. Contrary to the Ellers and Jervis (2003) prediction for synovigenic parasitoids, initial egg load and total egg production increase in the same proportion with increasing body size, in addition to a constant relationship between the OI and size. This suggests that there would be no differential resource allocation to these traits as proposed, by these authors, for synovigenic parasitoids. This might be the consequence of a lower proportion of immature eggs constraining the expression of increasing metabolic reserves with body size (in the same vein as predicted for proovigenic parasitoids).

The high OI encountered in *I. leucospoides* compared to some other synovigenic parasitoid species (Jervis et al. 2001) represents an adaptation to the stochasticity in oviposition opportunities; for instance, in terms of host abundance and distribution in accordance with theory (Ellers et al. 2000; Ellers and Jervis 2003). The Ellers et al. (2000) evolutionary model of optimal parasitoid fecundity predicts that in poor habitats, stochasticity in host encounter should lead to an increase in egg load. The adaptive significance of this output of the model, is that parasitoids gain more under good circumstances by producing more eggs than they are expected to be able to lay, in comparison to what they can lose under bad conditions. In the case of *I. leucospoides*, this is a parasitoid that attacks hosts with an unpredictable pulse-like outbreak population dynamics, and consequently it should be able to use all oviposition opportunities when hosts are available. In

this way, by over producing eggs at the beginning of adult life, whenever the occasion appears, females may have the chance of exploiting any opportunity to lay an unusually large number of eggs. Clearly, the benefit derived from the occasional match between egg load and host availability should offset the potential cost of an initial resource allocation to eggs, as well as the cost of reduced reproductive plasticity.

Concerning adult nutrient acquisition with relation to longevity, there is sufficient evidence of its positive effects, at least in laboratory conditions, in several species (Leius 1961; Lee et al. 2004; Fadamiro et al. 2005). For example, a single sugar meal can reduce the risk of death by starvation by up to 73 % in the parasitoid *Cotesia rubecula* (Siekmann et al. 2001); while *Aphidius ervi* females need at least two food intakes per day to attain maximum longevity (Azzouz et al. 2004). In turn, *Meteorus pulchricornis* fed continuously with a 30 % (w/v) 1:1:1 sucrose–glucose–fructose-mixture, lives six times longer than unfed parasitoids (Heping et al. 2008), and similarly, sugar feeding can increase longevity 15-fold in *Cotesia glomerata* (Wackers 2001; Lee and Heimpel 2008b) and 10-fold in *Mastrus ridibundus* (Bezemer et al. 2005). In this study, honey intake had a positive effect on *I. leucospoides* longevity, but only when food was provided ad libitum. Namely, females with full access to honey meals, an unrealistic phenomenon, increased their lifespan by 10 days, approximately 40 %. However, if later in life (*f14d*) females had one day access to food, longevity only increased by 4.61 days, as compared to individuals fed much earlier in adult life (*fEd* and *f7d*). These additional days did not give rise to a significant difference regarding unfed wasps longevity. We may conjecture that on the one hand, *I. leucospoides* may have physiological or morphological constraints (i.e. very low rate of nutrient metabolism; unspecialized mouthparts for feeding; Gilbert and Jervis 1998), and thus a single day of access to food is not sufficient to acquire enough nutrients to allow for differences in comparison with the nutritional state of starved individuals. On the other hand, it is possible that the lack of influence of limited access to food stems from adaptation of parasitoids to habitats reduced in food availability. That is, females may have evolved to emerge with robust energy reserves, enough to support maintenance needs without external food intakes.

It is not a clear-cut fact that the augmentation of lifespan of females fed ad libitum translates in an increase of lifetime reproductive success and consequently improves parasitoid performance in the field. Firstly, this depends on the extent to which this species is time-limited. That is, if *I. leucospoides* is able to deposit almost all mature eggs within a period of time shorter than their lifespan in the wild, 15 days approximately (Fernández-Arhex and Corley 2003), then an increase in longevity does not imply an additional gain in fitness. Secondly, the benefits of feeding may be irrelevant if a strong predation pressure exists; therefore killing parasitoids before starvation becomes a limiting factor. Likewise, other extrinsic mortality factors such as extreme temperatures or storms can have a strong impact on insect longevity (Dyer and Landis 1996; Fadamiro et al. 2005).

Nutrient acquisition and allocation to egg production during adult life in parasitoids has been also widely studied (see for review Thompson 1999; Jervis et al. 2008). Generally, a large proportion of the resources required for egg production by adult females that do not host-feed is obtained during larval development. These resources are then carried over to the adult stage as a component of fat body storage (i.e. mainly glycogen and lipids), and adult females metabolize them for egg production and/or somatic maintenance. In the presence of a carbohydrate-rich source of food, the catabolic drain that somatic maintenance exerts upon these stored resources could be attenuated. That is, feeding may enable parasitoids to keep fat body resources that promote egg maturation. There is much empirical evidence of the positive role of sugars on egg maturation (Olson and Andow

1998; Tylianakis et al. 2004; Bezemer et al. 2005), but there are also cases in which feeding does not influence this process (Sisterson and Averill 2002). In *I. leucospoides*, feeding has no effect on egg load. However, egg numbers increase with age and remain constant after 14 days. Additionally, female wasps emerge with more than 75 % of its total egg complement mature. We may assume that the nutritional strategies of *I. leucospoides* would be closer to those of proovigenic parasitoids than to those of synovigenic ones; that is why, this species may not depend on adult feeding for egg maturation. Strict proovigenic species emerge with all eggs mature and hence do not need supplementary food for egg production. Basically, only somatic functions (survival and locomotion) demand to be fuelled after emergence (Rivero and Casas 1999; Rivero et al. 2001; Jervis and Heimpel 2005). Due to the insignificant impact of adult nutrition on egg production and longevity in *I. leucospoides*, it can be expected that this parasitoid may have evolved a strategy during larval development to optimize the uptake and allocation of limited host resources to these two competing fitness-related functions, so as not to have to rely on adult food acquisition.

The high OI in *I. leucospoides* can be understood as a reproductive strategy that mirrors adaptation to any environment with low quality or scarce food availability (Boggs 1997), as well as to habitats with significant stochasticity in host encounter [e.g. either due to the distribution of host patches in space, the distribution of the number of hosts per patch, or host abundance variability (Ellers et al. 2000, Ellers and Jervis 2003)]. Theoretically, proovigeny (OI = 1) is related to uniform distributions of host patches in space (i.e. no stochasticity, which is a rare scenario in nature) whereas synovigeny (OI < 1) is associated with random or aggregated distributions of host patches (i.e. different degrees of stochasticity). In addition, within a synovigenic species, higher OI values are associated with higher stochasticity (Ellers and Jervis 2003, 2004). Most certainly the high number of mature eggs at emergence in *I. leucospoides* can be a consequence not only of host population dynamic but also of the habitat characteristics where this species has evolved. Perhaps pine plantations in Patagonia, where nowadays *I. leucospoides* inhabits, exhibit conditions that magnify those found in their native environment. This must have been a factor that helped this species to establish successfully in this region.

Lipids, glycogen and protein are the main components of fat body reserves (Bernstein and Jervis 2008). In this study, the decline of total sugar content between birth and death, of approximately 40 %, was displayed late in life of starved laboratory reared individuals (i.e. around 14 days old). Once again, it is shown that females may emerge with robust capital reserves (e.g. lipids mainly) making it possible to begin late in life to burn carbohydrates. Feeding behaviour in parasitoids is at the cost of time and energy investment, especially when hosts and food are found spatially separated. Sirot and Bernstein's (1996) and Bernstein and Jervis's (2008) models concerning food foraging in parasitoids suggest that if food is scarce and not extremely needed, it should be more profitable not to search for it. In cultivated pine forests, *I. leucospoides* females emerge within host patch aggregates, where the probability of finding food is low or food is unavailable. Although sample is limited to few individuals, we found that only one of the nine field captured wasps showed a high level of body sugars. This may reflect either a failure in food search or most likely, that females are reluctant to undertake search for food and probably remain close to host patches. We can conjecture that *I. leucospoides* food foraging behaviour is in accordance with model predictions.

Research on life-history traits of parasitoids with particular reference to resource acquisition and allocation strategies, is important from both fundamental and practical perspectives. From a fundamental perspective, knowledge on intraspecific and interspecific variability in egg production and longevity—two major components of parasitoids lifetime

reproductive success—may lead to a better understanding of the evolution of reproductive strategies in organisms, such as insects, with complex life-cycles. From an applied point of view, variability in such traits can make the difference between the success or failure of an insect as a biological control agent. Finally, ovigeny can be a reliable predictor of life-history diversity that reflect environmental characteristics but also may give additional baseline knowledge to appropriately manipulate holding conditions in the context of insect mass-rearing and release protocols.

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