The effect of host nutritional quality on multiple components of *Trichogramma brassicae* fitness

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Short title: effect of host age on adult *Trichogramma brassicae*

Abstract:

For parasitoids, the host represents the sole source of nutrients for the developing immature. Subsequently, host quality is an important factor affecting immature development and the resulting fitness of the emerging parasitoid, with impacts on fecundity, longevity and offspring sex ratio. Host age is an integral component of host quality and a key factor in host selection by the female parasitoid. The current study aimed to investigate the effect of decreasing host quality (determined by increasing host age) on adult life history traits (size, wing loading, longevity, and fecundity) and nutritional reserves (protein, lipid and glycogen concentrations) of the parasitoid *Trichogramma brassicae*. Higher quality hosts resulted in the production of larger offspring with increased resource reserves and enhanced mobility. One day old eggs contained significantly more protein and triglyceride than 25 and 45 day old eggs. Quality of host and fitness of reared wasps decreased due to host aging. Parasitoids reared on one day old hosts were larger, with greater fecundity and longevity, a reduced wind loading index, and produced a higher proportion of female offspring when compared to those reared on 25 and 45 day old hosts. In addition, wasps reared on one day old hosts contained higher energy resources, as determined by triglyceride, glycogen and protein reserves, which are essential to successful offspring production. One day old hosts can therefore be considered as the best age for producing wasps with greater fitness since they contain the highest amount of protein, glycogen and triglyceride. This has implications for the mass rearing of *T. brassicae* and enhancing the efficacy of this biological control agent.

Keywords:

Protein, Triglyceride, Glycogen, Life History Trait, Fecundity, Energy Reserves, Developmental Requirements

Introduction:

Host quality is a critical factor in determining developmental rate and success of parasitoids (Liu et al. 2013). For the immature parasitoid developing within the host, the host represents the sole source of nutrients. As a result, evaluation of host quality by the parental female parasitoid is vital to her reproductive success and offspring fitness, and a host selection trade-off results due to variation in host quality and the developmental requirements of the offspring (Harvey and Strand, 2002, Beckage & Gelman, 2004). The life stage of the host is an important factor in determining host quality and, as such, plays a key role in host selection (Godfray, 1994, Colinet et al., 2005, Kishani Farahani & Goldansaz 2013). Different host stages may represent qualities and quantities of various resources due to variation in size, physiological, behavioral and immunological status (Chong & Oetting, 2006). Many studies suggest that host quality preference by parasitoids affects adult size and reproductive performance of progeny (Harvey, 2005, Lampson et al., 1996), female egg load at emergence (Liu, 1985, Mills & Kuhlmann, 2000), as well as sex allocation, percent parasitism and immature developmental time of parasitoids (Godfray, 1994, Schmidt, 1994, Kishani Farahani and Goldansaz 2013).

The major nutritive components involved in development are triglycerides, carbohydrates and proteins. Essential amino acids are necessary for viability, thus imbalances in dietary amino acids can lead to significant effects upon development and fitness of both immatures and adults (Dadd 1985) leading to dietary restrictions on lifespan (Grandison et al. 2009). Carbohydrates provide the required energy for development and also represent the mechanism by which energy is stored for future use (Dadd 1985). Lipids, primarily triglyceride, are storage lipids in insects and have several roles in energetic biological demands such as flight and reproduction, both of which are imperative in the efficiency of parasitoids (Bauerfeind and Fischer 2005, Fischbein et al. 2013). Visser and Ellers (2012) believed that the addition of a lipid source improved or maintained nutrient availability for parasitoids and increased their effectiveness as biological control agents within agro-ecosystems. Thus, studying the content of these resources in adults may provide an index to correlate trade-offs in decision making during the host selection process by mothers and the obtained benefits by offspring.

Numerous environmental factors including humidity, photoperiod and temperature (Pizzol et al. 2012), in addition to biotic factors such as host age or size (Berrigan 1991, Martel et al. 2011) are known to influence effective parasitism by Trichogramma parasitoids. To date, limited studies have documented the potential effects of host egg age on Trichogramma wasp fitness (Pak et al. 1986, Moreau et al. 2009). However, the effect of host nutritional quality on adult wasp fitness across multiple life history traits has not been well studied. This study represents the first study to investigate the impact of host nutritional quality on multiple aspects of wasp fitness within a single study. Assessing multiple life history traits within a single study will provide valuable, comparative information on how and which traits are impacted by host nutritional quality, enabling us to elucidate the optimal host age to maximize wasp fitness.

The study species of the current research is *Trichogramma brassicae* Westwood (Hym.: Trichogrammatidae). Species belonging to the *Trichogramma* genus are endoparasitoids of lepidoperan eggs, although some have the potential to attack eggs of other insect taxa such as Diptera and Coleoptera (Mansfield and Mills, 2002). *Trichogramma brassicae* is a biological control agent which has been used against various pests (van Lenteren, 2000, van Lenteren and Bueno, 2003, Bigler *et al.*, 2010, Parra *et al.*, 2010, Ebrahimi et al. 1998, Poorjavad et al. 2012) and is thus of great importance within agro-ecosystems. The current study aims to investigate the effect of host quality on adult fitness using *T. brassicae* as a study organism. By understanding how and which traits are impacted by host nutritional quality, we may determine the optimal host age for maximum wasp fitness, with such knowledge feeding into the mass rearing of wasps for biological control purposes. More specifically, the study aims to test the following hypotheses: (1) hosts of different ages vary in nutritional quality, (2) parasitoids reared on hosts of different ages will be provided with different amounts of protein, triglyceride and glycogen during immature development and, this in turn will affect multiple aspects of their life history, including body size, longevity and fecundity.

Materials and methods

Parasitoids and their host

Parasitoids were obtained from cultures maintained at the Biological Control Research Department (BCRD) of the Iranian Research Institute of Plant Protection (IRIPP). The original source of the cultures were parasitoids obtained from parasitized eggs of *Ostrinia nubilalis* Hübner (Lep.: Pyralidae), collected from northern Iran (Baboulsar Region, South of the Caspian Sea) in 2014. Parasitoids were reared at 25±1ºC, 50±5% RH, and 16:8 L: D on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Eggs were obtained from a culture, reared at 25±1ºC on wheat flour and yeast (5%), maintained at the Insectary and Quarantine Facility of University of Tehran. Approximately 20 mated female moths were kept in glass containers (500 ml) to provide eggs for experiments.

To produce adult wasps for experiments, one hundred one day old eggs (high quality eggs), 25 day old (intermediate quality eggs) and 45 day old eggs (low quality eggs) were exposed to one day old females for 24 hours to rear wasps on different host qualities. After 24 hours, the eggs were removed and kept under controlled conditions of 25±1ºC, 16L: 8 D, and 50±5% RH in a growth chamber and checked until emergence of adult wasps. The twenty-five day old host treatment was performed separately to show the intermediate host age effects on adult wasp fitness.

Determination of glycogen, triglyceride and protein concentration

To determine the resources obtained from high, intermediate and low quality hosts by adult wasps, 50 newly emerged wasps were exposed separately to one day old, 25 day old and 45 day old hosts for 24 hours, maintained in tubes (10×1 cm) and prepared with 100 host eggs glued on cardboard. To avoid superparasitism by adults, only one female was introduced to each tube. Females were fed with a 10% honey solution, and maintained under controlled conditions of 25±1ºC, 70±10 RH and 16:8 (L: D). Wasps reared on each host quality were used for the extraction of macromolecules utilizing the methods detailed below.

Glycogen determination

Fat bodies of 30 adults per treatment were removed and immersed in 1 ml of 30% KOH w/Na_2SO_4. Tubes containing the samples were covered with foil to avoid evaporation and boiled for 20-30 min. Tubes were subsequently shaken and cooled in ice. Two ml of 95% EtOH was added to precipitate glycogen from the digested solution. Samples were again shaken and incubated on ice for 30 min. Following the incubation on ice, tubes were centrifuged at 13000 rpm for 30 min. Supernatant was removed and pellets (glycogen) were re-dissolved in 1 ml of distilled water and shaken. Standard Glycogen (0, 25, 50, 75 and 100 mg/ml) was prepared before adding phenol 5%. Incubation was performed on an ice bath for 30 min. Standards and samples were read at 492 nm (Microplate reader, Awareness Co., USA) and distilled water was used as a blank (Chun and Yin, 1998).

Triglyceride determination

A diagnostic kit from PARS-AZMOON® Co. was used to measure the amount of triglyceride in the adult parasitoids. One hundred wasps from each treatment group were used for triglyceride measurements. Reagent solution contained phosphate buffer (50 mM, pH 7.2), 4-chlorophenol (4 mM), Adnosine Triphosphate (2 mM), Mg^{2+} (15 mM), glycerokinase 0.4 kU/L), peroxidase (2 kU/L), lipoprotein lipase (2 kU/L), 4-aminoantipyrine (0.5 mM) and glycerol-3-phosphate-oxidase (0.5 kU/L). Samples (10 μL) were incubated with 10 μL distilled water and 70 μL of reagent for 20 min at 25 °C (Fossati and Prencipe, 1982). The optic density (ODs) of samples and reagent as standard were read at 546 nm. The following equation was used to calculate the amount of triglyceride:

$$mg/dl = \frac{OD\ of\ sample}{OD\ of\ Standard} \times 0.01126$$

Protein determination

Protein concentrations were assayed according to the method described by Lowry et al. (1951). The method recruits reaction of Cu^{2+}, produced by the oxidation of peptide bonds with Folin–Ciocalteu reagent. In the assay, 20 μL of the sample was added to 100 μL of reagent, and incubated for 30 min prior to reading the absorbance at 545 nm (Recommended by Ziest Chem. Co., Tehran-Iran). One hundred adult wasps from each treatment were used in this experiment.

Morphometric measurements:

Body size

To correlate body size with fitness parameters, the length of the left hind tibia of each individual was measured using a binocular microscope (0.5×6.3, Olympus SZ-CTV) connected to a video camera (JVC KY-F). Tibia length is a commonly used indicator of body size in parasitoid wasps and correlates strongly to other measures such as dry mass (Godfray1994). From photographed images, tibia length was determined using Image J software.

The wing loading value was obtained by calculating the ratio between the body mass and the wing area. Wing loading of females establishes a good index of their flight capacity. Lower wing loadings are considered to represent better dispersal capacities for individuals (Gilchrist and Huey 2004, Vuarin et al. 2012). Using weight as an index of size, for each treatment reared on high, intermediate and low quality hosts, a minimum of 40 females were selected randomly and frozen in liquid nitrogen on emergence to be weighed on a microbalance to ±0.1 µg (Mettler Toledo XP2U) (Ismail et al. 2012). At least 40 females for each host quality treatment were photographed under a binocular microscope (0.5×6.3, Olympus SZ-CTV) connected to a video camera (JVC KY-F). The Image J software was used to determine the area of the left wing.

Longevity

Following wasp emergence, adult longevity without food (but with access to water) was measured to estimate longevity with only capital resources available (n= 40 females reared on high, intermediate or low quality hosts, i.e. a total of 120 females). This represents the amount of energy reserves within the body after development. Individual adults were placed in small tubes (1.5 cm in diameter and 10 cm long) and were monitored hourly until death after the first 12 hours of life.

Fecundity

To compare parasitoid fecundity among treatments, 120 randomly selected newly emerged wasps (40 per each host quality) were maintained in tubes (10×1 cm) prepared with 100 host eggs glued on cardboards. The females were fed with a 10% honey solution. Egg cards were replaced every 12 hours (until the wasp died) and maintained under controlled conditions of

25±1ºC, 70±10 RH and 16:8 (L: D). The preliminary test showed that adults oviposited the majority of eggs in the first 6 hours of life. Subsequently, 40 newly emerged wasps from each treatment group (a total of 120 females) were selected and exposed individually to 100 host eggs for 1 h before removing the egg cards. This was repeated for the first 6 hours of an individual wasp's life. Lifetime fecundity was determined by counting the number of parasitized (blackened) eggs. Parasitoids were sexed according to antennae morphological differences (Pinto 1998), providing sex ratios associated with different types of hosts.

Statistical analysis:

Numerical data were analyzed by Generalized Linear Models (GLM) based on a Poisson distribution and log-link function. Likelihood ratio tests were used to assess the significance of the 'host age' factor. The rate of produced females was analyzed by GLM based on a Binomial Logit distribution (Crawley 1993, Le Lann et al. 2014). All the recorded times were compared with Cox Proportional Hazards models. When a significant effect of the treatment was found, the tests were followed by Bonferroni's *post hoc* multiple comparison tests, and the two-by-two comparisons were evaluated at the Bonferroni-corrected significance level of $P = 0.05/k$, where k is the number of comparisons. Data are presented as means ±SE. All statistical analyses were performed using SAS software (SAS Institute Inc. 2003).

Results:

Host eggs

Host age significantly affected the protein content of the host (χ^2= 94.79, p<0.0001), with the results showing that protein amount dropped significantly in response to egg aging. One day old eggs contained significantly more protein than 25 and 45 day old eggs respectively ($\chi^2_{1\ vs\ 25}$=396.8, p<0.0001, $\chi^2_{1\ vs\ 45}$=327.9, p<0.0001), and 45 significantly more than 25 day old eggs

(χ^2=9.42, p=0.009). The amount of triglyceride in hosts was also significantly affected by host age (χ^2=28.27, p<0.0001). One and 25 day old eggs showed no significant difference in the amount of triglyceride (χ^2=1.36, p=0.51), while one and 45 day old eggs were significantly different (χ^2=7.47, p=0.02), as were 25 and 45 day old eggs (χ^2=11.98, p=0.0025). Finally, the glycogen content of the host was also significantly affected by host age (χ^2=12.62, p=0.0004). One and 25 day old eggs (χ^2=15.19, p<0.0001) and one and 45 day old eggs (χ^2=12.57, p=0.0004) significantly differed with regards to glycogen content. However, no significant difference was revealed between 25 and 45 day old eggs (χ^2=0.13, p=0.72) (Figure 1).

Adult parasitoids

Host age significantly affected the protein content of the emerging wasps (χ^2= 121.53, p<0.0001) (Figure 1). Wasps reared on 1 day old eggs contained significantly more protein than 25 and 45 day old respectively ($\chi^2_{1vs\ 25}$=35.6, p<0.0001, $\chi^2_{1\ vs\ 45}$=30.4, p<0.0001) while no significant differences were observed between 25 and 45 day old eggs (χ^2=0.14, p=0. 93). Host age significantly affected triglyceride amount in wasps reared on different host ages (χ^2=36, p<0.0001). One and 25 day old eggs showed significant differences in triglyceride (χ^2=8.29, p=0.015) as did one and 45 day old eggs (χ^2=15.6, p=0.0004). In addition, triglyceride content differed between 25 and 45 day old eggs (χ^2=7.61, p=0.022). According to our findings, glycogen amount in the emerging wasps was not affected significantly by host age (χ^2=0.37, p=0.544). The glycogen content of wasps reared on one and 25 day old (χ^2=1.15, p=0.56), one and 45 day old (χ^2=1.46, p=0.48) and 25 and 45 day old eggs (χ^2=0.06, p=0.96) did not show significant differences (Figure 1).

Host age showed significant effects on wasp fecundity (χ^2= 5.67, P =0.01). Adult wasps reared on one-day-old hosts produced the same offspring number when compared to wasps reared on 25 day old hosts (Figure 2). However, adult wasps laid more female eggs in one day old hosts with a sex ratio of 1:3 (M: F), whereas the wasps laid more male eggs in 25 and 45 day old hosts with a sex ratio of 2:1 and (M: F). Adult wasp longevity was significantly affected by host age (χ^2= 19.47, P <.0001), with wasps reared on high quality hosts living longer than those reared on 25 and 45 day old eggs respectively (Figure 2). Survival curves of wasps reared on different host qualities are shown in Figure 3.

Tibia length (χ^2= 61.83, P <.0001) and weight (χ^2= 6.58, P=0.01) were significantly affected by host age. Wasps reared on 1 day old eggs showed higher tibia length (χ^2= 7.75, P =.0054) and weight (χ^2= 61.83, P <.0001) than wasps reared on 25 day old eggs (Figure 4).

Wing area was significantly affected by host age (χ^2= 53.94, P<.0001), with this parameter decreasing with host age (1 to 25 days old) (Figure 5). Furthermore, wing loading index was significantly affected by host age (χ^2= 7.03, P=0.009) (Figure 5).

Discussion:

The current study provides the first study to investigate the effect of host quality across multiple fitness parameters within a single study. The study thus provides comparative information, enabling us to elucidate how host quality affects multiple life history traits (body size and wing loading, longevity, fecundity and adult energy reserves) of parasitoid wasps, and ultimately wasp fitness. From a biological control perspective, this knowledge can inform the commercial mass rearing of parasitoid wasps, informing which age of host should be utilized to maximize both the proportion of female offspring and the fitness of the emerging parasitoids, and ultimately their efficacy as biological control agents.

Host eggs of different ages were shown to provide differing nutritional resources for the developing immature, thus supporting our first hypothesis. Results showed that host age, acting as a proxy for host quality, significantly affected life history traits and the nutritional reserves of *T. brassicae* adults. Wasps reared on high quality hosts were bigger, with greater fecundity and longevity, and produced more female offspring compared to those reared on intermediate and low quality hosts. Furthermore, wasps reared on high quality hosts showed lower wing loading index compared to wasps reared on low quality hosts. Wasps reared on high quality hosts also contained greater energy reserves, as determined by the body content of triglyceride, glycogen and protein.

For many endoparasitic Hymenopterans such as *Trichogramma* spp., their eggs possess no yolk and, as such, the parasitoids lay their eggs inside the body of a host which subsequently provides all nutrients for both embryonic and larval development (Chapman, 2012). In the body of insects, glycogen, triglyceride and protein represent the three main storage macromolecules responsible for several energetic demand processes. Phosphorylation of glycogen and triglyceride, as well as transamination of protein molecules, provides intermediate components for the electron transport system providing energy, oxygen and water (Nation, 2008, Arrese & Soulages 2010). The presence of these components, as obtained from the egg host, is thus essential for embryo development. In particular, it is the fatty acids stored as triglyceride, and fat reserves that are the most important reserve used by insects to provide energy for the developing embryo (Athenstaedt & Daum 2006, Ziegler & Van Antwerpen 2006). Reserves are subsequently carried through to adulthood and are depleted during periods of starvation or reproduction. In larval stages, glycogen is stored in fat bodies followed by active feeding by wasps larvae. In addition, glycogen represents the primary source of energy fuel for biological activity of larvae (Chapman 2012, Klowden 2007). Due to the precise processes behind the utilization of storage macromolecules, changes in the amounts of triglyceride, protein and glycogen may alter the suitability of the host for the development of parasitoid offspring, and host acceptance by the parental parasitoid. This is supported by a previous study by Barrett and Schmidt (1991) which investigated discrepancies in the amino acid content of the egg hosts of *Trichogramma minutum*. Whilst variation in amino acid content was evident, variation was greater in the egg hosts than in the emerging parasitoids, suggesting that metabolic compensation is occurring, although at a detriment to development. Furthermore, ovipositing females are believed to allocate eggs in accordance with the nutritional quality of the host, allocating proportionately fewer eggs to low quality hosts (Barrett and Schmidt 1991).

The nutritional content of host eggs is known to vary with age, as the chemical composition of the insect eggs changes rapidly from a more fluid medium to complex tissues as the egg develops. Our results showed that the total amount of protein and triglyceride in 45 day old eggs (low quality eggs) significantly decreased as a result of egg aging. Such changes to egg composition can further exert a negative effect on parasitism via pre-imaginal mortality, most likely the result of poorer resource availability (Brodeur & Boivin 2004, Da Rocha et al. 2006). According to Benoit and Voegele´ (1979) Trichogramma parasitoids do not oviposit in old host eggs, with modification to the host tissues offering an explanation as to why Tricogramma wasps do not accept older hosts within which to oviposit.

The present study revealed that host quality significantly affected life history traits of the emerging parasitoids. Adults of *T. brassicae* reared on high quality hosts (one day old eggs) displayed higher longevity than those reared on low quality hosts (45 day old eggs). Several studies have reported a relationship between host quality and parasitoid survival (Lauzière et al. 2001, Sagarra et al., 2001, Li & Sun 2011, Kishani Farahani & Goldansaz 2013). In parasitoids, like other insects, large adult body size is often related to an increase resource carry-over from the larval stage, and is manifested in higher energy reserves (Lopez et al. 2009, Kant et al. 2012). Our results support this, indicating that host age at oviposition affects adult survival because larger hosts provide more resources for the larval stages of the parasitoid. Lopez et al. (2009) stated that host quality influenced the life expectancy of *Diachasmimorpha longicaudata* (Hym.: Braconidae) as starved females and males emerging from high quality hosts lived significantly longer than wasps emerging from lower quality hosts.

In addition to longevity, host quality was also shown to affect gross and net fecundity of the parasitoid, with females emerging from high quality hosts being the most fecund. According to our results, female fecundity was affected by host age, with the most fecund wasps emerging from high quality hosts (1 day old eggs) than low quality hosts (45 days old eggs). Host egg age is known to affect the fecundity and parasitism rate of Trichogramma parasitoids (Brand et al. 1984, Calvin et al. 1997, Pizzol 2004, Pizzol 2012, Moreno et al. 2009). In female parasitoids, fecundity is often correlated with the adult body size and quality of the food resources available to the parasitoid during development (Jervis et al., 2008, Saeki & Crowley 2013). According to obtained results, low quality hosts contained less protein. Large amounts of proteins, such as storage proteins are used as an amino acid reservoir for morphogenesis, lipophorins responsible for the lipid transport in circulation, or vitellogenins for egg maturation (Guo et al. 2011, Fortes et al. 2011). Total amount of available protein during adulthood strongly affects reproduction (vitellogenins) (Fortes et al. 2011). Cônsoli and Parra (2000) showed that rearing *Trichogramma galloi* Zucchi and *T. pretiosum* Riley on artificial diets containing high amounts of protein led to an increased number of produced eggs. It seems that lower fecundity of low quality reared wasps may be due to less protein available during embryo growth and adulthood. Our results therefore show that there is a direct relation between the protein content of host eggs and the resultant number of eggs produced by adult wasps. As a consequence, rearing wasps on hosts with greater protein content, which can provide enhanced protein resources carried over into adulthood, may result in more fecund wasps. This finding has implications for biological control programs, since more fecund wasps would result in greater rates of parasitization, thus enhancing the efficacy of natural biological control.

Most parasitoid wasps, including *T. brassicae*, have a haplo-diploid sex determination system (Beukeboom & van de Zande 2010, Quicke, 1997). This system allows the ovipositing female to control the sex of her offspring by controlling sperm access to eggs. In fact, the adult females of many parasitoid species respond to a number of environmental variables by changing offspring sex ratio. Among the variables, host type (e.g. host size, age, and species) is one of the most important factors influencing the offspring sex ratio of parasitoid wasps (Kishani Farahani et al. 2015, Ueno 2015, Kraft and Van Nouhuys 2013). The relationships between offspring sex ratio and host quality has been investigated in many parasitoid wasps (Godin & Boivin 2000, Kishani Farahani & Goldansaz 2013, Ueno 2015, Ode and Heinz 2002). Host age or quality is considered a major factor affecting offspring sex ratio (King, 1993, Ueno 2015). A correlation between host quality and offspring sex ratio has commonly been demonstrated for solitary parasitoids (King, 1993, van Baaren et al. 1999, Ode and Heinz, 2002), a higher proportion of female offspring tend to emerge from higher-quality hosts compared to low-quality hosts. Accordingly, we showed that increased host quality results in a bias towards female production in *T. brassicae*. In the mass rearing of biological control agents, the number of produced females is a key factor in the success of mass release programs (Ode and Heinz 2002). As such, utilization of higher quality eggs in the mass rearing of biological control agents such as *T.brassicae* would result in the production of a higher proportion of females, thus increasing the efficiency of biological control programs.

Previous work has suggested that wing size and shape may increase parasitoid fitness and dispersal ability in the field (Kölliker-Ott et al., 2003, 2004) and as such, could act as a predictor of field performance of mass reared parasitoids. In the current study, we investigated the effect of host quality on parasitoid wing loading and the potential implications for parasitoid mass rearing. Results revealed that the wing loading index of *T. brassicae* reared on high quality hosts was reduced when compared to wasps reared on low quality hosts. Wing loading corresponds to the pressure exerted by the wings on the surrounding air (Gilchrist & Huey, 2004). Thus, the cost of transport is influenced in an important way by the wing surface area, which supports the body mass (Starmer & Wolf, 1989, Duthie et al. 2015). The lower the wing loading, the less costly the act of flight is to the individual. This reduced wing loading may facilitate flight (Gilchrist & Huey, 2004, Duthie et al. 2015) in an environment where females have to move over large distances to find hosts that are patchy in distribution. Flying over large distances to find hosts is an energy demanding activity (Ruohomaki 1992, Ellers *et al*., 1998). A study by Kalcounis and Brigham (1995) investigated the relationship between wing loading and habitat usage in bats. Results showed that bats with a higher wing loading index foraged in less cluttered areas. In the current study, the wing index suggests a higher maneuverability of wasps when reared on high quality hosts, which will enable them to forage in environments further afield to exploit new patches, whilst utilizing less energy resources. From a biological control perspective, an enhanced dispersal activity may allow wasps to cover a greater area for foraging and searching. This in turn could increase the efficiency of mass reared wasps by increasing the potential to parasitize more hosts.

In conclusion, our results show how host nutritional quality impacts adult wasp fitness by affecting wasp life history traits. Wasps reared on high quality hosts are provided with higher food resources (protein, glucose and triglyceride) during immature development, resulting in enhanced adult resource reserves. Higher amounts of protein and triglyceride will enhance the production of offspring, while higher glycogen amount will enhance energy reservoirs. This in turn has implications for adult fitness, resulting in larger body sizes, increased longevity, greater fecundity, and lower wing loading index. A reduced wind loading has the potential to increase adult maneuverability, aiding dispersal ability and thus access to patchy resources. Such individuals could be at an evolutionary advantage, providing their offspring with increased energy and structural resources during development. According to our results, the optimum host age for the mass rearing of this parasitoid is one day old eggs of *E. kuehniella*, which offer greater nutritional resources, enhancing wasp fitness and, in turn, their efficiency in biological control programs.

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Figure legend:

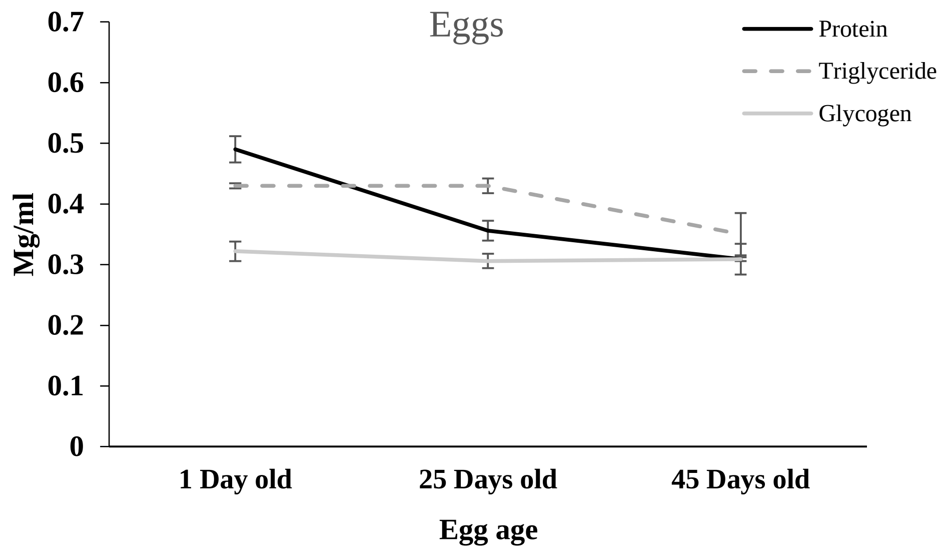
Figure 1. Total concentration (Mean± SE) of protein, triglyceride and glycogen in 1, 25 and 45 day old eggs of *E. kuehenliea* and the adult wasps reared on these hosts.

Figure 2. Longevity (H) and fecundity (Mean± SE) of 50 wasps, *T. brassicae*, reared on 1, 25 and 45 day old eggs of *E. kuehenliea*. Different letters indicate significant differences between the treatments after Bonferroni correction (P=0.0166).

Figure 3. Survival curves of 50 wasps, *T. brassicae*, reared on 1, 25 and 45 day old eggs of *E. kuehenliea*.

Figure 4. Mean (±SE) weight (µg), tibia length (mm) of 50 wasps, *T. brassicae,* reared on 1, 25 and 45 day old eggs of *E. kuehneilla.* Different letters indicate significant differences between the treatments after Bonferroni correction (P=0.0166).

Figure 5. Mean (±SE) wing area (mm^2) and wing loading index (mg/m^2) of 50 wasps, *T. brassicae*, reared on 1, 25 and 45 day old eggs of *E. kuehneilla.* Different letters indicate significant differences between the treatments after Bonferroni correction (P=0.0166).


Eggs
Protein
Triglyceride
Glycogen
Mg/ml
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 Day old
25 Days old
45 Days old
Egg age

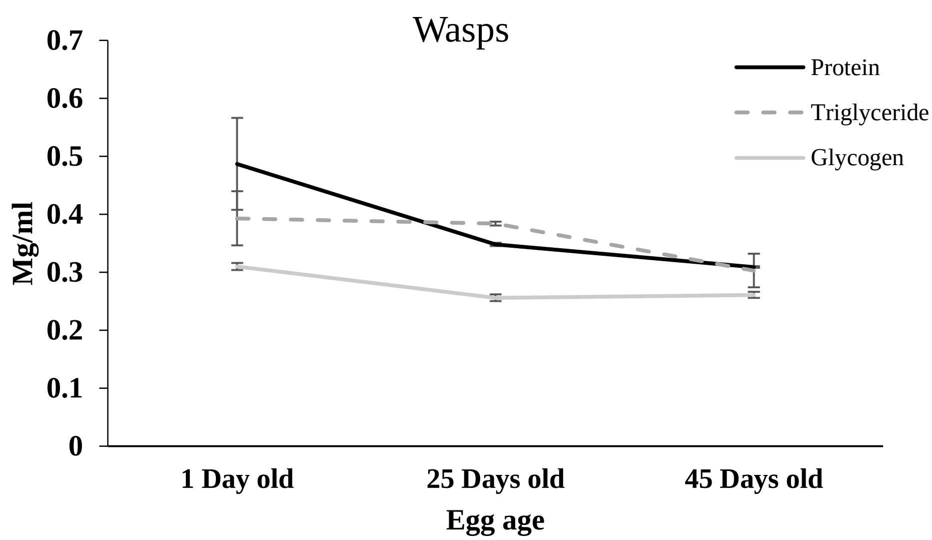
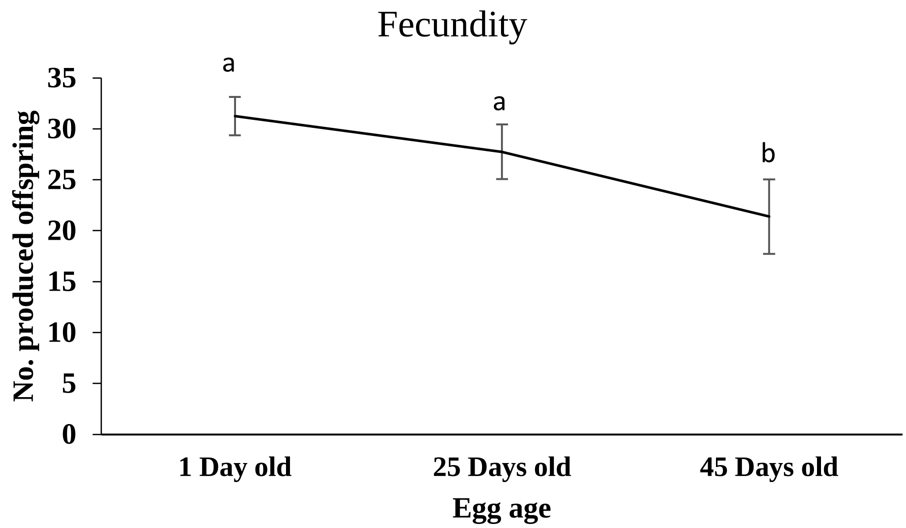

Wasps
Protein
Triglyceride
Glycogen
Mg/ml
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 Day old
25 Days old
45 Days old
Egg age

Figure 1.


Fecundity
a
a
b
No. produced offspring
35
30
25
20
15
10
5
0
1 Day old
25 Days old
45 Days old
Egg age

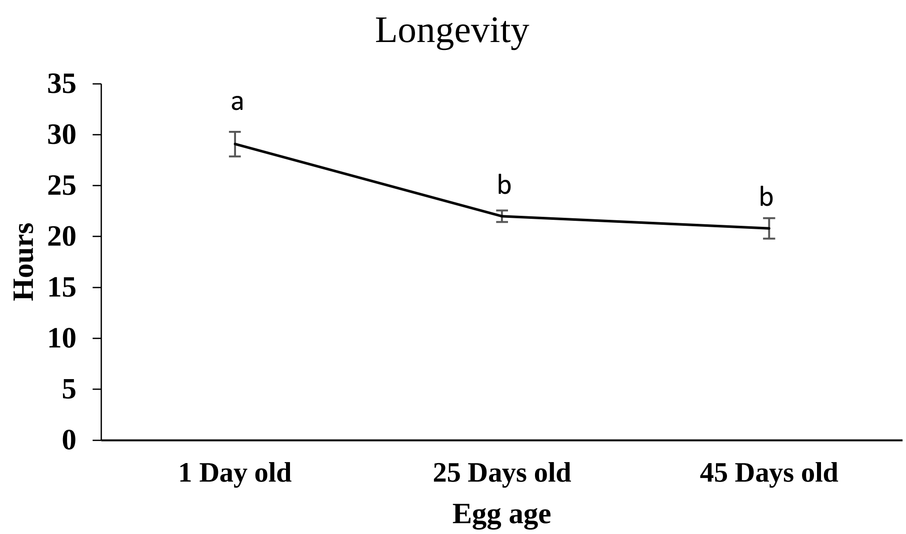

Longevity
a
b
b
Hours
35
30
25
20
15
10
5
0
1 Day old
25 Days old
45 Days old
Egg age

Figure 2.

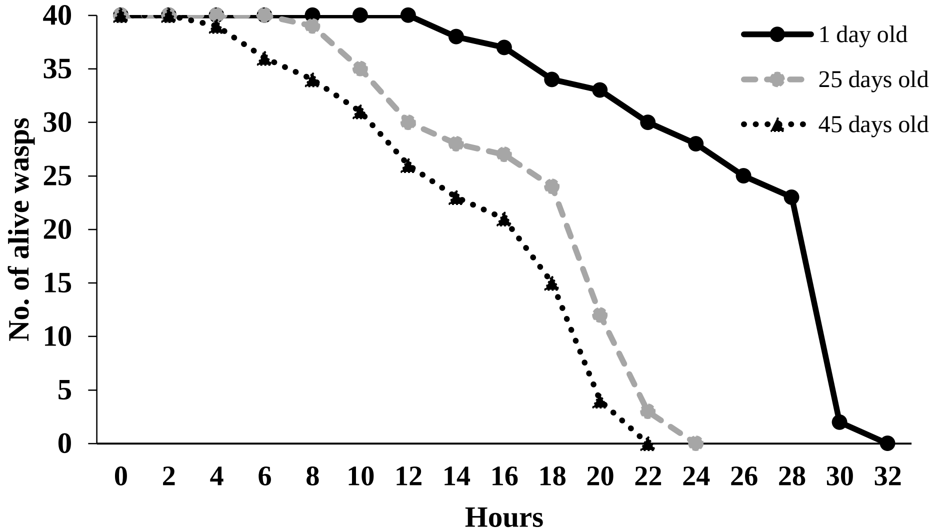
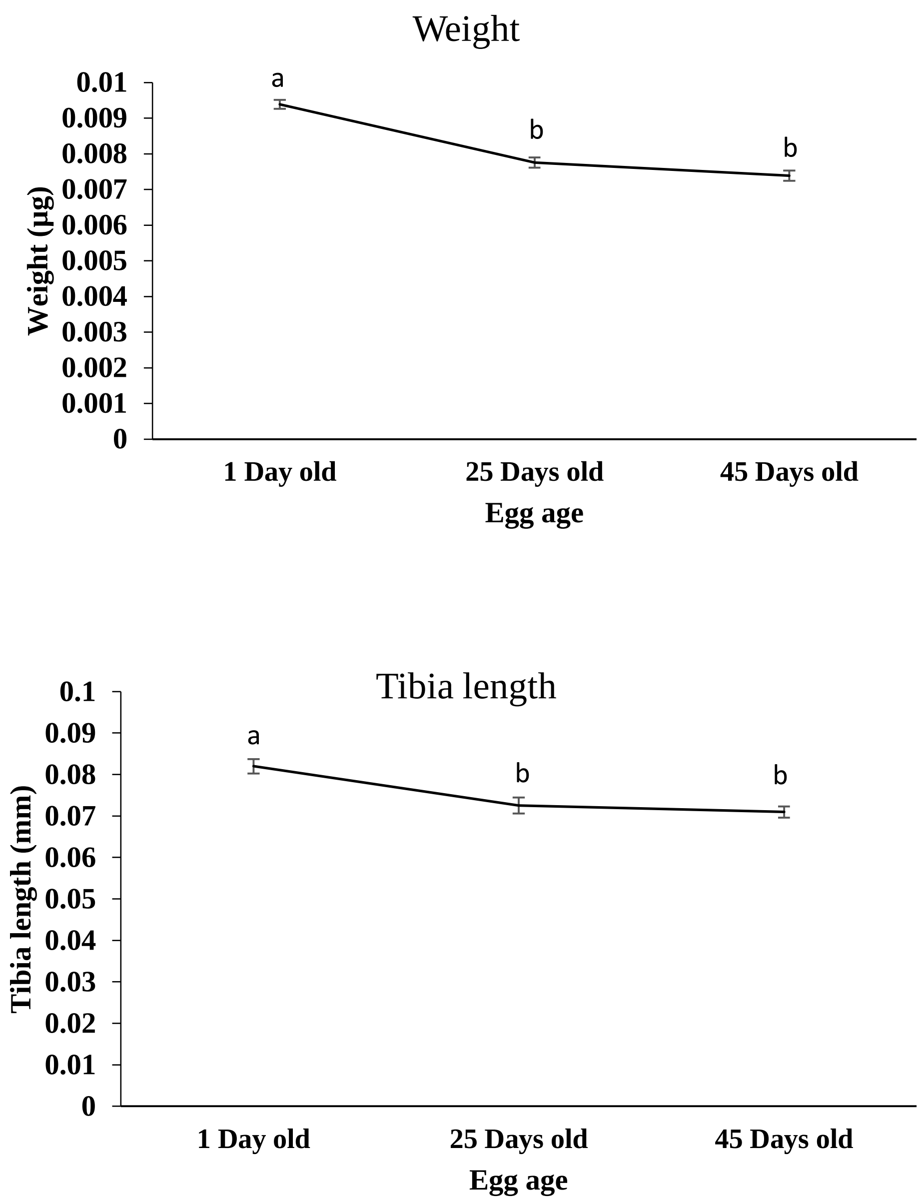

40
35
30
25
20
15
10
5
0
No. of alive wasps
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Hours
1 day old
25 days old
45 days old

Figure 3.



Weight
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Weight (µg)
a
b
b
1 Day old
25 Days old
45 Days old
Egg age
Tibia length
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Tibia length (mm)
a
b
b
1 Day old
25 Days old
45 Days old
Egg age

Figure 4.

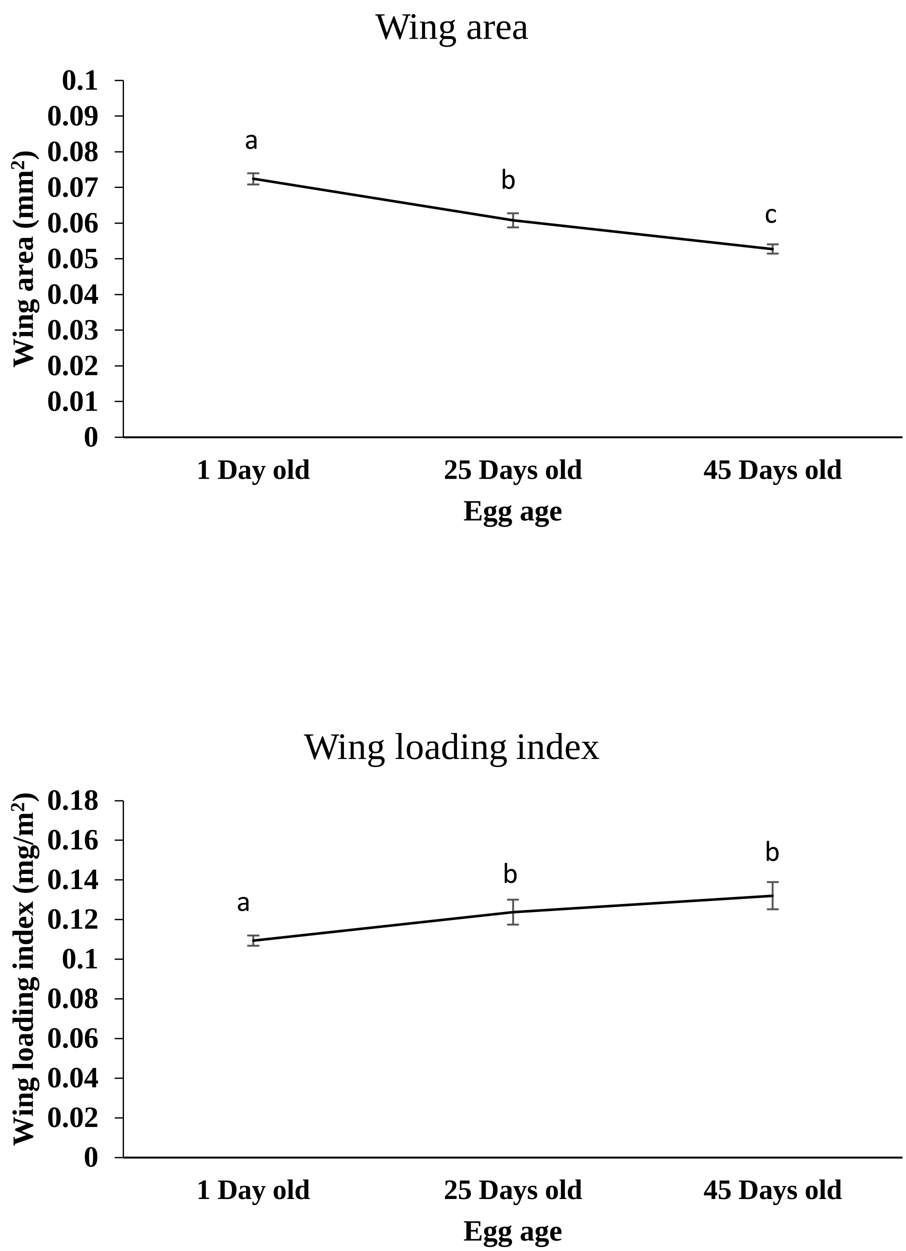

Wing area
Wing area (mm2)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
a
b
c
1 Day old
25 Days old
45 Days old
Egg age
Wing loading index
Wing loading index (mg/m2)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
a
b
b
1 Day old
25 Days old
45 Days old
Egg age

Figure 5.