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Early life nutritional quality effects on adult memory retention in a parasitic wasp

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Running Title: capital resources effects on memory of Trichogramma brassicae
Abstract:

Nutritional quality during early life can affect learning ability and memory retention of animals. Here we studied the effect of resource quality gained during larval development on the learning ability and memory retention of two sympatric strains of similar genetic background of the parasitoid *Trichogramma brassicae*: one uninfected and one infected by *Wolbachia*. Wolbachia is a common arthropod parasite/mutualistic symbiont with a range of known effects on host fitness. Here we studied, for the first time, the interaction between resource quality and *Wolbachia* infection on memory retention and resource acquisition. Memory retention of uninfected wasps was significantly longer when reared on high quality hosts when compared to low quality hosts. Furthermore, uninfected wasps emerging from high quality hosts showed higher values of protein and triglyceride than those emerging from low quality hosts. In contrast, the memory retention for infected wasps was the same irrespective of host quality, although retention was significantly lower than uninfected wasps. No significant effect of host quality on capital resource amount of infected wasps was observed, and infected wasps displayed a lower amount of protein and triglyceride than uninfected wasps when reared on high quality hosts. This study suggests that the nutritional quality of the embryonic period can affect memory retention of adult wasps not infected by *Wolbachia*. However, by manipulating the host’s obtained capital resource amount, *Wolbachia* could enable exploitation of the maximum available resources from a range of hosts to acquire suitable performance in complex environments.

Keywords:

Learning, memory, capital resources, *Trichogramma brassicae*, *Wolbachia*
Introduction:

Nutrient availability and quality during development can greatly affect adult phenotype, such as body mass, fecundity and longevity, directly affecting the fitness of the individual (Metcalf and Monaghan 2003, Schwartz and Morrison 2005). In addition, it has been reported that nutrient availability may also impact adult behavior such as dominance status, food preference or defensive behaviors, (Mevi-Schütz and Erhardt 2003, Lindstedt et al. 2009, Araújo et al. 2012,). For example, in *Nicrophorus vespilloides* burying beetles, adult size is influenced by the size of the carcass on which the individual develops; beetles reared on small carcasses are smaller than beetles reared on larger carcasses giving them less success in contest success Hopwood et al. 2014). Recently, the possible connections between quality of nutrition in early development and learning ability and memory capacity in adults has received considerable research attention in vertebrates (Fisher et al. 2006, Stangl and Thuret 2009). To address this question in invertebrates, parasitic wasps represent an ideal model since nutritional resources available during development are constrained by the host, and by the efficiency of the parasitic wasps to convert host body mass to their own body mass (Jervis and Kidd 1986, Kant et al. 2012 Sanders et al. 2016). Since the resources gained by parasitoid larvae (Capital resources) are a primary factor in determining the future fitness of the adult life stage (Jervis and Kidd 1986), host quality subsequently plays an important role in adult fitness and has been show to affect development, mortality, longevity, fecundity and adult size (Godfray 1994, Sampaio et al. 2008).

In insects, alteration in diet quality during development has various effects on adults’ fitness, generally called as the silver spoon effect (Chapman et al. 2012, Hopwood et al. 2014). For example, Runagall-McNaull et al. (2015) found that male and female lifespan of *Telostylinus angusticollis* was shortest when larvae were fed with a protein restricted diet. Restriction of
nutrients in insect larval instars is generally considered to negatively influence adult fitness by increasing development time and reducing adult size, male secondary sexual trait expression, and both male and female fecundity (Zwaan et al. 1991, Tatar and Tatar 2003, Boggs and Freeman 2005, Bonduriansky 2007). The major nutrients involved in development are proteins, carbohydrates and lipids. Proteins play essential roles in viability, vitelogenesis, neural differentiation and mating behavior. Thus imbalances in dietary amino acids can significantly affect survival, development and fitness of adults (Joachim-Bravo et al. 2009, Even et al. 2012, Bjorum et al. 2013). Carbohydrates act as body fuel and provide energy to development and represent the mechanism by which energy is stored for future use (Rivero and Casas 1999, Tenhumberg et al. 2006).

Wolbachia is an obligate intracellular endosymbiont, belonging to a-proteobacteria group which includes a number of important arthropod-transmitted bacterial agents such as Rickettsia rickettsii, R. felis, Anaplasma platys, Ehrlichia canis, E. chaffeensis, and E. ewingii. (Taylor and Hoerauf 1999, Bowman 2011). It is estimated to infect 66% of arthropod species, although often at a low prevalence within a species (Hilgenboecker et al. 2008). Recently, Wolbachia infections have been shown to influence the fitness of their hosts in various ways, positively and negatively, via altering patterns of reproduction and resistance to microbial infections (Hedges et al. 2008, Teixeira et al. 2008). The spread of Wolbachia in arthropod populations depends on the continuity of Wolbachia transmission across generations. Wolbachia causes parthenogenesis to spread, thus providing an advantage to their hosts in terms of female offspring production (Stouthamer 1997). Species belonging to the Trichogramma genus are gregarious endoparasitoids and are potential hosts to Wolbachia (Kishani Farahani et al. 2015). Most Trichogramma species are associated with the eggs of Lepidoptera, although some can attack
dipteran, coleopteran, and symphytan eggs (Hoffmann et al. 1995, Pinto 1998, Mansfield and Mills 2002). One particular species, *Trichogramma brassicae*, is widely used as a biological control agent and has been used in biological control of some key pests (Smith 1996, Ebrahimi et al. 1998, Lundgren et al. 2002). In nature, one uninfected and one infected strain of *T. brassicae* coexists, with the infected strain only producing female offspring (Farrokh et al. 2010, Poorjavad et al. 2012). It has been shown that both strains are genetically similar and that their differences are due only to *Wolbachia* infection (Kishani Farahani et al. 2015). However, a recent study revealed that *Wolbachia* infected *T. brassicae* possess a lower ability to evaluate host quality compared to uninfected wasps, leading authors to conclude that such behavior was the result of host manipulation (Kishani Farahani et al., 2015).

The current study was carried out to investigate whether nutritional quantity during immature development affects memory retention of adult wasps of both the infected and uninfected strain of *T. brassicae*. We hypothesized that (1) uninfected wasps, which need high quality hosts to develop (Kishani Farahani et al., 2015) will present less proteins, triglycerides and lipids when developing in low quality hosts (2) uninfected wasps with less nutrients will be affected in their learning capacities (3) infected wasps, which oviposit equally in high and low quality hosts (Kishani Farahani et al., 2015) will not present differences in nutrients and (4) they will not be affected by host quality for their learning ability.

**Material and Methods:**

**Parasitoids: choice of the strains, collection and rearing**

We compared two naturally infected and uninfected strains for which we have shown similar genetic background, which proved that all behavioral differences between these two strains are linked to *Wolbachia* prevalence (Kishani Farahani et al. 2015). Another possibility to conduct
this experiment would have been to use antibiotic treatment to obtain an uninfected strain, but some studies have shown the negative effects of antibiotics on *Wolbachia* treated arthropods (Dedeine et al. 2001, Timmermans and Ellers 2009). Removing *Wolbachia* by antibiotic treatment may affect physiology and behavior of *Wolbachia* infected arthropods and thus the observed difference between *Wolbachia* infected and antibiotic treated individuals may be due to negative effects of antibiotic treatment. In conclusion, comparing natural strains may show natural differences in all biological aspects of infected and uninfected individuals and may represent a better method by which to observe the effects of *Wolbachia*.

Parasitoid strains were derived from cultures maintained by 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 2012. Parasitoids were reared at 25±1°C, 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±1°C on wheat flour and yeast (5%), maintained at the Insectary and Quarantine Facility, University of Tehran. Approximately 20 mated female moths were kept in glass containers (500 ml) to provide eggs for experiments involving uninfected and infected wasps. Wasps were reared for 15 generations on *E. kuehniella* prior to use in experiments.

To rear wasps on different host qualities, one hundred fresh eggs (less than one day old) (high quality eggs) and old eggs (more than 45 days old) (low quality eggs) were exposed to one day old wasp females for 24 hours. Old eggs were smaller, had a scarious chorion and a lower weight, as low-quality reward. This was carried out for both strains. After 24 hours, the eggs
were removed and kept under controlled conditions of 25±1°C, 16L: 8 D, and 50±5% RH in a growth chamber and checked until adult wasp emergence.

**Experimental design:**

In the current research, effects of *Wolbachia* presence and host quality during immature developmental time on adult wasps learning ability and memory retention was studied in a factorial design for four total treatments: T1: uninfected wasps emerging from high quality hosts, T2: uninfected wasps emerging from low quality hosts, T3 infected wasps emerging from high quality hosts, T4: infected wasps emerging from low quality hosts).

**Determination of Glycogen, Triglyceride and Protein amounts**

For the four treatments T1 to T4, the amount of glycogen, triglycerides and proteins was determined from x newly emerging females.

**Glycogen determination**

Fat bodies of 30 adults, from each line, were removed and immersed in 1 ml of 30% KOH w/Na$_2$SO$_4$. 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. 2 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 490 nm 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 parasitoid. Reagent solution contained phosphate buffer (50 mM, pH 7.2), 4-chlorophenol (4 mM), Adenosine 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:

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

**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).

**Learning ability:**

**Conditioning**

To study the innate preference of the wasps towards one odor (peppermint and lemon odors were tested) against a filter paper without odor, 50 naïve wasps of each strain were exposed to either the odor of peppermint or the odor of lemon (98% purity, by Adonis Gol Darou Group, Iran) in
the wind tunnel as previously described by Yong et al. (2007) and their responses were recorded. Wasps were also exposed to odor versus clean air. To do this, single naïve female wasps were introduced into the flight chamber. The tested odor was presented on strips of filter paper (1×2 cm) on which 1 μl of one or other solution (peppermint or lemon) had been placed on one filter paper, and no odor on the second filter paper. Each filter paper was attached to a glass pipette placed vertically on a stand and spaced 10 cm apart. Twenty-five of the 50 wasps underwent this procedure using the peppermint odor (at least 97% pure) and the other 25 underwent the procedure using the lemon odor (at least 97% pure). The responses of the wasps to the odors were observed in the flight tunnel during a flight time of 15 min. Any individual that landed or hovered on an odor site for more than 2 minutes was recorded as a responder wasp. Females that did not complete a flight or did not fly over the start area in the flight chamber were scored as displaying no response.

The ability of wasps to learn was determined using a Pavlovian conditioning procedure whereby an odor stimulus was associated with the reward of ovipositing (Bleeker et al. 2006). Several studies have shown that oviposition is a reward for parasitoids (for example van Baaren et al. 2005, Bleeker et al. 2006). A flight chamber was subsequently used to observe the behavior of conditioned wasps. Sixty five one-day old naïve females, reared on high quality hosts and 65 reared on low quality hosts, were exposed individually to host eggs for 15 minutes to gain oviposition experience. This was to avoid the variability in sequence and the retention of behavioral events associated with learning from the first host encountered (Mills and Kuhlmann 2004). Since some wasps died, were lost or did not oviposit during the manipulation, approximately sixty wasps per treatment were used. Half of the test individuals (n=30) were conditioned using peppermint odor and the remaining half using lemon odor. For conditioning,
one adult wasp was introduced to a vial (2×10 cm) containing 100 host eggs (one day old eggs, glued on a cardboard) and was transferred into the conditioning tank (25×25×25 cm). Subsequently, peppermint or lemon odor (98% purity, by Adonis Gol Darou Group, Iran) were used to condition the wasps. During experiments, the conditioning odor (either peppermint or lemon) was pumped into the tanks with a speed of 1 m/s speed. The conditioning process lasted a total of 2 hours and was repeated for both uninfected and infected females reared on high and low quality hosts (60 females of each of the treatments T1 to T4). The conditioning time of 2 hours was set based on the average time of patch leaving of 100 adult wasps exposed to 100 eggs. This time was recorded by direct observation.

Test of odor preference after conditioning

Fifteen minutes after conditioning, infected and uninfected female wasps reared on high and low quality hosts were placed individually in the flight chamber. The responses of 50 female wasps (randomly selected from the surviving wasps of the 60 conditioned), 25 conditioned on peppermint and 25 conditioned on lemon, were tested for the four treatment groups (totaling 4 x 50 females). The responses of the wasps to the conditioned odors were observed in the flight tunnel during a flight time of 15 min. If females displayed a preference towards the conditioned odor (i.e. the individual landed or hovered on the conditioned odor site for more than 2 minutes), it was assumed that associative learning between the odor and the reward of oviposition had occurred. The number of rejection responses and non-responding wasps were recorded to determine behavioral response variation by both strains. Females that did not complete a flight or did not fly after 5 min were scored as displaying no response. All flight responses were tested at 25°C, 50% RH, and a light intensity of 2000 lux.
Test of memory retention duration:

Memory retention was assumed when wasps showed a significant preference for the conditioned odor (peppermint/lemon). To determine the duration of memory retention, experimental wasps of both strains, reared on low and high quality hosts, were observed 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, and 30h after training in a choice test experiment between the conditioned and unconditioned odor in the flight tunnel. For each time interval, 50 new wasps of each strain (25 conditioned with each odor) reared on high quality hosts and 50 reared on low quality hosts were observed in the wind tunnel as described, totaling 2400 tested wasps.

Statistical analysis:

Comparison of Glycogen, Triglyceride and Protein amounts

Amount of protein, triglyceride and glycogen were analyzed in a two way ANOVA in GLM Proc. by SAS ver. (9.1). In all cases, the factors were the strain and the host quality. When a significant effect of treatment was found, Tukey post hoc test was performed. Data are presented as means ± SE and statistical analyses were performed using SAS software (SAS Institute Inc. 2003).

Learning and memory

The innate responses of both strains were compared by Chi-Square tests using SAS software (SAS Institute 2003). To compare the responses of the two strains before and after conditioning, a Generalized Linear model implemented in the procedure GENMOD of SAS software (ver. 9.1) was used with the binomial family error and logit link. The least square estimates of the proportions in each level were then compared by the Chi-square approximation (an option offered by GENMOD).
The estimation of forgetting relies on a series of observations recorded at different times $t_1; t_2; \ldots t_n$ after conditioning. At each time, a set of $n_t$ subjects was subjected to a choice test with three possible responses: $a; b; c$, which correspond respectively to a preference for the odor side of the flight chamber, a rejection (choice of the opposite side of the chamber), and a null choice, i.e. no preference of chamber side and a null response. The forgetting of conditioning results in a switch from a high level to a lower level of positive responses, a simultaneous switch from a low level to a high level of null responses, and a switch from a very low to a moderate level of negative responses. A constraint links the three responses as $n_a + n_b + n_c = n_t$ or $n_c = n_t - n_a - n_b$.

The course of these three responses over time can be described by two logistic functions written here as probabilities,

$p_a, p_b, p_c$, constrained by $p_a + p_b + p_c = 1$:

\begin{align*}
(1) \quad p_a &= k_a - \frac{k_a - a_a}{1 + e^{(-b_a(t-t_0))}} + a_a \\
(2) \quad p_c &= k_c - \frac{k_c - a_c}{1 + e^{(-b_c(t-t_0))}} + a_c \\
(3) \quad p_b &= 1 - p_a - p_c
\end{align*}

$k_a$, respectively $k_c$, and $a_a$, respectively $a_c$ define the sill and baselines of the logistic models (1) and (2): the baselines are $a_a$ and $a_c$, and the seals are $k_a + a_a$ in model (1), $k_c + a_c$ in model (2). $k_a + a_a$ estimates the initial state in model (1), and $a_c$ the final state. It is the inverse in model (2), where $a_c$ is the initial state and $k_c + a_c$ the final state.

A supplementary restriction lies in the fact that, as $t_0$ represents the mean time to forgetting, i.e. the inflection time point of the logistics functions; it has to be the same in all three equations.

The data consist of a vector of three counts: $V_i = (n_{a_i}, n_{b_i}, n_{c_i})$ the respective number of subjects
responding \( a; b \) or \( c \) at time \( t \). An \( R \) script was written to enable this. The experimental design was a balanced factorial design with two factors: the type of strain, with two levels (uninfected and infected), and the number of conditioning experiments, with five levels. This design resulted in ten crossed levels. The model, defined by equations 1 to 3, was fitted individually on each set of ten data. The maximization of the likelihood cannot be fully automatic, and requires an initial guess of the seven parameters \( k_a; a_a; b_a; k_c; a_c; b_c; t_0 \). This was done by a visual evaluation of each graphic representation of the crossed levels. After maximization, we obtained seven estimates of each parameter, along with an estimate of their standard error through the hessian matrix, which is provided on demand by the \textit{optim} function of \( R \) (\( R \) core team 2013). All correlations between pairs of coefficients were lower than 0.9, a threshold commonly accepted as critical for the correct inversion of the hessian matrix (Bouvier 1996 by Bouvier and Huet 1994).

As our main focus was \( t_0 \), we will give only the results for this parameter, which was accurately estimated with a standard errors ranging from 0.3 to 1.8 hours. Recorded forgetting times between high and low quality reared uninfected and infected wasps were compared by Cox proportional hazard models.

\textbf{Results:}

\textbf{Amounts of Glycogen, Triglyceride and Protein}

For all physiological measurements, there was a difference between wasps reared on high quality food vs. low quality food only when they were uninfected with \textit{Wolbachia}.

Effects of strain, host quality and their interaction on amount of acquisitioned resource by uninfected and infected wasps are shown in Table 1. Uninfected wasp protein amount did not show significant difference by infected wasps in high and low quality host reared wasps (Uninfected wasps: \( p= <.0001; \) Infected wasps: \( p= 0.211 \)). Triglyceride amount in uninfected
wasps was significantly higher in wasps reared on high quality hosts (p=0.006) while infected wasps showed similar amount (p= 0.587) (Figure 1). Glycogen amount of uninfected wasps reared on high quality hosts differed significantly (p=0.009) (Figure 1) whereas infected wasps showed no significant difference (p= 0.837).

Test of odor preference after conditioning

Naïve females of both the uninfected ($\chi^2=0.13$, $p=0.93$, $df= 2$, $N=50$) and the infected ($\chi^2=0.69$, $p=0.70$, $df= 2$, $N=50$) strains displayed no significant innate preference for the odor of lemon or that of peppermint against the filter paper with no odor or for no response wasps ($\chi^2=0.95$, $p=0.62$, $df= 4$, $N=100$). Furthermore, when uninfected (peppermint vs air: $\chi^2=2.31$, $p=0.12$, $df= 2$, $N=50$; lemon vs air: $\chi^2=0.63$, $p=0.42$, $df= 2$, $N=50$) and infected wasps (peppermint vs air: $\chi^2=1.44$, $p=0.22$, $df= 2$, $N=50$; lemon vs air: $\chi^2=0.02$, $p=0.86$, $df= 2$, $N=50$) were tested with odor and clean air showed no significant difference in their behavior.

Effects of strain, conditioning and their interaction on the response of uninfected and infected wasps are shown in table 2. The rate of no response of uninfected wasps reared on high quality hosts decreased significantly after conditioning for both odors (GENMOD of SAS, lemon odor: $\chi^2=6.36$, $p=0.0117$, $df=1$, $N=25$; peppermint odor: $\chi^2=5.67$, $p=0.0173$, $df=1$, $N=25$) (Fig. 2a). The rate of no response of uninfected wasps reared on low quality hosts was not significantly different before and after conditioning (lemon odor: $\chi^2=2.18$, $p=0.14$, $df=1$, $N=25$; peppermint odor: $\chi^2=1.37$, $p=0.241$, $df=1$, $N=25$) (Fig. 2a). For individuals of the uninfected strain emerging from high and low quality hosts, the rate of positive response towards the odor increased significantly after conditioning (Proc GENMOD of SAS: lemon odor: $\chi^2=10.59$, $p=0.0011$, $df=1$, $N=25$; peppermint odor: $\chi^2=9.08$, $p=0.0026$, $df=1$, $N=25$ for high quality host reared wasps
(Figure 3a); lemon odor: $\chi^2=4.94$, $p=0.026$, df=1, N=25; peppermint odor: $\chi^2=4.94$, $p=0.026$, df=1, N=25 for low quality host reared wasps, (Figure 3a).

The number of null responses of infected wasps reared on high and low quality hosts did not decrease significantly after conditioning (lemon odor: $\chi^2=0.37$, $p=0.5451$, df=1, N=25; peppermint odor: $\chi^2=0.1$, $p=0.7578$, df=1, N=25 for high quality host reared wasps (Figure 2b); and lemon odor: $\chi^2=0.39$, $p=0.529$, df=1, N=25; peppermint odor: $\chi^2=0.4$, $p=0.53$, df=1, N=25 for low quality host reared wasps) (Figure 2b). In both cases (wasps reared on high and low quality hosts) approximately 30% of individuals did not respond to the odor (Figure 2b). Infected wasps reared on high and low quality hosts significantly associated the presence of a conditioned stimuli with oviposition (Lemon odor: $\chi^2=6.18$, $p=0.0129$, df=1, N=25; peppermint odor: $\chi^2=7.55$, $p=0.006$, df=1, N=25 for high quality host reared wasps; lemon odor: $\chi^2=7.56$, $p=0.007$, df=1, N=25; peppermint odor: $\chi^2=7.55$, $p=0.006$, df=1, N=25 for low quality host reared wasps) (Figure 3b).

Memory retention

To reveal significant differences in memory retention of both uninfected and infected wasps reared on high and low quality hosts, we carried out a three-factor analysis of variance to verify the conclusions. Differences between the strains were highly significant ($F= 0.23$, $p= 0.006$), as were the host quality ($F = 49.05$, $p= 0.0003$), with odor type ($F =0.015$, $p =0.09$) and their interaction ($F=7.63$, $p=0.02$) being significant. For the uninfected strain, the memory duration was longer for wasps reared on high quality hosts than for wasps reared on low quality hosts ($p= 0.029$ for lemon odor; $p= 0.030$ for peppermint odor) (Fig. 4). For the infected strain, the memory duration did not vary significantly with host quality ($p= 0.84$ for lemon odor; $p= 0.42$ for peppermint odor) (Fig. 4). The memory retention of infected wasps was lower than
uninfected wasps when reared on high quality hosts (p=0.009 for lemon odor; p=0.009 for peppermint odor). However, memory retention did not significantly differ between infected and uninfected wasps when reared on low quality hosts (p=0.52 for lemon odor; p=0.84 for peppermint odor).

Discussion:

In accordance with our main hypotheses, host quality affected learning ability and memory retention of uninfected wasps, while memory retention of infected wasps was not affected by host quality. Host quality significantly affected memory retention of uninfected wasps, with uninfected wasps reared on high quality hosts displaying greater memory retention than those reared on low quality hosts. For the amount of resources, uninfected wasps reared on high quality hosts obtained significantly more proteins, triglycerides and glycogen from their host during their development whereas infected wasps obtained the same amount of resources on both high and low quality hosts. We will first discuss the case of the uninfected wasps and then the case of the infected wasps.

Recent studies have shown the effects of nutrition during the early life stages on adult cognition and learning capacity in different vertebrates such as rats, pigs, primates and humans (Tveden-Nyborg et al. 2009, Anwar Zainuddin and Thuret 2012, Nyaradi et al. 2013). In most cases, it was shown that nutritional quality strongly affects neurogenesis in organs which is involved in learning and memory formation during adulthood due to lack of minerals, vitamins, amino acids and fatty acids (Lucassen et al. 2013, Nyaradi et al. 2013, Hoeijmakers et al. 2015). Our results showed that uninfected wasps reared on low quality hosts displayed shorter memory retention than wasps reared on high quality hosts and this may be due to a lack of the requirements in low quality hosts. For insect parasitoids, the host represents the sole nutritional and physiological
environment during immature development (Jervis and Kidd 1986). Many endoparasitic Hymenopterans like *Trichogramma* spp. have no yolk in their eggs and so lay their eggs inside the body of hosts, which subsequently provides the nutrients for both embryonic and larval development (Chapman 2012). Consequently, host quality is important for overall parasitoid growth and development and may influence the developmental time, mortality rate, longevity, fecundity and behavior (Harvey and Strand 2002, Sampaio et al. 2008). In adult wasps, it was shown that storage macromolecules have critical roles in searching behavior, flying, and reproduction. Since efficient parasitoids must have appropriate searching behavior, these macromolecules provide required energy by processing carbohydrates, proteins and lipids (Lee and Park 2004, Bauerfeind and Fischer 2005, Plácido-Silva et al. 2006, Jordão et al. 2010). Moreover, most parasitic species (and those of this study) are incapable of lipogenesis as adults (Visser et al. 2010), and, as such, their lipid resource consists solely of lipids obtained from the host during development. Thus acquisitioned energetic and structural resources during immature development of parasitoid wasps play an important role in adult behavioral and physiological fitness. Glycogen, triglycerides and proteins are the three main storage macromolecules in the body of insects responsible for several energetic demanding processes. Phosphorylation of glycogen and triglycerides, as well as transamination of protein molecules, provides intermediate components for electron transport system providing energy, oxygen and water (Nation 2008). In eggs, presence of these components is essential for development of the embryo. Learning and storing memory is a costly process which affects animal fitness (Niven et al. 2003, Jones and MacLarnon 2004, Isler and van Schaik 2006 a b, Niven et al. 2007, Niven and Laugiiun 2008,). The brain is a specialized tissue in which functionality depends upon the generation of electrical potentials and their conduction through long axonal components of cell-bodies and through the
synaptic gaps between these cell-bodies (Rosales et al. 2009). These special functions of brain are reflected in a higher need for nutrients such as fats and glycogen, and nutrition plays a critical role at the cross-roads of the biological and nurturing factors that mediate brain growth and development. As our results showed that uninfected wasps reared on high quality hosts contained more glycogen, which is the most readily available energy source, this enables the wasps to spend more energy resources on daily activities such as movement or memory formation.

Triglycerides are a major form of lipids stored into the fat body, and when triglycerides are transformed into diglycerides, they are transported by a specific lipoprotein to various organs/tissues, including the brain to provide the required energy. Octopamine, as an involved neurotransmitter in learning (Unoki et al. 2006), does play a role in metabolism because activating octopaminergic cells increases triglycerides (Erion et al. 2012). Thus extra resources of triglycerides in uninfected wasps reared on high quality hosts can be used as energy resources needed to consolidate longer memory. In conclusion, the lack of appropriate nutrients seemed to be the cause of the decrease of memory retention, although the precise physiological mechanisms remain to be elucidated.

For the infected strain, there was no effect of host quality, either for the learning ability or for the amount of nutrients at the end of the development. There is evidence in the literature that Wolbachia may utilize the host’s recourses like a parasite. For example, Caragata et al. (2014) determined competition for nutrients between Wolbachia and Aedes aegypti. Infection of mosquitoes with the virulent Wolbachia strain wMelPop caused a significant reduction of fecundity and egg viability. The authors explained the finding by competition between A. aegypti and the symbiont for essential blood meal nutrients. Moreover, they found cholesterol and amino acids had critical roles in egg structural development, with Wolbachia infection reducing total
cholesterol levels in mosquitoes by 15-25% (Caragata et al. 2014). As such, both Wolbachia
and host may be involved in competition over nutritional resources, which will strongly affect
the amount of available capital resources in adults. Interestingly, results of our study suggest that
this is not the case, with infected wasps reared on low quality hosts displaying the same amount
of nutrients as infected wasps reared on high quality hosts.

In our study, Wolbachia may manipulate and maximize resource acquisition in its host,
resulting in a similar amount of nutritional resources of Wolbachia infected wasps reared on high
and low quality hosts. Wolbachia infected wasp energy resources contained more triglycerides,
meaning that these wasps save their energy under the form of triglycerides instead of glycogen as
with uninfected wasps. Kishani Farahani et al. (2015) stated that infected wasps spend more time
in host feeding during their adult life compared to uninfected wasps. This host-feeding behavior
provides them with more proteins and glycogen. As shown by Kishani Farahani et al. (2015),
superparasitism rate in infected wasps was higher than in uninfected wasps; a behavior which
helps Wolbachia to disperse between uninfected individuals via horizontal transfer. As such, a
shorter memory retention may lead to a higher superparasitism rate by forgetting host parasitized
marking cues. It seems that Wolbachia is using an adaptive strategy to enhance dispersal
between individuals by shortening memory retention and maximizing the same energy resource
amount from high and low quality hosts. However, at present, it cannot be concluded whether the
differences observed in the current study represent a strategy employed by Wolbachia or are
simply a physical or physiological side effect of Wolbachia infection.

Finally, it seems that differences between the learning ability and memory retention of
uninfected and infected wasps may be derived from the amount of energy which can be utilized
daily for brain function, since these organs are energetically expensive. Further research is
therefore required to investigate the effects of *Wolbachia* infection on the Central Nervous System and other vital organs involved in lifetime information acquisition.
References:


Araújo MS, Herman L, Gill S, Silva AA. 2012. Larval food quantity affects development time, survival and adult biological traits that influence the vectorial capacity of Anopheles darling under laboratory conditions. Malaria J. 11:261-270.


Table 1. Effects of *Wolbachia* presence, host quality and the interaction of these two factors on the amount of protein, triglyceride and glycogen of wasps. Significant results are shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>Host quality</th>
<th></th>
<th>Wolbachia presence</th>
<th></th>
<th>Interaction</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F value</td>
<td>Pr &gt; F</td>
<td>F value</td>
<td>Pr &gt; F</td>
<td>F value</td>
<td>Pr &gt; F</td>
</tr>
<tr>
<td>Protein</td>
<td>25.71</td>
<td><strong>0.001</strong></td>
<td>6.01</td>
<td><strong>0.039</strong></td>
<td>48.87</td>
<td><strong>0.0001</strong></td>
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<tr>
<td>Triglyceride</td>
<td>8.87</td>
<td><strong>0.017</strong></td>
<td>2.65</td>
<td>0.14</td>
<td>4.75</td>
<td>0.060</td>
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<tr>
<td>Glycogen</td>
<td>5.10</td>
<td>0.053</td>
<td>3.39</td>
<td>0.103</td>
<td>6.54</td>
<td><strong>0.033</strong></td>
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</tbody>
</table>
Table 2. Effects of host quality, conditioning and the interaction of these two factors on responses of uninfected and infected wasps. Significant results are shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>Host quality</th>
<th>Conditioning</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>P value</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td><strong>Uninfected</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peppermint</td>
<td>No response</td>
<td>2.51</td>
<td>0.1128</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1.03</td>
<td>0.3098</td>
</tr>
<tr>
<td>Lemon</td>
<td>No response</td>
<td>6.30</td>
<td>0.0121</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0.44</td>
<td>0.5077</td>
</tr>
<tr>
<td><strong>Infected</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peppermint</td>
<td>No response</td>
<td>0.11</td>
<td>0.7396</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0.00</td>
<td>1.0000</td>
</tr>
<tr>
<td>Lemon</td>
<td>No response</td>
<td>0.11</td>
<td>0.7363</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0.10</td>
<td>0.7554</td>
</tr>
</tbody>
</table>
Figure legends:

Figure 1. Mean± SE of protein, triglyceride and glycogen amount in uninfected and infected wasps in low- (LQE) and high-quality (HQE) hosts. Different letters indicate significant differences between the treatments after Bonferroni correction (P=0.0125).

Figure 2. Percentage of null responses by uninfected wasps (a) and infected wasps (b) reared on high or low quality hosts after and before conditioning by peppermint and lemon odor. Responses of 25 wasps (uninfected, only females, and infected) to peppermint/lemon odor were compared. Different letters indicate significant differences. N.S.: Not Significant.

Figure 3. Percentage of recorded Positive Responses by uninfected wasps (a) and infected wasps (b) reared on high or low quality hosts after and before conditioning by peppermint and lemon odor. Responses of 25 wasps (uninfected, only females, and infected) to peppermint/lemon odor were compared. Different letters indicate significant differences. N.S.: Not Significant.

Figure 4. Differences in the forgetting mid-time depending on the host quality and the type of strain. Vertical notched bars indicate the 95% confidence interval of the estimates.
Fig 1.
Fig 2.
Fig 3.
Fig 4.