The effects of a meridic diet on the sex ratio of offspring, on glycogen and protein content, and on productivity and longevity of adult *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) for five generations

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ABSTRACT. In this study, we determined the effects of a meridic diet on adult emergence, glycogen and protein level of the endoparasitoid *Pimpla turionellae*, and investigated female lifetime, adult emergence rate, egg numbers and hatching for five generations. The meridic and control diets were fed to *P. turionellae* for 28 days. The meridic diet was associated with a significant increase in the total adult emergence, which reached 93.33% on day 16; 100% females were produced from eggs laid on day 25. The meridic diet significantly decreased glycogen level in *P. turionellae* on days 13 and 16, while it significantly increased the protein level on days 13, 16 and 19 in comparison to the control natural diet. The meridic diet did not negatively affect total adult emergence, lifetime and egg numbers over the first and second generations when compared to the control. However, female lifetime and egg numbers were significantly decreased in F3, F4 and F5 generations. This manuscript is the first to report rearing the adult stages of *P. turionellae* on meridic diets for five generations. It shows that a chemically-defined meridic diet may be beneficial in improving the biological and biochemical fitness of the first couple of generations of these parasitoids reared for use in biological control programs.

KEY WORDS : *Pimpla turionellae*, sex ratio, synthetic diet, glycogen, protein, adult emergence.

INTRODUCTION

Chemical pest management has been widely used, even though it can have a significant negative effect on the ecosystem. Chemicals affect not only the target pests, but also collaterally damage beneficial insects such as predators, parasitoids, and pathogens (SINGH, 1977).

Species of Hymenoptera are still widely used in biological pest control, and are easy to rear in *in vitro* culture by using an artificial diet (YAZGAN, 1981; EMRE, 1988). Entomophagous parasitic hymenoptera have a long life cycle, and the nutrients required for their survival and reproduction, such as proteins, lipids, carbohydrates, vitamins and inorganic salts, are supplied from plant juice, pollen and host haemolymph (LEUIS, 1961).

One of the main problems for continuous mass culturing of parasitic hymenopterans is obtaining the required hosts. Theoretical and empirical evidence suggest that according to the qualitative and quantitative combinations of the food source, the stored glycogen and protein play a key role in affecting egg number, emergence ratio, and transition time to adulthood (DADD, 1985; CANGUSSU & ZUCOLOTO, 1997). For a successful and efficient mass production system, egg number and sex ratio are important (ORR & BOETHEL, 1990; RAMADAN et al., 1995), and work on the latter has often been successful (WERREN, 1987; WEST et al., 2002).

In biological control, the target populations of pests should be kept at an acceptable level, and at the same time, side effects on the ecosystem should be minimized; thus a sufficient number of biological control agents must be introduced to the area where the pests species are.

Because parasitoid hymenopterans can determine the sex of the adult insect by controlling the sperm entrance to the egg with their haplo-diploid sex determination (FLANDERS, 1956), they are the ideal organisms for sex ratio studies (GODFRAY, 1994). The sex ratio can be affected by host size, sperm morphology, coupling rate, temperature, photoperiod, and qualitative and quantitative nutritional requirements (WILKES, 1964; HOLSCHER & VINSON, 1971; SANDLAND, 1979; HAGLEY & BARBER, 1992; ALLEN et al., 1994; KAZMER & LUCK, 1995; COŞKUN et al., 2005). As well as keeping the sex ratio at the optimum level, it is important to ensure diets and ambient conditions appropriate to the insect species (ETZEL & LEGNER, 1999).

The ichneumonid endoparasitoid *Pimpla turionellae* L. is used in biological control against numerous species of Lepidoptera, including the larvae or pupae of the black-veined white *Aporia crataegi* (L) (Pieridae), the gypsy moth, *Lymantria dispar* (L) (Lasiocampidae), and the Mediterranean flour moth, *Ephestia kuehniella* Zell. (Pyralidae) (THOMPSON, 1957). The ichneumonid lays its eggs in the lepidopteran species, thus assuring the continuation of its population. The effects of changing the synthetic diet developed by EMRE (1988), or of adding nutrients, on egg number, hatching, lifespan, sex ratio, glycogen and protein amount in the insect, have been documented only for the first generation (ÖZALP & EMRE, 2001; SULANÇ & EMRE, 2000; BAYKAL et al., 2005; COŞKUN et al., 2005).

It is important to study the effects of changes in reproduction and development on subsequent generations for mass rearing programs. In the present study, we quantified the effect of a meridic diet on *P. turionellae*, focusing on sex ratio, glycogen and protein levels in the first generation, and on sex ratio, egg number laid by adult females, hatchability, and lifespan for five generations.

TABLE 1

Composition of the chemically-defined synthetic diet fed to *P. turionellae* adults (EMRE, 1988)

Constituent	mg/100ml diet	Constituent	mg/100ml diet
L-Amino acid mixture	3000.00	Water soluble vita-min mixture	284.38
Alanine	210.00	Ascorbic acid	10.6105
Arginine-HCl	150.00	Biotin	0.0379
Aspartic acid	195.00	Ca-Panthothenate	2.8042
Cysteine	39.00	Choline chloride	246.3158
Glutamic acid	315.00	Folic acid	0.1137
Glycine	192.00	Inositol	17.0526
Histidine	120.00	Nicotinic acid	5.6842
Hydroxproline	57.00	Pyridoxine-HCl	0.2842
Isoleucine	156.00	Riboflavin	1.3263
Leucine	231.00	Thiamine-HCl	0.1516
Lysine	159.00		
Methionine	90.00	Inorganic salt mixture	75.00
Phenylalanine	165.00	$FeCl_3 \, 6H_2O$	2.1583
Proline	246.00	K_2HPO_4	45.0129
Serine	195.00	$Na_2HPO_4 \, 12H_2O$	6.2201
Threonine	165.00	$MgSO_4 \, 7H_2O$	15.7853
Tryptophane	60.00	$MnSO_4 \, H_2O$	0.0479
Tyrosine	120.00	$CoCl_3 \, 6H_2O$	0.5798
Valine	135.00	$CuSO_4 \, 5H_2O$	0.6721
		$CaCl_2$	3.6684
Lipid mixture	540.96	$ZnCl_2$	0.8552
Cholesterol	138.8430		
Linoleic acid	8.0331	Miscellaneous	
Linolenic acid	25.5537	Ribonucleic acid	75.00
Oleic acid	10.5950	Sucrose	14000.00
Palmitic acid	0.6777	2N KOH	280.00
Stearic acid	0.2314	2N K_2HPO_4*	14.03
Tween 80	357.0248	Distilled water to 100 ml	

*: Added into the water soluble mixture solution

MATERIALS AND METHODS

Maintenance of adult wasps

Wasps of *P. turionellae* were reared in the laboratory on the pupae of the greater wax moth, *Galleria mellonella* (L) (Lepidoptera: Pyralidae), and fed a diet of 50% honey solution, *G. mellonella* pupae and synthetic diet (EMRE, 1988). The composition of the chemically-defined synthetic diet, consisting of amino acids, lipids, vitamins, inorganic salts, sucrose and other nutrients, is shown in Table 1. To prepare the synthetic diet, the L-amino acid mixture, inorganic salt mixture, ribonucleic acid and sucrose, were dissolved in 50ml 90ºC distilled water. The solution was allowed to cool, the lipid and vitamin mixtures added, and the pH of the diet adjusted to pH 6.5 with 2N KOH, and finally the volume was brought to 100ml with distilled water.

For the experiments, we used recently matured *P. turionellae,* which had not been fed or mated. They were held in a cage approximately 20x25x25cm. The experimental group then received a liquid synthetic diet at regular intervals on 3x3cm aluminium foils that were put into the bottom of the cages. The control group received equal parts liquid honey and water, absorbed on a piece of cotton, along with five *G. mellonella* pupae every three days; the pupae were kept in the cage for an hour and then removed. This was repeated every three days at the same time throughout the experiment.

Determination of sex ratio

The method that was used to evaluate the effect of the meridic diet on *P. turionellae* sex ratio is described by COŞKUN et al. (2005). Ten females and five males that matured on the same day, were transferred to each experimental cage. Simultaneously, 10 *G. mellonella* pupae were provided for 1 hour, in which the wasps laid their eggs. After oviposition, all pupae were removed, placed in a beaker, and held until the adults emerged. The number of emerging male and female wasps was recorded, and pupae were dissected to determine the number that failed to produce an adult wasp. The female emergence ratio is the number of females emerging compared to the total number of emerging adults. This experiment was repeated three times and data were pooled for statistical analysis.

Adult emergence

Adult emergence was determined by calculating, as a percentage, the number of emerged individuals compared to the total number of pupae that were placed in the cages to be parasitized.

Determination of glycogen and protein levels

Four females, on each day, were placed in a 1000ml beaker covered with a highly porous cloth. The insects were removed to evaluate the synthesis of glycogen and protein beginning 10 days after the start of the experiment and then every three days until day 28. The anthrone test was used for determination of glycogen, and the quantitative Biuret test was used for determination of protein (ROE et al., 1961; PLUMMER, 1971).

For the protein and glycogen extraction, insects were placed in 10% trichloroacetic acid (TCA) solution and homogenized for five minutes. The homogenate was centrifuged at 3500g for 15min. Total proteins were determined spectrophotometrically at a wavelength 540nm. In order to determinate the total glycogen, aliquots of the supernatant were mixed with 96% ethyl alcohol for 24h at 37ºC, and then centrifuged at 3500g for 30 minutes. Total

glycogen of the pellet was determined spectrophotometrically at a wavelength 620nm.

All of the experiments were repeated at least three times under laboratory conditions at 24±2ºC, 75±5% humidity and 12 hours of light photoperiod. The statistical analyses were done with "Independent Samples Test" (T test). Differences between groups were considered to be significant at p≤0.05

Rearing adult *P. turionellae* for five generations with a meridic diet

Experiments were conducted on five successive generations to determine the effects of a meridic diet on (i) female emergence, (ii) number of eggs produced per female during her lifespan, (iii) the percentage of eggs hatched during a lifespan (hatchability), and (iv) the average female lifetime corresponding to each generation (lifetime). Generations were named as F1, F2, F3, F4, and F5.

To evaluate the effect of meridic diet on sex ratio and adult emergence in each generation, 10 female and 5 male were placed in cages and until the insects were 28-days-old data were gathered as described above for one generation.

To determine the effect of the meridic diet on egg number, hatchability and lifetime in each generation, four unfed and unmated females and two males were placed into a 1000cc beaker which was then closed by cheesecloth. The females were allowed to parasitize *G. mellonella* pupae after 10 days and at subsequent three day intervals until day 28. The provision of pupae for parasitism took about 30min, and at the end of this period, pupae were collected from the beakers and held for 24 hours for embryonic development. Pupae were then dissected in a petri dish with 0.8% NaCl, and the eggs gently transferred into another petri dish with 0.8% NaCl. After 24h incubation, numbers of laid and hatched eggs were counted under a stereomicroscope. In all replicates (3), the mean numbers of eggs per female were calculated by dividing the total number of eggs by the number of females. Hatchability was calculated by dividing the hatched eggs by the total number of eggs laid.

To calculate the lifetime of the female *P. turionellae*, feeding was continued until all insects died. The cages were checked daily for mortality, and after all females died, the average longevity of wasps was determined for each generation. The corresponding procedure was repeated for the control group.

Statistical analyses

Data from the effect of the meridic diet over the five generations were compared with the control. Statistical analysis was done with "Student-Newman Keuls Test (SNK)" (SOKAL & ROHLF, 1969). Differences between groups were considered to be significant at a probability level of p<0.05%.

TABLE 2

Effects of meridic diet on the sex ratio of *P. turionellae*

| | Adult Emergence (%) | |
| | Total | Female |
Diets	Mean±S.D *	Mean±S.D *
Meridic diet	74.28±0.82 a	72.34±0.72 a
Natural (control)	68.57±1.43 b	67.36±0.12 b

*Values followed by the same letter are not significantly different from each other (P<0.05, T test)

TABLE 3

Effects of meridic diet on the emergence ratio of *P. turionellae* according to days

| | Emergence adult (%) | | | |
| Day eggs laid | Total | | Female | |
	Meridic diet Mean±S.D *	Control diet Mean±S.D *	Meridic diet Mean±S.D *	Control diet Mean±S.D *
10	70.00±5.77 a	66.67±3.33 a	45.79±2.85 a	39.57±3.29 a
13	83.33±3.33 a	53.33±3.33 b	68.05±3.67 b	78.25±3.55 a
16	93.33±3.33 a	80.00±5.77 b	67.78±1.11 b	74.74±1.84 a
19	76.67±3.33 a	66.66±3.33 b	73.81±1.19 b	84.91±0.79 a
22	73.33±3.33 a	76.67±3.33 a	72.62±1.19 a	60.71±1.78 b
25	76.67±3.33 a	70.00±0.00 a	100.00±0.00 a	71.43±0.00 b
28	46.67±3.33 a	60.00±5.77 a	78.33±1.66 a	61.27±2.82 b

* Values followed by the same letter in the same line are not significantly different from each other (P<0.05, T test)

TABLE 4

Effects of meridic diet on total protein and glycogen levels of adult *P. turionellae*

| | Average Weight of insect (g) | | Total protein (%) | | Total glycogen (%) | |
Day eggs laid	Meridic diet	Control diet	Meridic diet Mean±S.D*	Control diet Mean±S.D*	Meridic diet Mean±S.D*	Control diet Mean±S.D*
10	0.0252	0.0317	5.45±0.03 a	5.50±0.03 a	0.090±0.03 a	0.095±0.04 a
13	0.0241	0.0319	5.91± 0.04 b	5.50±0.02 b	0.130±0.06 a	0.290±0.03 b
16	0.0261	0.0311	6.30±0.02 b	4.90±0.02 b	0.129±0.03 a	0.310±0.03 b
19	0.0278	0.0289	6.94±0.02 b	6.44±0.02 b	0.140±0.05 a	0.165±0.04 a
22	0.0309	0.0284	7.25±0.03 a	7.30±0.01 a	0.158±0.06 a	0.162±0.05 a
25	0.0321	0.0235	6.20±0.02 a	6.10±0.02 a	0.169±0.04 a	0.153±0.06 a
28	0.0327	0.0212	5.96±0.01 a	6.00±0.02 a	0.185±0.12 a	0.129±0.05 a

* Values followed by the same letter across the same line are not significantly different from each other (P<0.05, T test)

RESULTS

Overall, total adult emergence (74%) and female emergence (73%) were significantly higher in wasps fed the experimental meridic diet than in wasps fed the control diet (68% and 67%) (Table 2).

When considered over time, total adult emergence was significantly higher in the experimental meridic diet group than in the control group from eggs deposited on days 13, 16 and 19 (Table 3); there were no significant differences on the other days. Maximum adult emergence (93.33%) occurred from eggs deposited on day 16 in the experimental group fed the meridic diet.

Although female emergence from eggs deposited on days 13, 16 and 19 was significantly lower in the experimental meridic diet group than in the control group, it was significantly higher on days 22, 25 and 28 (Table 3). Maximum female emergence (100%) occurred in the experimental group on day 25.

The effects of meridic and natural diets on the protein and glycogen levels of *P. turionellae* are shown in Table 4. The total percentage of protein in wasps fed the experimental meridic diet was significantly increased, on days 13, 16 and 19, compared to wasps that received the control diet. Glycogen levels were not significantly different between the wasps receiving the meridic and control diets for most of the study period, the exception being on days 13 and 16 when levels were significantly lower in the meridic diet groups compared to the control.

The effect of a meridic diet on total adult emergence of *P. turionellae* over the five generations is shown in Table 5. For wasps fed a meridic diet, there was a significant decrease in total adult emergence in the F4 and F5 generations compared to the preceding generations, and compared to the control. Maximum adult individual emergence occurred in the first generation. In percentage female emergence, a significant decrease was observed only in the F5 generation compared to the preceding generations, and compared to the control.

The effects of a meridic on the total egg numbers, hatchability and the lifetime of the female of *P. turionellae* over the five generation are shown in Table 6. The female lifetime was significantly decreased in the F3 (34 days), F4 (32 days) and F5 (32 days) generations compared with preceding generations (F1, 43 days; F2, 41 days) and with the control (45 days). The number of eggs per female was significantly higher in F1 and F2 generations (41 and 38 respectively), than in subsequent generations (F3, 29; F4, 25; F5, 24), but was not significantly different from the control. In percentage hatchability there were no significant differences between the five generations, or between any of the generations on the meridic diet and control wasps.

DISCUSSION

The main findings of this study are that the meridic diet significantly increased the sex ratio of *P. turionellae* in favour of females, and that it also significantly increased the protein content of the individuals. Thus this study improves our understanding of the role of synthetic diets

TABLE 5

Effects of meridic diet on Adult Emergence
of *P. turionellae* over five generation

	Adult Emergence (%)	
Generation	Total Mean±S.D *	Female Mean±S.D *
Control	71.42±2.47 ab	69.89±1.85 a
F1	83.33±3.71 a	77.05±1.15 a
F2	72.38±1.71 b	70.31±4.20 a
F3	78.09±1.26 b	77.40±0.97 a
F4	57.08±2.53 c	73.75±2.10 a
F5	55.00±1.44 c	59.96±2.85 b

* Statistical analyses were done separately for each generation and for the control group. Values followed by the same letter are not significantly different from each other, P<0.05

TABLE 6

Effects of meridic diet on lifetime, egg number and fertility
of adult female *P. turionellae* over five generations

	Survival (Days)	Eggs Number (No of egg/ female)	Hatchability (%)
Generation	Mean±S.D *	Mean±S.D *	Mean±S.D *
Control	44.56±1.10 a	36.14±1.58 a	82.66±1.74 a
F1	43.44±1.11 a	41.39±3.23 a	81.14±2.50 a
F2	41.18±1.00 a	37.67±0.46 a	76.03±0.78 a
F3	34.42±1.04 b	28.69±1.55 b	80.99±4.71 a
F4	32.46±1.76 b	24.86±3.36 b	80.44±0.65 a
F5	32.36±0.49 b	24.25±0.52 b	80.54±2.69 a

* Statistical analyses were done separately for each generation and for the control group. Values followed by the same letter are not significantly different from each other, P<0.05

in maintenance of insects required for biological control. It is also the first study to report rearing the adult stages of *P. turionellae* on meridic diets for five generations.

The growth, development, reproduction, and behaviour of insects are closely related to the quantity and quality of their food, and it is crucial that dietary components are well balanced (PARRA, 1991; IDRIS & GRAFIUS, 1997; CHANG, 2004; MAGRO et al., 2006). The nutrients that larvae receive affect not only the growth rate, development, weight, and survival of the larvae, but also the fitness of the adult (PARRA, 1991).

Our study shows that the meridic diet increased both total and adult female emergence ratios. Diet has previously been shown to affect the reproductive performance of *P. turionellae* (EMRE, 1988), and in *Itoplectis conquistor,* increase in amino acid amount in an artificial diet was related to an increase in male emergence (YAZGAN, 1972).

A decrease in adult emergence (evident in our study on both the control and meridic diets) is an unavoidable result of aging. However, in our study the meridic diet did eliminate the effect of aging on female emergence between days 22 and 28.

Protein and glycogen are used as the main energy sources in many insect species. Proteins directly affect the reproductive performance of the insects (DADD, 1985),

and CANGUSSU & ZUCOLOTO, (1997) showed that the amount of protein that insects store affects egg number, adult emergence and adult size. In our study of *P. turionellae* we showed that protein and glycogen levels may correlate with adult emergence.

In many insect species, the relationship between available energy reserves and coupling success and stability in reproductive behaviour is very important (PETERSSON, 1989). In our study the meridic diet decreased the glycogen levels on days 13 and 16 compared to the control; this could be a result of nutritional stress while the insects were adapting to the meridic diet, and glycogen from their own reserves was required to compensate for it.

Considerable amounts of glycogen are needed by many adult insects for flying, movement, searching for hosts and parasitizing them. For example, *Anopheles freeborni*, consumed over 50% of their current energy reserves for a flight of 40 minutes. Efficient restocking of energy reserves therefore may contribute to mating success (YUVAL et al., 1994). Glycogen reserves of insects change according to age, temperature, rearing and photoperiod. It is known that there is a significant relationship between the change of glycogen amount based on age, diet and mating (YUVAL et al., 1994). Levels of total sugars and glycogen in sugar-fed flies are positively correlated with wing length, possibly indicating higher accumulation of storage sugars by larger flies. These results are generally in agreement with previous reports on nutrient levels in *Pseudacteon tricuspis* (FADAMIRO & CHEN, 2005; FADAMIRO et al., 2005).

In our study, a lower female individual emergence rate on the meridic diet for days 13 and 16 was correlated with lower glycogen levels than the controls on those days. This is similar to the decrease in egg numbers associated with a decrease in glycogen levels reported for *P. turionellae* by ŞEKER & YANIKOĞLU (1999).

Amino acids are essential for the growth and development of insects (CHEN, 1985), and many researchers stress their key role in the reproduction of parasitoids under laboratory conditions (THOMPSON, 1983; VINSON, 1994; NETTLES, 1987; HU et al., 1998). The effect of different concentrations of amino acids on insect development has been extensively studied (FRIEND et al., 1957; CANGUSSU & ZUCOLOTO, 1997; CHANG, 2004). The differences in egg numbers in insects such as *Dacus olea* (TSIROPOULOS, 1980), *Acheta domesticus* (MCFARLANE, 1988), and *Melanogryllus desertus* (BASHAN & BALCI, 1994) depending on diet, illustrates the importance of the nutritional balance on the reproduction period of *P. turionellae*.

The data obtained from generations F1 to F5 show that there is a linear relationship between lifetime and egg number. The fact that longer survival in the control, F1 and F2 generations resulted in greater total egg numbers produced, shows that there is a requirement for diets that prolong the lifetime as much as possible. Previous studies have shown that diet, particularly carbohydrate, affects insect lifetime (ÖZALP & EMRE, 2001; JACOB & EVANS, 2004; ONAGBOLA et al., 2007). In the synthetic diet we used for *P. turionellae,* sucrose, which is a strong phagostimulant, was used as a carbohydrate source because ÖZALP & EMRE (2001) had shown sucrose to be the most

beneficial among the 23 different carbohydrates tested for their effect on *P. turionellae* lifetime. We suggest that future studies should determine the optimal amount of sucrose, as the carbohydrate source, in meridic diets for *P. turionellae*.

We observed that although there was no decrease of egg number, female emergence rate or lifetime for the first two of five generations, there were decreases in the fourth and fifth generations compared to the control. We suggest that adding vitamin E to the diet may rectify these problems, since EMRE & YAZGAN (1990) observed that 0.0010% vitamin E in the meridic diet increased egg number, and COŞKUN et al. (2005) observed that adding 0.0010 and 0.0015% vitamin E markedly increased female individual emergence.

ACKNOWLEDGMENTS

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