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Research Article

Protein and lipid amounts of the parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) at constant and alternating temperatures

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Abstract: *Bracon hebetor* Say (Hymenoptera: Braconidae) is a gregarious synovigenic parasitoid that attacks late stage larvae of many important lepidopterous species. The purpose of the current study was to evaluate the influence of 3 constant (18, 25, and 35 °C) and 1 alternating temperature (incubator programmed to vary daily between 18, 25, and 35 °C at 8 h intervals) on protein and lipid amounts of female and male parasitoids. Protein amounts of female parasitoids were highest when exposed to alternating temperature. At all the temperatures tested, 1-day-old males had similar amounts of protein, but 5- and 10-day-old males had higher amounts of protein at alternating temperature than at other temperatures. One-day-old females had slight fluctuations in lipid levels depending upon rearing temperature, but no consistent trend was found resulting in either increasing or decreasing between these values. In 1-day-old males, lipid levels were significantly lower at 18 and 25 °C than at 35 °C and alternating temperatures. *B. hebetor* adults reared at different temperatures were found to utilize their original lipid reserves during the first 10 days of adult life.

Key words: *Bracon hebetor*, temperature, age, protein, lipid

Parazitoit *Bracon hebetor* Say (Hymenoptera: Braconidae)'un sabit ve değişmeli sıcaklıklarda protein ve lipit miktarları

Özet: *Bracon hebetor* Say (Hymenoptera: Braconidae) birçok önemli Lepidopter türünün olgun larvalarına saldıran gregar, sinovigenik bir parazitoittir. Bu çalışmanın amacı, 3 sabit (18, 25 ve 35 °C) ve 1 değişmeli (8 saatlik aralıklarla günlük olarak 18, 25 ve 35 °C değişecek şekilde programlanmış inkübatör) sıcaklığın dişi ve erkek parazitoitlerin protein ve lipit miktarları üzerine etkisini belirlemekti. Değişmeli sıcaklıkta yetiştirilen dişi parazitoidin protein miktarı en fazlaydı. Test edilen tüm sıcaklıklarda, 1 gün yaşlı erkekler benzer protein miktarına sahipti, fakat 5 ve 10 gün yaşlı erkekler değişmeli sıcaklıkta diğer sıcaklıklardan daha yüksek protein miktarına sahipti. Bir günlük dişilerin lipit seviyelerinde, yetiştirildikleri sıcaklığa bağlı olarak zayıf dalgalanmalar gözlendi, ancak bu değerler arasında artma yada azalma şeklinde sabit bir eğilim belirlenmedi. Bir gün yaşlı erkeklerde lipit seviyesi 18 ve 25 °C'de, 35 °C ve değişmeli sıcaklıklardan önemli derecede düşüktü. Farklı sıcaklıklarda yetiştirilen *B. hebetor* erginlerinin ergin hayatın ilk 10 günü boyunca orijinal lipit kaynaklarını kullandıkları belirlendi.

Anahtar sözcükler: *Bracon hebetor*, sıcaklık, yaş, protein, lipit

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Introduction

Temperature is an important environmental factor that affects development and reproduction in insects because it had a direct effect on physiological and biochemical processes (Brévault and Quilici, 2000; Urbaneja et al., 2002; Maceda et al., 2003).

Insect development does not occur below a low-temperature threshold; above this, the rate increases with temperature until an optimum is reached. It has been demonstrated that development is prolonged and reproduction fails when an insect is exposed to extreme high or low temperatures, and moreover death may occur if exposed for sufficiently long (Geden, 1997; Schöller and Hassan, 2001; Liu et al., 2002; Kalaitzaki et al., 2007).

Bracon hebetor Say (Hymenoptera: Braconidae) is a gregarious synovigenic parasitoid that attacks late stage larvae of many important lepidopterous species (Baker and Fabrick, 2000; Darwish et al., 2003; Gündüz et al., 2008). The importance of this parasitoid species as a biological control agent of stored product moths has been recognized for a long time (Heimpel et al., 1997; Baker and Fabrick, 2000; Dweck et al., 2008).

It is known that considerable amounts of carbohydrate, protein, and lipid are needed by most parasitoids for survival and reproduction. Nutritional reserves for adult parasitoids may be either accumulated during larval development, or may be synthesized by intake of precursor substances via nutrition during the adult stage (Wheeler and Buck, 1992).

Previous studies have examined the effects of temperature on development (NaKyoung et al., 2000; Qiu et al., 2006), longevity (Kyawt and Takasu, 2004), survival rate (Uwais et al., 2006), functional response (Shojaei et al., 2006), parasitizing efficiency, and fecundity of *B. hebetor* (Al-Tememi and Ashfag, 2005).

Although these studies have provided some essential information on the effects of temperature on some vitality parameters of *B. hebetor*, the effect of constant and alternating temperatures on protein and lipid levels of parasitoids is not well known. It is particularly important because *B. hebetor* depends on protein and lipid to sustain egg production and maturation. Therefore, the goal of the present study was to assess the effect of constant and alternating temperatures upon the protein and lipid amounts of female and male *B. hebetor.*

Materials and methods

Insects

Bracon hebetor and late stage larvae of *Ephestia kuehniella* were used as parasitoid and host species, respectively, in the experiments. Stock cultures of *E. kuehniella* were prepared in glass jars (500 mL) containing sterilized corn flour. Host cultures were kept at 26 ± 2 °C, 60 ± 5% RH, and continuously illuminated laboratory conditions. To prepare the stock cultures of parasitoid under different temperatures, 25- to 30-day-old host larvae were used (Darwish et al., 2003).

For this purpose, newly emerged (<24 h) adults were confined in pairs to the test tubes (16 × 160 mm). A piece of cotton ball soaked with 50% honey solution and one host larva were placed inside the tubes. They were then allocated to 4 experimental groups. Three of them were exposed to constant temperature regimes (18, 25, and 35 °C), while one of them was subjected to a variable-temperature regime (incubator programmed to vary daily between 18, 25, and 35 °C at 8 h intervals). The tubes were then checked every day until the emergence of the adults.

Biochemical analysis

To compare protein and lipid amounts of adult parasitoids at different temperatures, they were analyzed at 1, 5, and 10 days of age. Unfed 1-day-old females and males were used to determine initial amounts of protein and lipid in adults. To obtain 5- and 10-day-old individuals, newly emerged adults were individually placed into a test tube (16 × 100 mm) containing a piece of cotton ball soaked with 50% honey solution. This cotton ball was changed twice a week. After 5 or 10 days had elapsed, 5 individuals from each sex were weighed, placed in a 1.5 mL Eppendorf tube, and stored at −50 °C until analysis.

Protein analysis

Parasitoids, containing 5 females or males, were homogenized in 250 µL of phosphate buffered saline (pH 7.4) and centrifuged at 1160 × *g* at room temperature for 15 min. After centrifugation, 100 µL of the resulting supernatant was transferred to a glass test tube (12 × 50 mm) and the amount of protein was determined using Folin-phenol reagent, as described by Lowry et al. (1951). Absorbance was read at 695 nm. Bovine serum albumin was used as the standard protein.

Lipid analysis

Lipid level was quantified using a series of biochemical tests originally developed by van Handel (1985a, 1985b) for mosquitoes, and adapted for parasitoids (Olson et al., 2000; Lee et al., 2004). Parasitoids were crushed with a plastic pestle in 100 µL of 2% sodium sulfate. The solved nutrient was then extracted with 900 µL chloroform-methanol (1:2), after which the tube was vortexed. The tube was then centrifuged at 14,000 × *g* for 2 min and 200 µL of the resulting supernatant was transferred to a glass test tube (12 × 50 mm) for the lipid assay. The tubes were heated at 90 °C until all solution had evaporated. Samples were then dissolved in 40 µL of sulfuric acid and heated at 90 °C for 2 min. To the tubes was added 960 µL of vanillin-phosphoric acid reagent. The solution in each tube was left to react at room temperature for 30 min, mixed, and then absorbance was read at 525 nm. Corn oil was used as the standard lipid.

Statistical analysis

For data analysis we used the mean quantity of protein or lipid per 100 mg of parasitoid mass. Differences in protein and lipid amounts of parasitoids were compared using SPSS for Windows (version 10.0). When the differences were significant, means were separated using the Student-Newman-Keuls (SNK) multiple range test at a probability level of $P \leq 0.05$.

Results

The effects of temperature on the amounts of protein in females and males are shown in Figure 1a and 1b, respectively. The mean quantity of protein obtained from 1-day-old parasitoids was regarded as the standard for the initial reserve in *B. hebetor* adults.

One-way ANOVA indicated that temperature had a significant effect on protein levels of 1-day-old females, but not on that of males (Figure 1a and 1b). It was determined as 6.32 mg/100 mg at 18 °C, 6.09 mg/100 mg at 25 °C, 5.93 mg/100 mg at 35 °C, and 7.17 mg/100 mg at alternating temperature in females ($P \leq 0.05$, Figure 1a), whereas males had 5.78 mg/100 mg at 18 °C, 6.04 mg/100 mg at 25 °C, 5.76 mg/100 mg at 35 °C, and 5.91 mg/100 mg protein at alternating temperature ($P > 0.05$, Figure 1b).

We detected significant differences among protein levels of 5-day-old females, with the highest values at alternating temperature, the lowest values at 25 and 35 °C, and intermediate values at 18 °C ($P \leq 0.05$, Figure 1a). However, 5-day-old males showed a different trend to females, with the highest values at alternating temperature, the lowest values at 18 °C, and intermediate values at 25 and 35 °C ($P \leq 0.05$, Figure 1b).

In 10-day-old females, significant differences were obtained among all temperatures ($P \leq 0.05$, Figure 1a), but 10-day-old males had more or less similar amounts of protein ($P > 0.05$, Figure 1b), except at alternating temperature.

The amounts of lipid obtained from female and male *B. hebetor* at different temperatures are depicted in Figure 2a and 2b, respectively.

The results showed that lipid levels of 1-day-old female parasitoids show some variations with temperature, but no consistent trend was found resulting in either increasing or decreasing between these values ($P \leq 0.05$, Figure 2a). In 1-day-old males, lipid levels were significantly lower at 18 and 25 °C than at 35 °C and alternating temperatures ($P \leq 0.05$, Figure 2b).

Five-day-old females and males showed similar patterns for lipid level. They had the highest amounts of lipid at 18 °C, intermediate amounts at 25 °C and alternating temperature, and the lowest amount at 35 °C ($P \leq 0.05$, Figure 2a and 2b).

In 10-day-old females and males, the highest lipid levels were obtained at 18 °C. For both sexes, it was significantly lower at the other 3 temperature regimes ($P \leq 0.05$, Figure 2a and 2b).

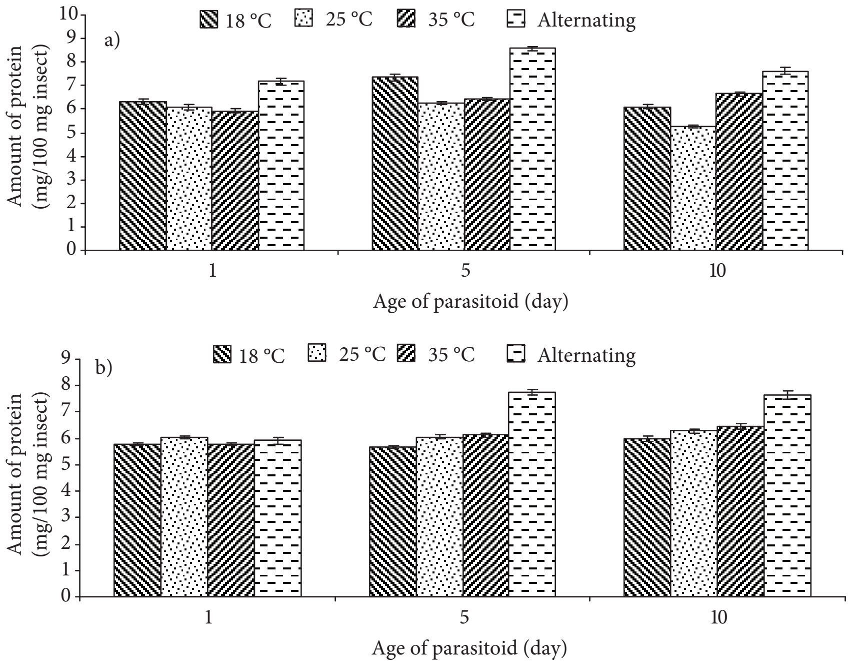

Figure 1. Mean amounts (mg/100 mg ± SE) of protein in female (a) and male (b) *Bracon hebetor* kept at different temperatures. Means for the same parasitoid age having different letters are significant (SNK, P ≤ 0.05).

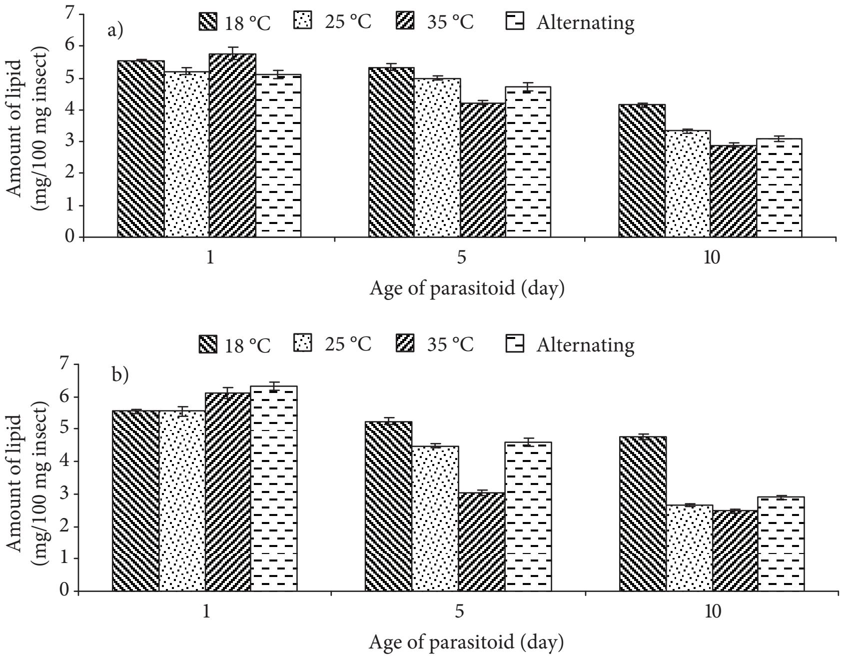

Figure 2. Mean amounts (mg/100 mg ± SE) of lipid in female (a) and male (b) *Bracon hebetor* kept at different temperatures. Means for the same parasitoid age having different letters are significant (SNK, P ≤ 0.05).

Discussion

We found that the protein levels of female and male *B. hebetor* were affected by temperature. For females, rearing at alternating temperature results in the accumulation of the highest amount of protein in all age groups. Under constant temperature regimes, in general, the amount of protein increased to a maximum at 18 °C and decreased as temperature increased in females, except at 35 °C. Males had more or less similar amounts of protein at the beginning of adult life at all temperatures. However, 5- and 10-day-old males had the highest levels of protein at alternating temperature.

B. hebetor is a synovigenic species, i.e. females emerge with a very limited number of mature eggs and produce yolk-rich eggs and mature them throughout their lifetime (Godfray, 1994). Protein and lipid are the 2 essential components of the insect eggs; thus the amounts of these 2 important nutrients in adults have been determinative in the biology of insect (Nijhout, 1994).

Benson (1973) showed that *B. hebetor* females emerge with zero mature eggs and mature approximately 16 eggs within 48 h. The material used for this initial egg maturation comes from fat body reserves. This may explain why significantly higher levels of protein are detected in 5-day-old females. Host-feeding is essential for egg production and maturation in *B. hebetor* during its lifetime (Benson, 1973) since it enables the female to obtain proteins to meet high amino acid demands associated with these 2 processes (Heimpel et al., 1997; Rivero and Casas, 1999; Giron et al., 2002). Females were held without hosts during the experiment. In the absence of hosts, mature eggs are resorbed in females. Through egg resorption, energy and materials contained in the eggs returned to the body of the wasp and can be used both for adult maintenance and for sustaining oogenesis. Although we did not investigate egg resorption in this species, Benson (1973) found that on a honey diet *B. hebetor*'s eggs are resorbed. Egg resorption behavior has also been reported for different parasitoid species such as *Aphytis melinus* (Heimpel et al., 1997), *Anaphes nitens* (Santolamazza Carbone et al., 2008), *Metaphycus flavus*, and *M. luteolus* (Kapranas and Luck, 2008).

B. hebetor emerges from its host with a limited amount of energy reserves, which are stored mainly in the form of lipids. In the present study, lipid amount was not improved by temperature increase and did not show any trend. In contrast, *B. hebetor* adults were found to utilize their original lipid reserves for egg production and for metabolic maintenance during the first 10 days of adult life at all temperatures (Figure 2a and 2b).

In a previous study, Gündüz and Gülel (2010) suggested that lipids are synthesized only during pre-adult stages and *B. hebetor* adults have no lipogenic capabilities. Similar results also have been reported for different parasitoid species such as *Macrocentrus grandi* (Olson et al., 2000), *Nasonia vitripennis* (Rivero and West, 2002), *Venturia canescens* (Casas et al., 2003), *Diadegma insulare* (Lee et al., 2004), and *Asobara tabida* (Visser and Ellers, 2008).

Since lipid reserves cannot be fully replaced through feeding in these species, a prudent use of lipids is required throughout their lifetime. In the present work, the higher lipid depletion rate at higher temperature occurs because their metabolic activity and energy requirements increase. In a previous work, Işıtan et al. (2010) observed that survival of *B. hebetor* adults decreased with increasing temperature. At 18 °C they survive longer than that at 25, 35 °C, and alternating temperature. Ahmed et al. (1985) reported the same situation for *B. hebetor* parasitizing the larvae of *Ephestia cautella* (Walker).

B. hebetor adults somewhat tolerated the 4 temperatures used in this study. Here, we examined only one aspect of the relationship between temperature and protein and lipid level of adults. Further studies need to be carried out to determine the effect of different temperatures on some life history parameters of *B. hebetor*. Such information would allow us to determine the optimum temperature for rearing of this species.

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