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# The effects of parasitism by the ectoparasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) on host hemolymph proteins in the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

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**Abstract:** Parasitism of lepidopteran larvae by braconid wasps has significant effects on host hemolymph proteins and the physiology of the host insect. In this study the post-parasitism effects of a gregarious idiobiont ectoparasitoid (*Bracon hebetor*) on hemolymph plasma proteins in the final instar larvae of the host (*Ephestia kuehniella*) were investigated. Hemolymph plasma proteins were analyzed using spectrophotometry and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total quantity of plasma proteins in the host's hemolymph decreased slightly 24 and 48 h after parasitization; however, SDS-PAGE analysis showed that the quantity of 12 proteins decreased, whereas that of 5 proteins increased in concentration in the hemolymph of parasitized host larvae 24 and 48 h post-parasitism. Therefore, we conclude that host regulation of *E. kuehniella* by *B. hebetor* involves only quantitative changes in host plasma proteins and does not lead to upregulation of novel proteins.

**Key words:** Host regulation, hemolymph proteins, ectoparasitoid

## Konak un güvesi *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) larvalarının hemolenf proteinleri üzerine ektoparazitoid *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae)'un parazitik etkileri

**Özet:** Braconid ve Ichneumonid parazitoidler tarafından konak böcek türlerin parazitlenmesi sonucunda konak hemolenf proteinlerinde ve konak fizyolojisinde önemli değişikler meydana gelmektedir. Bu çalışmada ise gregar, idiobiont ve ektoparazitoid *Bracon hebetor* Say (Hymenoptera: Braconidae)'un, konak *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) olgun larvalarının hemolenf plazma proteinleri üzerindeki parazitik etkileri araştırılmıştır. Hemolenf plazma proteinleri spektrofotometrik ve sodyum dodesil sülfat-poliakrilamid jel elektroforez yöntemleri ile araştırılmıştır ve protein bantları densitometrik olarak analiz edilmiştir. Hemolenfteki total plazma protein miktarı parazitlenmeden 24 ve 48 saat sonra giderek azalmıştır. Ancak, SDS- PAGE analizine göre, 24 ve 48 saatlik parazitlenmeye bağlı olarak konak hemolenfindeki 12 proteinin miktarı azalırken, beş proteinin miktarı artmıştır. Böylece, *B. hebetor*'un *E. kuehniella*'nın hemolenf proteinleri üzerindeki parazitik etkisinin sadece kantitatif yönden olduğu ve yeni proteinlerin sentezine neden olmadığını düşünmekteyiz.

**Anahtar sözcükler:** Konak düzenlemesi, hemolenf proteinleri, ektoparazitoid

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## Introduction

The braconid *Bracon hebetor* Say is a gregarious, idiobiont arrhenotokous ectoparasitoid that parasitizes lepidopteran larvae and is an important biological control agent for several stored product moth pests (Gül and Gülel, 1995; Heimpel et al., 1997; Darwish et al., 2003). *B. hebetor* has been widely used in various studies related to host-parasitoid interactions because of its high reproductive rate, short generation time, and considerable range of host species (Yu et al., 2002; Gündüz and Gülel, 2005).

The success of biological control programs is highly dependent on the nature of the host-parasitoid interaction, which has led to many host-parasitoid systems being investigated in great detail (Vinson and Iwantsch, 1980; Rolle and Lawrence, 1994; Baker and Fabrick, 2000; Salvador and Consoli, 2008). Knowledge of the physiological and biochemical interactions in host-parasitoid systems is important for the efficient production of biological control agents (Hentz et al., 1998; Vinson et al., 2001; Nakamatsu and Tanaka, 2003). In vitro rearing techniques have been used to determine the quantitative and qualitative needs, as well as the nutritional biochemistry of natural enemies. However, improvement of these techniques depends on knowledge and understanding of the physiology, nutrition, genetics, and behavior of parasitoids (Magro and Para, 2003).

Parasitism of arthropods by nematodes, trematodes, insects, and pathogens frequently elicits both qualitative and quantitative changes in hemolymph proteins and detectable amino acids in the host (Vinson and Iwantsch, 1980; Beckage et al., 1987). It is well known that parasitism by ichneumon and braconid endoparasitoids causes multiple alterations in the physiology of their insect hosts, such as endocrine and developmental programming (Lawrence and Lanzrein, 1993), metabolism (Thompson, 1985), immunocompetence (Stoltz et al., 1988), behavior (Horton and Moore, 1993), and pigmentation (Beckage et al., 1990). When endoparasitoids parasitize their hosts, especially lepidopteran species, qualitative and quantitative changes occur in the profile of host plasma proteins (Rolle and Lawrence, 1994; Vinson et al., 2001; Consoli and Vinson, 2002; Rahbe et al., 2002). In the latter case, proteins may be negatively regulated or suppressed in the host larvae by the developing parasitoid (Beckage and Kanost 1993). For example, the concentration of arylphorin in *Manduca sexta* hemolymph decreased after parasitism by *Cotesia congregata* (Beckage et al., 1987). On the other hand, parasitism-specific proteins (Soldevila and Jones, 1994) or polydnaviruses (Beckage et al., 1987) may be derived from parasitoid larvae, co-injected into the host together with the eggs, or derived from serosal cells (teratocytes) that dissociate after eggs hatch (Vinson and Iwantsch, 1980). In many instances these components provide protection pathways and host regulation for the developing endoparasite by interfering with various defenses (Asgari et al., 1998). Recently, Salvador and Consoli (2008) reported that 2 parasitism-specific host hemolymph proteins (125 and 48 kDa) were upregulated by the endoparasitoid *Cotesia flavipes*.

In contrast, only a few studies on the effects of parasitism by ectoparasitoids on the hemolymph proteins of their insect hosts have been published (Coudron et al., 1997; Richards and Edwards, 1999; Baker and Fabrick, 2000). Like endoparasitoids, adult ectoparasitoids introduce their venom and/or other fluids into their host at oviposition; however, ectoparasitoid venom often causes paralysis of the host and alters development of the host by arresting the larval molting cycle. Unlike endoparasitoids, their eggs are deposited on the outside of their host, where they develop into larvae (Shaw, 1981). Additionally, little is known about the effect of ectoparasitoid secretions on the plasma protein profiles in their hosts (Quistad et al., 1994; Doury et al., 1997; Asgari et al., 2003; Nakamatsu and Tanaka, 2003; Parkinson et al., 2003). The present study aimed to investigate if parasitization by *B. hebetor* affects the concentration and profile of plasma proteins in the host *E. kuehniella*.

## Materials and Methods

### Insect rearing

The Mediterranean flour moth *E. kuehniella,* used as the host, and the ectoparasitoid *B. hebetor* were provided as gifts by Dr. Eylem Akman Gündüz (Ondokuz Mayıs University, Samsun, Turkey).

A laboratory culture of *E. kuehniella* was reared in 500-mL glass jars in constant darkness at 25 ± 2 °C and relative humidity of 55 ± 2%. The jars contained whole-wheat flour (2:1) that was previously heated in order to ensure the absence of any infestation. Every week female moths were allowed to oviposit by transferring them into new oviposition jars for 24 h, so that host larvae would be constantly available during the experimental period.

A stock culture of *B. hebetor* was reared on 25- to 30-day-old final larval stadium hosts in cylindrical 250-mL glass jars under the same environmental conditions described for the host culture. In addition, adult wasps were fed a 50% aqueous honey solution absorbed onto a cotton pad and were allowed to mate in a glass tube (16 × 100 mm) for 24 h. Female wasps were then separated from males and used to parasitize hosts.

**Host parasitization**

Host parasitization was performed under the same environmental conditions described above. Newly ecdysed *E. kuehniella* final instars were selected from the culture and parasitized individually by exposure to a single mated *B. hebetor* female in a glass tube for at least 24 h. Larvae of the same age and weight as those exposed to parasitoids were selected as controls. Wasps were removed from the tube immediately after laying eggs.

**Haemolymph collection**

Hemolymph samples were obtained from parasitized larvae 24 h and 48 h after parasitization, and from the unparasitized control insects of the same age. Hemolymph was collected from 10 insects at each time point and 5 µL of hemolymph was obtained from each larva. Unparasitized larvae were cleaned of adhering food material and anesthetized on ice for 10 min. The parasitized larvae were not anesthetized due to the relaxing effect of the wasp's paralyzing venom. Parasitized and unparasitized larvae were dropped into 70% ethanol for 2-3 min for surface sterilization. Hemolymph was collected by cutting the third proleg with micro-scissors while lightly massaging the larval body with forceps. Care was taken to avoid rupturing the larval gut or dislodging the fat body. Hemolymph was drawn into a 10-µL micropipette and transferred to a microcentrifuge tube placed on ice. Subsequently, a few crystals of 1-phenyl-2-thiourea were added to each sample to prevent melanization of the hemolymph. When a sufficient quantity of hemolymph was collected the samples were centrifuged at 10,000 ×g for 5 min at 4 °C to pellet cell debris. Plasma samples were stored at –20 °C until analysis.

**Measurement of the hemolymph protein concentration**

Plasma samples were diluted 1:20 with distilled water and protein concentrations were measured according to Bradford (Bradford, 1976), using Coomassie blue G250 protein-assay reagent at 595 nm (Eppendorf BioPhotometer). A standard curve was prepared using bovine serum albumin as the standard. Each sample was analyzed in triplicate and the experiment was repeated at least 3 times.

**SDS-PAGE analysis**

The protein profiles of hemolymph obtained from parasitized and unparasitized *E. kuehniella* larvae were analyzed using 11% SDS-PAGE, according to Laemmli (1970). Briefly, 20 µg of protein from the each sample was mixed with sample buffer (0.125 M Tris, 4% SDS (w/v), 20% glycerol (v/v), 10% 2-mercaptoethanol (v/v), 0.2% bromophenol blue (w/v, pH 6.8) at a ratio of 1:3, and then was boiled for 4 min and kept on ice until loading onto the gel. Wide- and low-range molecular weight markers (Sigma) were also loaded into parallel lanes on the gel. After electrophoresis at 120 V, the gel was stained with 0.04% Coomassie blue R 250 in 50% methanol/10% acetic acid overnight and destained in 10% methanol/10% acetic acid solution. The gel was subsequently scanned using a UVitec gel documentation (BioLab) system and the optical density (OD) of the bands was analyzed using a Gel-Pro analyzer (software v.4.0, United Bio., NJ, USA). Each experiment was repeated at least 3 times. The percentage of OD values of the protein bands from the parasitized host were determined as follows:

% OD of protein = (OD value of parasitized protein)/(OD value of unparasitized protein) × 100.

**Statistical Analysis**

The quantity of hemolymph total protein was inferred using one-way analysis of variance (ANOVA). Subsequently, means were separated using Tukey's honestly significant difference (HSD) post hoc test (SPSS Inc., 1999).

## Results

Spectrophotometric analysis of total plasma protein present in the hemolymph of *E. kuehniella* 24 and 48 h after parasitization showed that there was a slight decrease in total protein, as seen in Table 1; the difference in total plasma protein between parasitized and unparasitized hosts was not statistically significant (P > 0.05).

Table 1. Total plasma protein values of the unparasitized and the parasitized *E. kuehniella* larvae.

| | Total Hemolymph Protein Mean ± SE (μg/μL) | |
|---|---|---|
| Hour | Control | Post-parasitism |
| 24 | 228.00 ± 35.56 | 235.67 ± 56.05 |
| 48 | 257.67 ± 34.82 | 210.00 ± 73.75 |

The mean ± S.E. of data is from 3 independent experiments (P > 0.05).

After spectrophotometric analysis total plasma protein was further analyzed by SDS-PAGE. As shown in Figure 1, 25 bands were observed in the hemolymph of parasitized host larvae, while 26 bands were produced by the blood of unparasitized larvae. The quantity of protein in the parasitized host larvae was generally quantitatively different than that in unparasitized larvae (Table 2). On the other hand, there were no qualitative differences in terms of novel protein bands, the one exception being a 14.5-kDa protein, which disappeared from the hemolymph of parasitized larvae.

Each protein band from 3 independent experiments was analyzed using a Gel-Pro analyzer, and then was evaluated by comparison with controls (Table 2.). Following parasitization, the quantity of 12 protein bands (55.3, 41.2, 39.4, 37.2, 35.6, 34.7, 33.9, 32.1, 29.6, 23.1, 15.1, and 14.5 kDa) significantly decreased (>40%). On the other hand, the quantity of

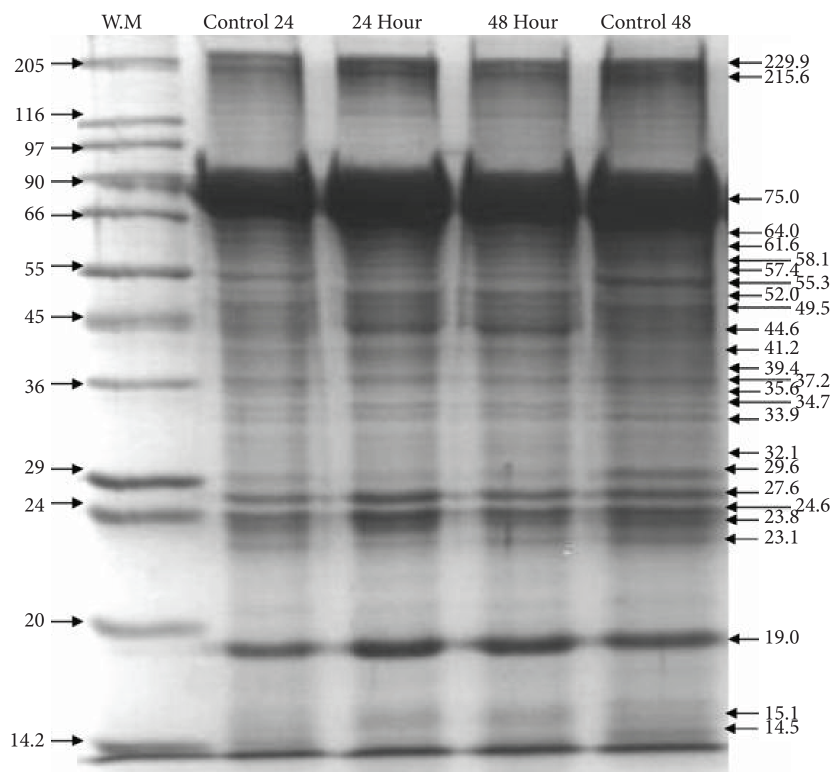


Figure 1. Protein profiles of hemolymph from unparasitized as control 24 and 48 h and post-parasitized *E. kuehniella* larvae after 24 and 48 hincubation were identified by SDS/PAGE using 11% gel. W.M: Wide range molecular weight marker (205-14.2 kDa). Equal protein amounts of 20 μg per lane were loaded. A representative result from at least 3 independent experiments is shown. Arrows indicate the molecular weight of protein bands.

Table 2. Changes in the level of hemolymph proteins from *E. kuehniella* parasitized with *B. hebetor* at different hours analyzed by Gel-Pro Analyzer system.

| Proteins (kDa) | (%) OD values of proteins post-parasitism | |
|---|---|---|
| | 24 h | 48 h |
| 229.9 | 16.62$^{+}$ | 3.98 |
| 215.6 | 12.20$^{+}$ | 4.81$^{-}$ |
| 55.3 | 40.60$^{-}$ | 41.58$^{-}$ |
| 41.2 | 40.63$^{-}$ | 41.48$^{-}$ |
| 39.4 | 54.79$^{-}$ | 60.74$^{-}$ |
| 37.2 | 52.62$^{-}$ | 57.37$^{-}$ |
| 35.6 | 80.13$^{-}$ | 76.44$^{-}$ |
| 34.7 | 63.13$^{-}$ | 68.19$^{-}$ |
| 33.9 | 72.16$^{-}$ | 72.89$^{-}$ |
| 32.1 | 80.83$^{-}$ | 80.83$^{-}$ |
| 29.6 | 72.07$^{-}$ | 70.03$^{-}$ |
| 27.6 | 12.84$^{+}$ | 14.22$^{-}$ |
| 24.6 | 28.91$^{+}$ | 1.46$^{-}$ |
| 23.1 | 60.49$^{-}$ | 46.85$^{-}$ |
| 19.0 | 35.60$^{+}$ | 34.65$^{+}$ |
| 15.1 | 61.35$^{-}$ | 64.13$^{-}$ |
| 14.5 | 100.00$^{-}$ | 100.00$^{-}$ |

Proteins increased (+) and decreased (-) in the parasitized host as compared to the control after 24 and 48 h.

5 protein bands (229.9, 215.6, 27.6, 24.6, and 19.0 kDa) increased in the hemolymph of parasitized larvae. These important differences in the quantity of proteins post-parasitism are illustrated in graph form in Figure 2; however, the level of some other proteins remained constant, regardless of parasitism.

## Discussion

Physiological and biochemical interactions within host-parasitoid systems are important to the success of biological control processes (Hentz et al., 1998; Uçkan and Gulel, 2002; Salvador and Consoli, 2008). Therefore, the effects of parasitization by hymenopteran species on the protein concentration and profile in lepidopteran hosts have been investigated extensively, especially in the case of endoparasitoids (Rolle and Lawrence, 1994; Bischof and Ortel, 1996; Vinson et al., 2001; Consoli and Vinson, 2002; Rahbe et al., 2002; Park et al., 2005; Kaleli et al., 2007; Salvador and Consoli, 2008). On the other hand, the effects of parasitism by ectoparasitoids on hemolymph proteins in their hosts have received comparatively less attention (Kanost et al., 1990; Coudron et al., 1997; Richards and Edwards, 1999; Nakamatsu and Tanaka, 2003).

Insect hemolymph contains a number of proteins involved in diverse developmental, physiological, and immunological processes. Some of these proteins have

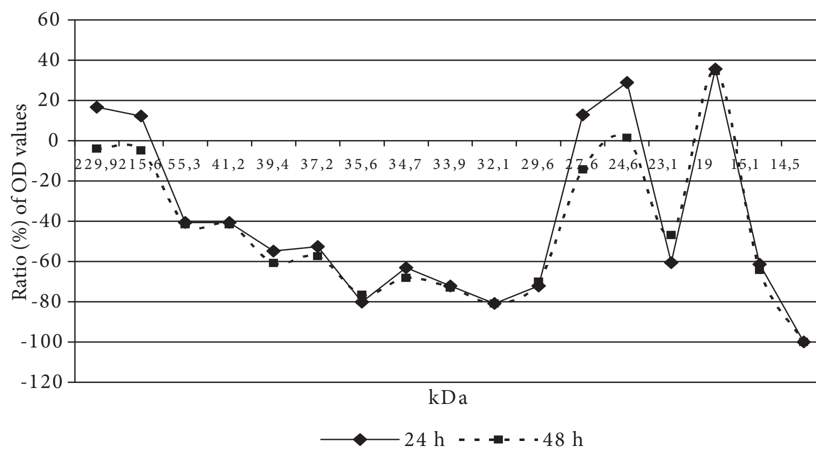


Figure 2. Significant changes in the level of some proteins post-parasitism given in Table 2.

been isolated and their dual functions in hemolymph were confirmed (Riddiford, 1983; Rosell and Coons, 1991). Previous studies reported that the total quantity of hemolymph protein is reduced during ectoparasitism (Coudron et al., 1997) and endoparasitism (Beckage and Kanost, 1993); however, according to the present results, the total quantity of hemolymph protein decreased slightly following parasitism.

Baker and Fabrick (2000) reported that parasitization of *Plodia interpunctella* last instar larvae by *Habrobracon hebetor* did not qualitatively change plasma proteins when analyzed by SDS-PAGE, indicating that parasitization did not induce any novel hemolymph proteins. The fact that we did not observe any novel hemolymph proteins in the present study supports the results of this earlier study. On the other hand, we observed for the first time that a 14.5-kDa protein disappeared in host hemolymph after parasitization; this protein might have been broken down or immunosuppressed by the feeding parasitoid larvae to ensure its normal development

Proteins between 75 and 55.3 kDa may include storage proteins, such as hexamerins (Kanost et al., 1990; Nakamatsu and Tanaka, 2003). Only one of the proteins in this size category, a 55.3-kDa protein, significantly decreased in concentration in the hemolymph of our parasitized hosts. Hexamerins are synthesized in the fat body, secreted into the larval hemolymph, and taken up again by the fat body shortly before pupation. Within the pupal fat body, these proteins are initially stored in protein granules, and are later proteolytically broken down to supply the amino acids necessary for the completion of adult development (Martins et al., 2008; Pick et al., 2008). Consequently, the 55.3-kDa protein may have been consumed by parasitoid larvae as an amino acid resource for development.

Arylphorin is another storage protein with several subunits that was affected by developing parasitoid larvae as a result of nutrient usage (Dong et al., 1996). According to Beckage and Kanost (1993), in the hemolymph of parasitized *Manduca sexta* larvae the level of some subunits of arylphorin, such as apolipophorin I (approximately 240 kDa), apolipophorin II (approximately 78 kDa), apolipophorin III (approximately 22 kDa), and serpin (approximately 75 kDa), were changed slightly. Furthermore, insecticyanin (approximately 19 kDa) was detected at higher levels in the hemolymph of parasitized host larvae. The results of the present study are similar in terms of the differences observed in the relative abundance of the 229-, 75-, 41.2-, 39.4-, 37.2-, 35.6-, 34.7-, 33.9-, 32.1-, 29.6-, 23.1-, and 19.0-kDa proteins. Additionally, the level of the 24.6- and 19-kDa protein bands increased significantly, which confirms the results of a previous study (Nakamatsu and Tanaka, 2003). These bands may be defensive proteins, such as insectacyanins, which are synthesized by the parasitized host as an immune reaction in response to ectoparasitoid larvae. An alternative explanation has been suggested by Nakamatsu and Tanaka (2003), who suggest that these proteins may be related to ectoparasitoid venom that contains a diverse range of proteins and peptides. A previous study demonstrated that the venom of the ectoparasitoids *Euplectrus comstockii* and *E. plathypenae* affected the level of hemolymph storage proteins in *Heliothis virescens* larvae (Coudron et al., 1997). Quistad et al. (1994) identified 6 paralytic toxins in the venom of the ectoparasitoid *B. hebetor*, with at least 2 of the isolated proteins demonstrating high insecticidal activity toward 6 species of lepidopteran larvae. In addition, 9 major reacting proteins (47.5-9.2 kDa) detected in the female ectoparasitoid *Eupelmus orientalis* were also found in the hemolymph proteins of the host larvae stung by *E. orientalis*. The quantity of protein synthesized in host larvae stung by female *E. orientalis* was maximal 12 h after envenomation (Doury et al., 1997). In other studies venom proteins are reported to have an immunosuppressive role (Asgari et al., 2003; Parkinson et al., 2003) or even induce host castration (Digilio et al., 2000). Similarly, in the present study elevation in the quantity of protein may have been the result of the effects of paralytic toxins in the venom.

The present study shows that parasitism had several effects on the host hemolymph. These could be related to parasitoid nutrition, development, and immunosuppression in the host. A decrease in some hemolymph proteins in host larvae following parasitism is predictable, while an increase in other proteins could be related to decomposition of the fat body and tissue proteins, or these proteins could also be affected by parasitoid venom for host regulation. Further investigations are required to identify these proteins and their activity using various assays.

## References

Asgari, S., Theopold, U., Wellby, C. and Schmidt, O. 1998. A protein with protective properties against the cellular defense reactions in insects. Proc. Natl. Acad Sci. USA. 95: 3690-3695.

Asgari, S., Zhang, G., Zareie, R. and Schmidt, O. 2003. A serine proteinase homolog venom protein from an endoparasitoid wasp inhibits melanization of the host haemolymph. Insect Biochem. Mol. Biol. 33: 1017-1024.

Baker, J.E. and Fabrick, J.A. 2000. Host haemolymph proteins and protein digestion in larval *Habrobracon hebetor* (Hym: Braconidae). Insect Biochem. Mol Biol. 30: 937-946.

Beckage, N.E., Templeton, T.J., Nielsen, B.D., Cook, D.I. and Stoltz, D.B. 1987. Parasitism-induced haemolymph polypeptides in *Manduca sexta* (L.) larvae parasitized by the Braconid wasp *Cotesia congregata* (Say). Insect Biochem. 17: 439-455.

Beckage, N.E., Metcalf, J.S., Nesbit, D.J., Schliefer, K.W., Zetlan, S.R. and Buron, I. 1990. Host haemolymph monophenoloxidase activity in parasitized *Manduca sexta* larvae and evidence for inhibition by wasp polydnavirus. Insect Biochem. 20: 285-294.

Beckage, N.E. and Kanost, M.R. 1993. Effects of parasitism by the braconid wasp *Cotesia congregata* on host haemolymph proteins of the tobacco hornworm, *Manduca sexta*. Insect Biochem. Mol. Biol. 23: 643-653.

Bischof, C. and Ortel, J. 1996. The effects of parasitism by *Glyptapantales liparidis* (Hym: Braconidae) on the haemolymph and total body composition of gypsy moth larvae (*Lymantria dispar*, Lymantridae: Lepidoptera). Parasitological Research. 82: 687-692.

Bradford, M.M. 1976. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding," Annu. Biochem. 72, 248-254.

Consoli, F.L. and Vinson, S.B. 2002. Haemolymph of reproductives of *Solenopsis invicta* (Hymenoptera: Formicidae) amino acids, proteins and sugars. Comp. Biochem. Physiol. B 132, 711-719.

Coudron, T.A., Brandt, S.L. and Raqib, A. 1997. Comparison of the response of *Heliothis virescens* to parasitism by *Euplectrus comstockii* and *E. plathypenae*. Comp. Biochem. Physiol. 116B (2): 197-202.

Darwish, E., El-Shazly, M. and El-Sherif, H. 2003. The choice of probing sites by *Bracon hebetor* (Say) (Hymenoptera: Braconidae) foraging for *Ephestia kuehniella* Zeller (Lepidoptera:Pyralidae). J. Stored Prod. Res. 39: 265-276.

Digilio, M. C., Isidoro, N., Tremblay, E. and Pennacchio, F. 2000. Host castration by *Aphidius ervi* venom proteins. J. Insect Physiol. 46:1041-1050.

Dong, K., Zhang, D. and Dahlman, D.L. 1996. Down-regulation of juvenile hormone esterase and arylphorin production in *Heliothis virescens* larvae parasitized by *Microplitis croceipes*. Arch Insect Biochem Physiol. 32: 237-248.

Doury, G., Bigot, Y. and Perguet, G. 1997. Physiological and biochemical analysis of factors in the female venom gland and larval salivary secretions of the ectoparasitoid wasp *Eupelmus orientalis*. J. Insect Physiol 43: 69-81.

Gül, M. and Gülel, A. 1995. Parasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae)'un biyolojisi ve konak larva büyüklüğünün verim ve eşey oranı üzerine etkisi. Turk. J. Zool. 19: 231-235.

Gündüz, A.E. and Gülel, A. 2005. Investigation of fecundity and sex ratio in the parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) in relation to parasitoid age. Turk. J. Zool. 29: 291-294.

Heimpel, G.E., Antolin, M.F., Franqui, R.A. and Strand, M.R. 1997. Reproductive isolation and genetic variation between two "strains" of *Bracon hebetor* (Hymenoptera: Braconidae). Biol. Control. 9: 149-156.

Hentz, M.G., Ellsworrth, P.C., Naranjo, S.E. and Watson, T.F. 1998. Development, longevity and fecundity of *Chelonus sp. nr. curvimaculatus* (Hymenoptera: Braconidae), an egg-larval parasitoid of pink bollworm (Lepidoptera: Gelechiidae). Environ. Entomol. 27: 443-449.

Horton, D. and Moore, J. 1993. Behavioral effects of parasites and pathogens of insects. In Parasites and Pathogens of Insects (Edited by Beckage N. E., Thompson S.N. and Federici B.A.), Vol.1, pp. 107-124. Academic Press, New York.

Kaleli, S., Aksoylar, M.Y., Aktümsek, A. and Çökmüş, C. 2007. Effects of parasitism by endoparasitoid *Pimpla turionellae* on haemolymph proteins of host *Galleria mellonella*. J. of Sci. Technol. 1: 146-156.

Kanost, M.R., Kawooya, J.K., Law, J.H., Ryan, R.O., Vanheusden, M.C. and Ziegler, R. 1990. Insect haemolymph proteins. Adv. Insect Physiol. 22: 299-396.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage. $T_4$. Nature. 227: 680-685.

Lawrence, P.O. and Lanzrein, B. 1993. Hormonal interactions between insect endoparasites and their host insects. In Parasites and Pathogens of Insects (Edited by Beckage N.E., Thompson S.N. and Federici B.A.), Vol.1, pp. 59-86. Academic Press, New York.

Magro, S.R. and Parra, J.R.P. 2003. Comparison of artificial diets for rearing *Bracon hebetor* Say (Hymenotera: Braconidae). Biol. Control. 29: 341-347.

Martins, J.R., Nunes, F.M.F., Simoes, Z.L.P. and Bitondi, M.M.G. 2008. A honeybee storage protein gene, hex 70a, expressed in developing gonads and nutritionally regulated in adult fat body. J. Insect Physiol. 54: 867-877.

Nakamatsu, Y. and Tanaka, T. 2003. Development of a gregarious ectoparasitoid, *Euplectrus seperatae* (Hym: Eulophidae), that parasities *Pseudaletia seperata* (Lep: Noctuidae). Arthropod Struct. Dev. 32: 329-336.

Quistad, G.B., Nguyen, Q., Bernasconi, P. and Leisy, D.J. 1994. Purification and characterization of insecticidal toxins from venom glands of the parasitic wasp, *Bracon hebetor*. Insect Biochem. Mol. Biol. 24: 955-961.

Park, S.Y., Kim, C.H., Jeong, H.W., Lee, J.H., Seo, S.J., Han, Y.S. and Lee, I.H. 2005. Effects of two haemolymph proteins on humoral defense reactions in the wax moth, *Galleria mellonella*. Dev. Comp. Immunol. 29: 43-51.

Parkinson, N.M., Conyers, C.M., Keen, J.N., MacNicoll, A.D., Smith, I. and Weaver, R.J. 2003. cDNAs encoding large venom proteins from the parasitoid wasp *Pimpla hypochondriaca* identified by random sequence analysis. Comp. Biochem. Physiol. 134C: 513-520.

Pick, C., Holler, H.S. and Burmester, T. 2008. Molecular characterization of hemocyanin and hexamerin from the firebrat *Thermobia domestica* (Zygentoma). Insect Biochem. Molec. 1-7.

Rahbe, Y., Digilio, M.C., Febvay, G., Guillaud, J., Fanti, P. and Pennacchio, F. 2002. Metabolic and symbiotic interactions in amino acid pools of the pea aphid, *Acyrthosiphon pisum*, parasitized by the braconid *Aphidius ervi*. J. Insect Physiol. 48, 507-516.

Richards, E.H. and Edwards, J.P. 1999. Parasitism of *Lacanobia oleracea* (Lepidoptera, Noctuidae) by the ectoparasitic wasp *Eulophus pennicornis*, result in the appearance of a 27 kDa parasitism-specific protein in host plasma. Insect Biochem. Molec. 29: 557-569.

Riddiford, L.M. and Law, J.H. 1983 Larval serum proteins of Lepidoptera: In the larval serum proteins of insects (Ed: Schelleri K.). Thieme Stuttgart. 75-85.

Rolle, R.S. and Lawrence, P.O. 1994. Characterization of a 24 kDa parasitism-specific haemolymph protein from pharate pupae of the Caribbean fruit fly, *Anastrepha suspensa*. Arch. Insect Biochem. Physiol. 25:227-244.

Rossel, R. and Coons, L.B. 1991. Purification and partial characterization of vitellin from the eggs of the hard tick, *Dermacentor variabilis*. J. Insect Biochem. 21: 871-885.

Salvador, G. and Consoli, L.F. 2008. Changes in the haemolymph and fat body metabolites of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) parasitized by *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). Biol. Control. 45: 103-110.

Shaw, M.R. 1981. Delayed inhibition of host development by the nonparalysing venoms of parasitic wasps. J. Invertebrate Path. 37: 215-221.

Soldevila, A.I. and Jones, D. 1994. Characterization of a novel protein associated with the parasitization of lepidopteran hosts by endoparasitic wasp. Insect Biochem. Mol. Biol. 24: 29-38.

Stoltz, D.B., Guzo, D., Belland, E.R., Lucarotti, C.J. and MacKinnon, E.A. 1988. Venom promotes uncoating in vitro and in vivo of DNA from a braconid polydnavirus. J. Gen. Virol. 69: 903-907.

Thompson, S.N. 1985. Metabolic integration during the host associations of multicellular animal endoparasites. Comp. Biochem. Physiol. 81B: 21-42.

Uçkan, F. and Gülel, A. 2002. Age-related fecundity and sex ratio variation in *Apantales galleriae* (Hym: Braconidae) and host effect on fecundity and sex ratio of its hyperparasitoid *Dibrachys boarmiae* (Hym: Pteromalidae). J. Appl. Entomol. 126: 534-537.

Vinson, S.B. and Iwantsch, G.F. 1980. Host regulation by insect parasitoids. Q. Rev. Biol. 55: 143-165.

Vinson, S.B., Pennacchio, F. and Consoli, F.L. 2001. The parasitoid-host endocrine interaction from a nutritional perspective. In: Edwards, J.P., Weaver, R.J. (Eds.), Endocrine Interactions of Insect Parasites and Pathogens. BIOS Scientific Publishers, Oxford, pp. 187-206.

Yu, S.H., Ryoo, M.I., Na, J.H. and Choi, W.I. 2002. Effect of host density on egg dispersion and the sex ratio of progeny of *Bracon hebetor* (Hymenoptera: Braconidae). J. Stored Prod. Res. 39: 385-393.

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