

Dietary Influence on Larval Storage Proteins of the Pharaoh's ant, *Monomorium pharaonis* (Hymenoptera: Formicidae)

by

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ABSTRACT

This preliminary study reported the influence of dietary protein levels on larval storage proteins of the Pharaoh's ant, *Monomorium pharaonis*. Small *M. pharaonis* colonies of 6 queens and 150 – 200 workers (without the presence of brood) were subjected to either high, normal or limited protein diets and allowed to breed until they became established colonies. Larvae from each colony were sampled and divided according to their stages ($L_1 - L_4$). The larval homogenates were subjected to silver-stained SDS-PAGE. Results indicated that dietary protein levels affected the patterns of larval storage proteins in *M. pharaonis*. In addition, there appeared to be variation in protein storages among different larval stages. Larger larval stages (L_3 and L_4) were seen to have a higher diversity of proteins and higher absolute protein contents than those of L_1 and L_2 . The possible implications of the findings on roles and responsibilities of the *M. pharaonis* larvae in a colony are discussed.

Keywords: Storage protein, dietary influence, *Monomorium pharaonis*, larva.

INTRODUCTION

The Pharaoh's ant is one of the world's most important tramp species. They possess specific characters that enable them to spread through human activities and settle successfully far from their original habitat (Børgesen 1995). Tramp ants are species with small sterile workers that are usually monomorphic, widely distributed throughout the world by human activities, and often live in close association with humans. Tramp ants are also polygynous where queens are equally fertile and live in unicolonial colonies. Sociotomy, or budding remains

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to be the main method of colony reproduction instead of nuptial flights (Passera 1994). However, very little information regarding diet and its effects on these ant colonies is available.

An ant colony's dietary condition might actually have effects on the production of reproductives. Wheeler & Martinez (1995) found that patterns of resource consumption, storage and use could be an important aspect of caste specialization. A combination of egg enrichment and rich diet induces queen determination (Gösswald & Bier 1954a, 1954b; Wheeler & Martinez 1995). Food supply was also demonstrated to be an important proximate influence on sex investment where fed colonies of *Formica podzolica* were female biased and unfed colonies were male biased (Deslippe & Savolainen 1995).

The body weight of Pharaoh's ant's queen was also found to be significantly affected by the presence or absence of larvae (Børgesen & Jensen 1995). In *Leptothorax acevorum*, 93% of the queens' liquid nourishment was obtained from oral secretions from larvae (Bourke 1991). Wilson (1974) and Tschinkel (1988) also made similar observations in *Leptothorax curvispinosus* and *Solenopsis invicta* respectively.

Numerous studies have shown the importance of larva in regulating colony nutrient flow and distribution (Wilson 1976; Børgesen 1989). In fire ants (*S. invicta*), larvae play an important role in the distribution of food within the colony via different levels of interactions with the workers (Cassill & Tschinkel 1999). Colony fecundity of the Pharaoh's ant (*Monomorium pharaonis*) was also reported to be dependent on transfer of nutrients from larvae to queens (Børgesen 2000). There seems to be a preference to feed on amino acids as compared to sucrose in fire ant larvae (Cassill & Tschinkel 1999). Important storage proteins needed for colony development and metamorphosis have also been isolated and identified from the larvae of several ant species (Wheeler & Buck 1995). All these point to the importance of elucidating the role of ant larva in the regulation of colony nutrient storage and transfer.

As a prerequisite to further the understanding of nutrient dynamics in Pharaoh's ant, we report here a study of the effects of different levels of protein supplementation on storage protein patterns in ant larval stages under laboratory conditions.

MATERIALS AND METHODS

This project was conducted with Pharaoh's ants that have been cultured in the Urban Entomology Laboratory, Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia since 1995. Ants were separated from the original stock colony into groups of 6 queens

and 150-200 workers without the presence of broods. Nine similar colonies were prepared in aluminum trays (40 x 24.5 x 8 cm) with flouon-coated inner sides.

Three colonies were subjected to a high-protein dietary treatment by feeding with proteinaceous food such as lobster cockroach (*Nauphoeta cinerea*), tuna fish, and egg yolk daily. Protein foods were given alternately to avoid satiation. Another three colonies were given a limited-protein dietary treatment with proteinaceous food given only once a week. The remaining colonies were treated to a normal protein dietary regime where they were given proteinaceous food similar to those of the stock cultures, once every three days. All colonies were given 10% sucrose solution *ad libitum*.

These experimental colonies were allowed to proliferate before protein sampling. We also identified and distinguished four stages of larvae (L_1 to L_4) according to size differences, similar to the classification used by Edwards (1986).

Larvae (5-50 μ g) were separated and carefully placed into Eppendorf[®] tubes, followed by homogenization in 10 μ l of cold deionized distilled water, and subsequent addition of 50 μ l of cold distilled water. Homogenate was centrifuged at 13,200 rpm, 4°C for 20 minutes. The resulting supernatant was transferred into a clean Eppendorf[®] tube and used for analysis. Protein concentration of the supernatant was carried out according to the method described by Bradford (1976). Concentrations were read at 595nm absorbance using a BioRad[®] Microplate Reader Model 680.

Electrophoretic separation of proteins was carried out using the denaturing SDS-PAGE method (Laemmli 1970). Briefly, supernatant was diluted in sample buffer (60mM Tris-HCl, pH 6.8; 25% glycerol, 10 % sodium dodecil sulphate, 14.4 mM 2-mercaptoethanol and 0.1 % bromophenol blue) at ratio of 5:1 (v/v). This mixture was then vortexed, followed by heating at 100°C for 10 min and centrifugation at 12,000 rpm for 5 min. A total of 5.30 μ g protein was loaded in 12.5% SDS PAGE gel and run at 200 V. Molecular weight markers from 10kDa to 250kDa (BIORAD[®]) were also loaded for molecular weight estimation of proteins. Gels were then stained with silver nitrate and scanned with a Densitometer GS800 (BIORAD[®]), followed by documentation and band analysis with Quantity One (BIORAD[®]) software.

RESULTS AND DISCUSSION

Bradford assay revealed differences in protein content among different larval stages from colonies receiving different dietary protein

highest protein content in both limited and high protein treatments as compared to the other 3 stages. The magnitude of this difference was also higher under limited protein supply condition. There did not seem to be any significant differences in protein content between any of the larval stages when an intermediate supply of protein was given.

Electrophoretic profiles (Figs. 1-4) revealed differences in intensity of several bands, indicating changes in protein expression resulting from different dietary protein treatment in respective stages of larvae. Since homogenized crushed larvae were used, our protein profile consisted of mainly structural proteins and hemolymph. Among the two, hemolymph protein content is more dynamic and readily influenced by factors such as developmental processes, temperature, water content and food quality (Mullins 1985; Consoli & Vinson 2002).

Differences in protein expressions resulting from different dietary protein treatments occurred mainly in the molecular weight region of 20kDa - 75kDa for all larval stages. In L_2 , L_3 and L_4 stages, expression of several proteins in this region was comparatively lower in colonies receiving limited dietary protein regime. This higher dietary protein

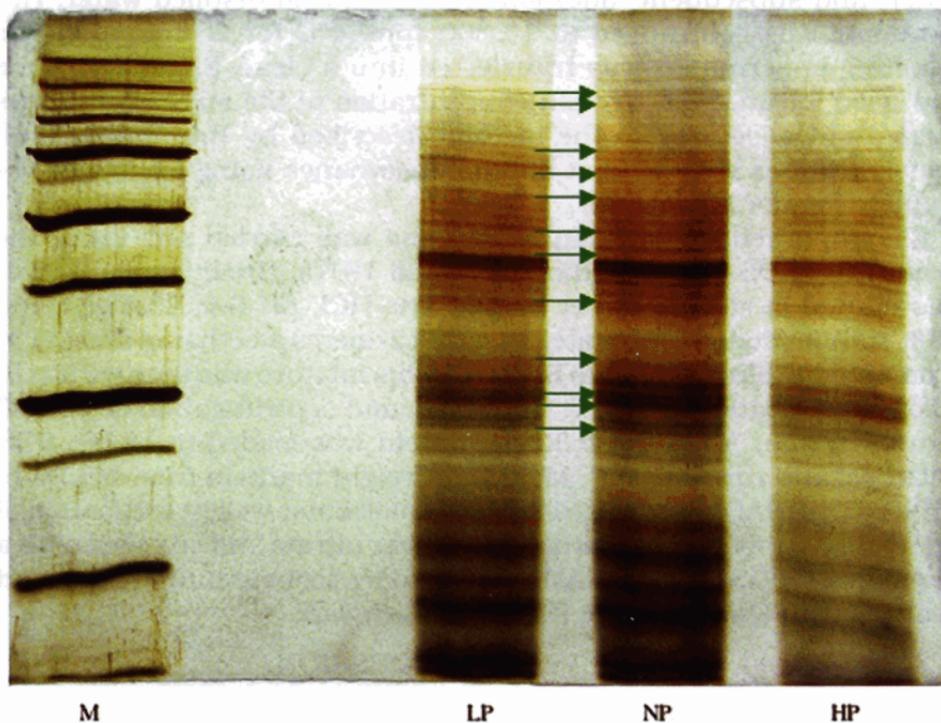


Fig. 1. Protein profiles of larval stage 1 after being subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows show bands with changed protein expression as a result of different dietary treatments. Green arrows indicate NP bands with the highest protein content.

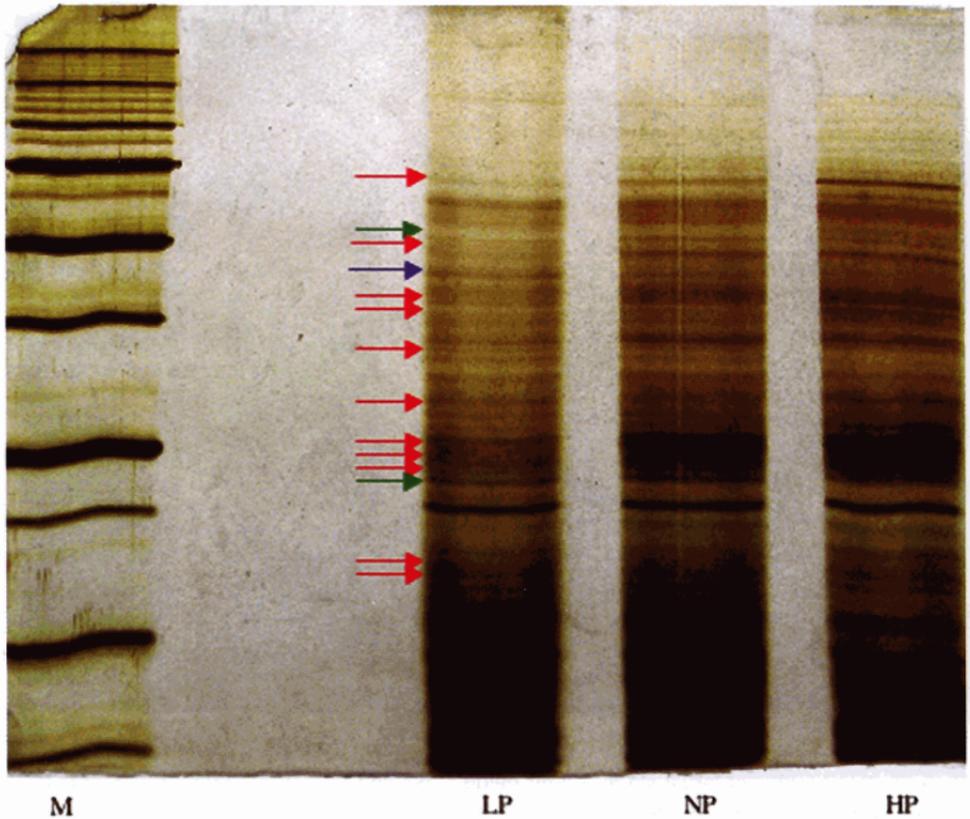


Fig. 2. Protein profiles of larval stage 2 after being subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows show bands with changed protein expression as a result of different dietary treatments. Green arrows indicate NP bands with the highest protein content, red arrows indicate upregulated protein content and blue arrows indicate downregulated protein content.

expression trend is clearly shown in the L_4 stage, suggesting the role of latter stage larvae in handling protein nutrient regulation within the colony. The role of larger larvae in colonial nutrient distribution has been shown in this species, where queens select large larvae to feed from their stomodeal secretions (Børgesen 1989). The same study also showed that removal of these large larvae resulted in decreased egg production. During our experiments, we observed that the larger larvae were usually the first to feed from foragers returning with food particles before workers distributed the nutrients to the rest of the colony through trophallaxis. We postulate that larger larva helped in digestion and even enrichment of nutrients, which are essential to colonial queens. In fire ants, the foragers are responsible for regulating the flow of food from the environment into the nest while other adults and larvae regulate food distribution inside the nest (Cassill & Tschinkel 1999). More relevantly, numerous studies have shown the ability of

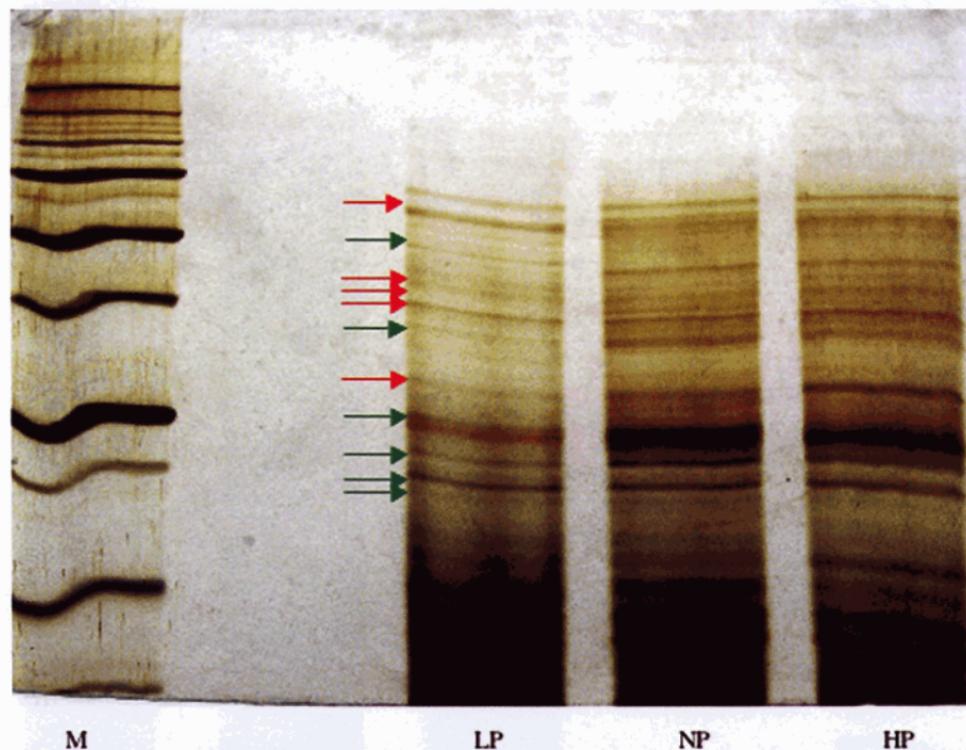


Fig. 3. Protein profiles of larval stage 3 after being subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows show bands with changed protein expression as a result of different dietary treatments. Green arrows indicate NP bands with the highest protein content and red arrows indicate upregulated protein content.

larva to regulate colonial nutrient distribution using various factors such as larval size, hunger level and even food quality as regulators (Cassill & Tschinkel 1995, Cassill *et al.* 1998).

A clear difference is seen in the L_1 stage, where highest density of protein was obtained with intermediate supply of protein (Fig. 1). This was most probably due to the fact that proteinaceous food given in this study was mostly solid or semi-solid. L_1 were most probably fed with secondary protein after older larvae regurgitated it back to more important members of the colony, i.e. the queens. An earlier experiment demonstrated that solid food primarily went to larger larvae (L_3 and L_4) while liquid food was given to smaller larvae (L_1 and L_2). Cassill & Tschinkel (1999) also reported that fire ant larvae preferred soluble proteins (amino acids) as compared to the solid form. Fig. 5 shows a photo of different stages of brood being fed with solid and liquid food respectively. The solid food was dyed tuna fish (blue) and liquid food was dyed sucrose (red).

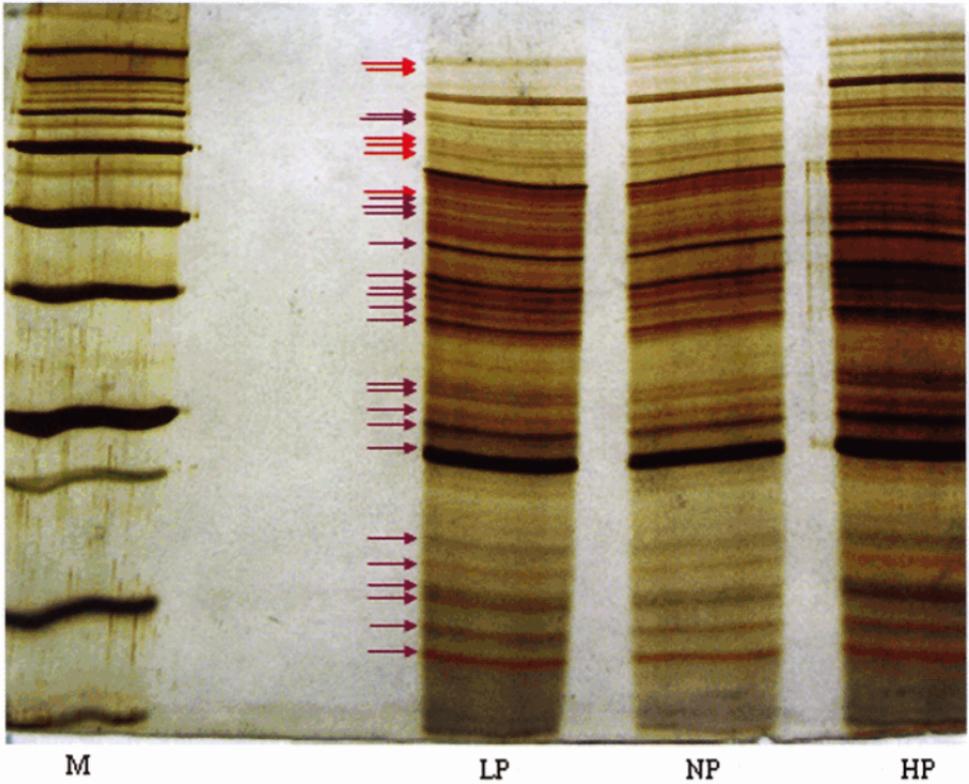


Fig. 4. Protein profiles of larval stage 4 after being subjected to dietary protein treatments (LP-low, NP-normal, HP-high). Arrows show bands with changed protein expression as a result of different treatments. Red arrows indicate upregulated protein content and purple arrows indicate NP bands with the lowest protein content.

Although our studies did not specifically identify the proteins, the changes in expression of high molecular weight proteins in L_4 larval could be associated with the large molecular weights storage proteins which are important for metamorphosis in numerous species of insects (Levenbook 1985, Shipman *et al.* 1987) and beetles (DeKort & Koopmanschap 1994; DeKort & Koopmanschap 1987; Duhamel & Kunkel 1983; Jamroz *et al.* 1996). Holometabolous insects in particular gather large quantities of protein during larval period as storage proteins, which are normally used during metamorphosis (Wheeler & Buck 1996). Metamorphosis in insects is a good example of a period when lack of food is coupled to a high demand for raw materials for building and remodeling tissues (Wheeler & Martinez 1995). These proteins are accumulated in times of dietary surplus and are subsequently used during shortfalls of protein supply. The lower expression of these proteins when treated with limited protein regime in our study could be due to intensified utilization of these proteins to ensure continuous

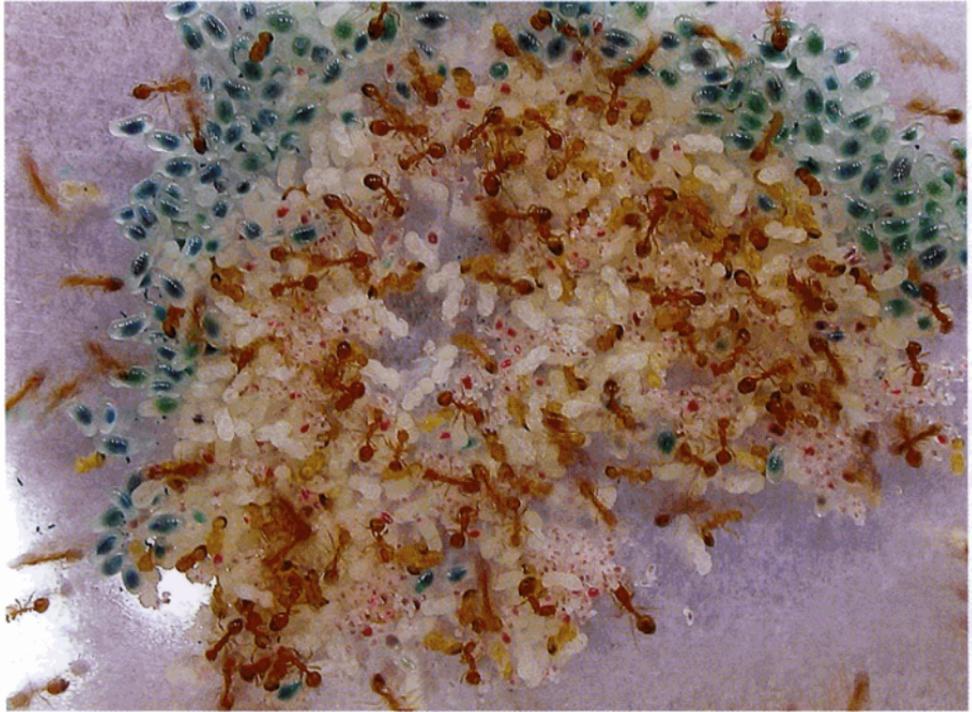


Fig. 5. This picture demonstrates varying nutrient distribution to different stages of larvae by worker ants, as indicated by the colors of the stained larvae.

supply of proteins. Telang *et al.* (2002) also showed increased levels of storage proteins along with dietary protein levels.

Results from this study showed that larval protein profile in Pharaoh's ants varies with different levels of dietary protein. Different stages of larvae may also have different roles and responsibilities in a colony. We foresee that colony conditions may also affect these proteins. These include the presence and absence of queens. However, further experiments are needed to verify these hypotheses.

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REFERENCES

- Børgesen, L.W. 1989. A new aspect of the role of larvae in the pharaoh's ant society (*Monomorium pharaonis* (L.) Formicidae, Myrmicinae): producer of fecundity-increasing substances to the queen. *Insectes Sociaux* 36: 313-327.

- Børgesen, L.W. 1995. Aspects of social regulation in the Pharaoh's ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae). Ph.D. thesis. University of Copenhagen.
- Børgesen, L.W. 2000. Nutritional function of replete workers in the pharaoh's ant, *Monomorium pharaonis* (L.). *Insectes Sociaux* 47: 141-146.
- Børgesen, L.W. & P.V. Jensen 1995. Influence of larvae and workers on egg production of queens of the pharaoh's ant, *Monomorium pharaonis* (L.) *Insectes Sociaux* 42: 103-112.
- Bourke, A.F.G. 1991. Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant, *Leptothorax acevorum*. *Animal Behaviour* 42: 295-310.
- Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein dye-binding. *Analytical Biochemistry* 72: 248-254.
- Cassill, D.L. & W.R. Tschinkel 1995. Allocation of liquid food to larvae via trophallaxis in colonies of fire ants, *Solenopsis invicta*. *Animal Behavior* 50, 801-803.
- Cassill, D.L., A. Stuy, & R.G. Buck 1998. Emergent properties of food distribution among fire ant larvae. *Journal of Theoretical Biology* 195: 371-381.
- Cassill, D.L. & W.R. Tschinkel 1999. Regulation of diet in the fire ant, *Solenopsis invicta*. *Journal of Insect Behavior* 12, 307-328.
- Consoli, F.L. & S.B. Vinson 2002. Hemolymph of reproductives of *Solenopsis invicta* (Hymenoptera: Formicidae)-amino acids, proteins and sugars. *Comparative Biochemistry and Physiology B* 132, 711-719.
- DeKort, C.A.D. & A.B. Koopmanschap 1987. Isolation and characterization of a larval hemolymph protein in *Locusta migratoria*. *Archives of Insect Biochemistry & Physiology* 4: 191-203
- DeKort, C.A.D. & A.B. Koopmanschap 1994. Nucleotide and deduced amino acid sequence of a cDNA clone encoding diapause protein 1, an arylphorin-type storage hexamer of the Colorado potato beetle. *Journal of Insect Physiology* 40: 527-535.
- Deslippe, R.J. & R. Savolainen 1995. Sex investment in a social insect: The proximate role of food. *Ecology* 76(2): 375-382.
- Duhamel, R. & J. Kunkel 1983. Cockroach larval-specific protein, a tyrosine-rich serum protein. *The Journal of Biological Chemistry* 258: 14461-14465.
- Edwards, J.P. 1986. The biology, economic importance, and control of the pharaoh's ant, *Monomorium pharaonis* (L.). In: Vinson, S.B. (ed) *Economic impact and control of social insects*, New York, Praeger Publishers, Pp. 257-271.
- Gösswald, K. & K. Bier 1954a. Untersuchungen zur Kastendetermination in der Gattung *Formica*. 3. Die Kastendetermination von *Formica rufa rufopratensis minor*. *Insectes Sociaux* 1: 229-246.
- Gösswald, K. & K. Bier 1954b. Untersuchungen zur Kastendetermination in der Gattung *Formica* 4. Physiologische Weisellosigkeit als Voraussetzung der Audzucht von Geschlechstieren im polygynen Volk. *Insectes Sociaux* 1: 305-318.

- Jamroz, R.C., J.J. Beintema, W.T. Stam, & J.Y. Bradfield 1996. Aromatic hexamerin subunit from adult female cockroaches (*Blaberus discoidalis*): molecular cloning, suppression by juvenile hormone, and evolutionary perspectives. *Journal of Insect Physiology* 42: 115-124.
- Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Levenbook, L. 1985. Insect storage proteins. In: Kerkut, G.A. & Gilbert, L.I. (eds). *Comprehensive insect physiology, biochemistry and pharmacology* vol 10. Pergamon Press, Oxford. Pp. 307-346.
- Mullins, D.E. 1985. Chemistry and physiology of the hemolymph. In: Kerkut, G.A., Gilbert L.I. (Eds). *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol 3. Pergamon Press, Oxford, pp 355-400.
- Passera, L. 1994. Characteristics of tramp species. In: Williams, D.F. (ed). *Exotic ants: Biology, impact and control of introduced species*. Boulder, CO. Westview Press. Pp. 23-43.
- Shipman, B.A., R.O. Ryan, J.O. Schmidt, & J.H. Law 1987. Purification and properties of a very density lipoprotein from the hemolymph of the honeybee, *Apis mellifera*. *Biochemistry* 26: 1885-1889.
- Telang, A., N.A. Buck, & D.E. Wheeler 2002. Response of storage protein levels to variation in dietary protein levels. *Journal of Insect Physiology* 48: 1021-1029.
- Tschinkel, W.R. 1988. Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta*. *Physiological Entomology* 13: 327-350.
- Wheeler, D.E. & T. Martinez 1995. Storage proteins in ants (Hymenoptera: Formicidae). *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology* 112(1): 15-19.
- Wheeler, D.E. & N.A. Buck 1996. Depletion of reserves in ant queens during claustral colony founding. *Insectes Sociaux* 43: 297-302.
- Wilson, E.O. 1974. The population consequences of polygyny in the ant *Leptothorax curvispinosus*. *Annals of Entomological Society of America* 67: 777-780.
- Wilson, E.O. 1976. *The insect societies* (Fourth edition). Bellknap press of Harvard University Press, Cambridge, Massachusetts and London. Pp. 548.

