Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 97, No. 5, September 1988, pp. 423–427.

Haemolymph protein profiles during the gonadotrophic period of *Gesonula punctifrons* Stal. (Orthoptera: Insecta)

K MURUGAN and J PRASANTH JACOB
Entomology Research Institute, Loyola College, Madras 600 034, India

MS received 8 June 1988

Abstract. Quantitative and qualitative aspects of the haemolymph proteins during the entire life span of both sexes of *Gesonula punctifrons* Stal. in relation to their variations during gonadotrophic period are discussed. The appearance of a female-specific protein in 8-day old female which was absent in freshly-emerged and senescent females and in males, is of interest.

Keywords. *Gesonula punctifrons*; protein profiles; gonadotrophic period.

1. Introduction

Haemolymph proteins in female insects are known to undergo quantitative and qualitative changes correlated with egg maturation (Hill 1962; Engelmann and Penney 1966). The fluctuations in yolk formation, as well as haemolymph concentration in relation to gonad maturation in both sexes, are believed to reflect the net balance between the variation in the rate of protein synthesis in the fat body and protein uptake by developing oocytes. The haemolymph protein pattern of male insects though more or less static, nevertheless shows variations in relation to the physiological state of the individuals. The female insect utilizes the available haemolymph protein for vitellogenesis (Engelmann 1970). Quantitative and qualitative differences in the haemolymph protein pattern existing between male and female insects, as well as, haemolymph protein pattern of female correlation of oocyte development (Engelmann and Penney 1966; Thomas and Nation 1966) have been confirmed through present observations on *Gesonula punctifrons*.

2. Materials and methods

The acridids used for the present studies were taken from a stock culture maintained in the laboratory. The insects were fed *ad libitum* on *Eichhornia crassipes* leaves. Haemolymph for quantitative studies and also for electrophoresis was drawn into a capillary tube from a puncture made on the cervical region and also by amputating the antennae. The samples were centrifuged at 10,000 *g* for 30 min. The clear supernatant was used as protein source for electrophoresis. Protein was quantitatively estimated by the method of Lowry *et al* (1951). For the qualitative profile of the protein, polyacrylamide gel electrophoresis was carried out, employing the method of Davis (1964). The gels were removed from their tubes and stained with Commassie brilliant blue in a mixture of methanol, acetic acid and water in the ratio of 25 : 7 : 68. After destaining, the gels were scanned using LKB 2202 Ultrascan laser densitometer. Haemolymph of each sample was repeated thrice and identically resolving protein fractions were ordered according to their increasing mobility as illustrated in figures 1 and 2.

3. Results

Figure 1 relates to the quantitative protein concentration in the haemolymph during the entire life span of normally reproducing female and male insects, and the corresponding oocyte length. The total protein content of haemolymph in freshly-emerged female was 23 μg/ml and increased to a maximum of 48·9 μg/ml on 14th day subsequently declining to 21 μg/ml on the 26th day. During the 2nd gonadotrophic period (28–44th day), an upward trend in haemolymph protein concentration from 28th to 36th day 31·2 μg/ml and dropped to 21·5 μg/ml on 44th day. From 44th day (second oviposition) to 52nd day (senescent period), the haemolymph protein level gradually declined to 10·5 μg/ml. The haemolymph concentration of male was 12·1 and 10·2 μg/ml on the 1st and 38th day respectively, and did not show drastic variations. Oocyte length varied from 1–4·8 mm (6–20th day; 1st oviposition) and 0·8–4·5 mm (28–44th day: 2nd oviposition). During the final stages of maturation (5·2–5·8 mm), the total protein content in haemolymph declined rapidly.

A comparison of densitometric scans of haemolymph proteins (figure 2) revealed interesting results. The newly emerged female (0 day) showed the presence of two major fractions, and as the oocyte maturation progressed, a new fraction with R_m value 0·221 (table 1) appeared and reached its peak on the 8th day. A sudden drop in the concentration of this protein fraction starting from the 12th day was clearly evident, and was almost eliminated after oviposition. Newly emerged and senescent females were completely devoid of such a protein fraction, and surprisingly males did not show the presence of this fraction throughout their life span.

4. Discussion

Cyclic fluctuations in the haemolymph protein concentrations of normal *G. punctifrons* can be correlated with the egg maturation process as in other species (Hill 1962;

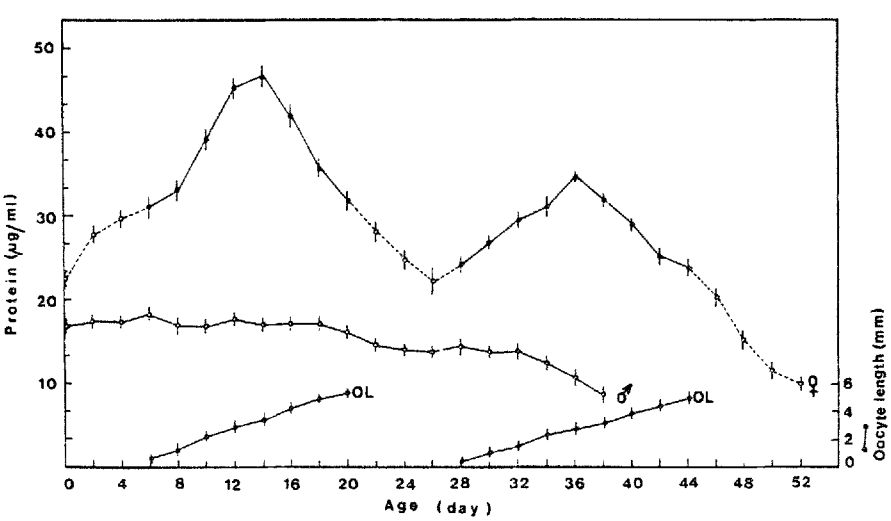

Figure 1. Quantitative haemolymph protein profiles and oocyte length of *G. punctifrons* (OL, oocyte length).

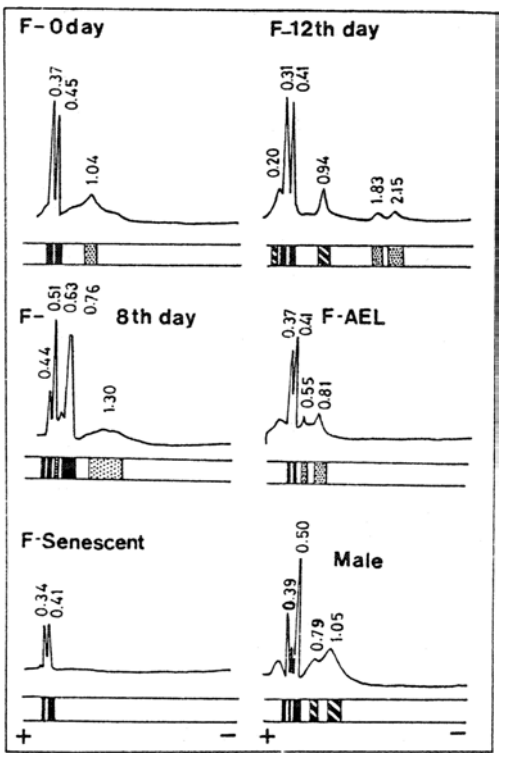

Figure 2. Densitometric scan of protein fractions of *G. punctifrons*.

Table 1. Electrophoretic analysis of the haemolymph protein profile of *G. punctifrons* during gonadotrophic period.

Protein	A	B	C	D	E	F	G	H	I	J	K	L
Ef	0·115	0·136	0·163	0·178	0·221	0·242	0·273	0·288	0·305	0·420	0·696	0·778
Stage/day												
Female												
0	28·391	20·843	50·766	—	—	—	—	—	—	—	—	—
8	13·648	—	17·981	0·878	53·899	—	—	—	—	13·774	—	—
12	—	—	12·754	—	32·520	28·852	—	—	—	14·686	4·485	6·230
Oviposited	—	—	—	—	—	—	7·097	7·719	1·446	83·738	—	—
Senescent	31·094	68·906	—	—	—	—	—	—	—	—	—	—
Male	1·395	0·720	64·874	33·011	—	—	—	—	—	—	—	—

Values represent area percentage.

Engelmann 1970; Gillott and Elliott 1976). However, the lesser protein concentration during the freshly-emerged female and 2nd day of adult life does not appear to be related to oocyte development because the oocytes are not competent to sequester yolk at this time. Hill *et al* (1968) suggested that haemolymph protein may be used for somatic growth in *Schistocerca gregaria* and large amounts of proteins were deposited in the cuticle and flight muscles during the somatic growth phase. Once

yolk deposition begins, a significant increase in the haemolymph protein concentration indicates that synthesis greatly exceeds uptake by the oocytes. However, during the final stages of vitellogenesis, the converse appears to be true as protein levels in the haemolymph are drastically reduced.

In *G. punctifrons* at oocyte length of 3–4 mm, protein concentration was 31·2 μg/ml and at the 4–4·8 mm stage, the maximum protein content of about 48·9 μg/ml was reached. Later, though the development of oocytes progresses protein concentration in the haemolymph declines. Similar fluctuation in protein concentration in the haemolymph was observed by Gillott and Elliott (1976) in *Melanoplus sanguinipes.* Scheurer and Leuthold (1969) have also suggested that in *Leucophaea maderae* the first and subsequent gonadotrophic periods are of sufficient similarity and they may be treated together. However, in *G. punctifrons* protein concentration in haemolymph is comparatively lower during second gonadotrophic period. In oviposited and senescent females, a further decrease in the concentration of the protein was noticed.

In *G. punctifrons*, formation of yolk starts after the age of 6 days (after previtellogenic period). If fluctuation in the concentration of protein fractions and the formation of yolk is correlated, the concentration of protein in the haemolymph falls when the yolk formation starts and the protein accumulates in the ovary. More protein accumulates in the haemolymph during production of the first batch of eggs. This is because of low food intake and stored energy materials are already utilized.

In *G. punctifrons* the qualitative protein profiles vary during development. The same trend was noticed in *Schistocerca gregaria* by Hill (1962). Further, he has reported that a sex-specific or vitellogenic or female specific protein is utilized during vitellogenesis. In *G. punctifrons* also, a sex-specific protein is evident in 8th day old female. In the present study except for the absence of female-specific protein, the pattern of mature males closely resembles that of vitellogenic female. This is in agreement with the report of Elliott and Gillott (1979) in *M. sanguinipes.*

Acknowledgements

The authors are thankful to Prof. T N Ananthakrishnan for suggesting the problem and critically going through the manuscript, to the Council of Scientific and Industrial Research, New Delhi for funding and to the COSIST for instrumentation facilities.

References

Davis B J 1964 Disc electrophoresis II. Method and application to human serum proteins; *Ann. N. Y. Acad. Sci.* **121** 404–427

Elliott R H and Gillott C 1979 An electrophoretic study of proteins of the ovary, fat body and haemolymph in the migratory grasshopper, *Melanoplus sanguinipes*; *J. Insect. Physiol.* **25** 405–410

Engelmann F and Penney D 1966 Studies on the endocrine control of metabolism in *Leucophaea moderae* (Blattaria)—I. Haemolymph proteins during egg maturation; *Gen Comp. Endocrinol* **7** 314–325

Engelmann F 1970 *The physiology of insect reproduction* (Oxford: Pergamon Press)

Gillott C and Elliott R H 1976 Reproductive growth in normal, allatectomized, median-neurosecretary-cell-cauterized, and ovariectomized females of *Melanoplus sanguinipes*; *Can. J. Zool.* **54** 162–171

Hill L 1962 Neurosecretary control of haemolymph protein concentration during ovarian development in the desert locust; *J. Insect. Physiol.* **15** 1067–1077

Hill L, Luntz A J and Steele P A 1968 The relationships between somatic growth, ovarian growth and feeding activity in the adult desert locust; *J. Insect. Physiol.* **14** 1–20

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with Folin Phenol reagent; *J. Biol. Chem.* **193** 265–275

Scheurer R and Leuthold R 1969 Haemolymph proteins and water uptake in female *Leucophaea maderae* during the sexual cycle; *J. Insect. Physiol.* **8** 609–619

Thomas K K and Nation J L 1966 Control of sex-limited haemolymph protein by corpora allata during ovarian development in *Periplaneta americana* L.; *Biol. Bull. Woods Hole Mass.* **130** 254–264