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ORIGINAL ARTICLE

ELECTROPHORETIC PROTEIN PATTERNS AND PROTEASES ACTIVITY DURING FEMALE ADULT DEVELOPMENT OF *CHILO PARTELLUS* (SWINHOE).

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Abstract:

The electrophoretic protein patterns and proteases activity during female adult development of Chilo partellus have been studied. Gradual increase in number of protein fractions was observed from1 to 3- day adults and sharp decrease in number of protein fractions from 3 to 4-day adults was observed. Maximum number of protein fractions was observed in 3- day adultsand minimum in 4-day adult.

The partial characterization of proteases (acidic, alkaline, and neutral) in female adult development has been studied.The maximum enzyme activity was noted in 1- day adults. Then gradual decrease in enzyme activity from 1 to 4-day adults was observed. The minimum enzyme activity was observed in 4-day adults.

KEYWORDS:

Electrophoresis, proteins, proteases, female adult development, Chilo partellus.

INTRODUCTION

The maize stem borer *Chilo partellus (Lepidoptera- Crambidae)* is very serious pest in India. The larvae feed and damage the inner vascular tissue of stem to form elongated tunnel leading to great loss in yield.

The female adult moth is medium sized yellowish brown insect measuring 25-30mm across the wings. The straw coloured forewings have a double row of black spots along their outer margins. The hind wings in male moth are whitish. The tip of the abdomen in female is dilated and tufted with hairs. The moths are nocturnal in habits. They were found hidden under plants, dry leaves, clods etc. during daytime. The adults live for four to five days. Generally mating occurs during night at second day. The adult female moth started egg laying from second day onward. The female continued egg laying for about two to three days. The female adult lived for about one day after their completion of egg laying. The number of eggs laid per female has been recorded to be 150-300.

The escape of adult insect from the cuticle to pupa or in hemi metabolous insects of the last larval instar is known as eclosion. To produce the split, the adult swallows air to increase its volume and then further increases its thoracic volume by pumping blood forwards from the abdomen (Chapman, 1969).

Of all known chemical compounds proteins are the most complex and at the same time most characteristic of living matter. They are the compounds which as nucleoproteins, are essential to the process of cell division and as enzymes and hormones control many chemical reactions in the metabolism of cells.

In addition to maintenance the dominant phenomenon in the life of adult insects is reproduction. In the female there is continuous deposition of yolk at the time of egg production (Chen, 1966). In addition to hormones, the quality of the diet may have also a definite influence on ovarian development. A variety of

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amino acids included in the synthetic diet are found to be indispensable for egg formation (House, 1962). Several investigations have demonstrated that egg proteins may be synthesized outside the ovary and then transferred without change to the ovary and in tissues outside it. In the ovary protein synthesis is initiated in the trophocytes and follicle cells. Outside the ovary proteins are synthesized in the fat body, released in to the haemolymph and taken up by the female gonads (Chen, 1966).

It is now evident that proteolytic reactions play a key role in the regulation of intracellular protein turnover.

A few studies have been carried out in the rate of proteins and activity of protease enzymes in the adult insects.

With regard to proteins much attention has been paid to the haemolymph proteins since the haemolymph serves as an important internal environment for the metabolism. The haemolymph proteins fluctuate much more widely than those of free amino acids. There is decrease in volume of haemolymph during adult development suggesting that the haemolymph protein serves as an important source for protein synthesis of adult organ (Chen, 1966). Hayes et. al. (1970) worked on electrophoretic patterns of proteins in haemolymph from the adult *medeira* cockroach, *Leucophaea maderae*. (Parker, 1971) compared the haemolymph proteins in two species of *Leptinotarsa* beetles. Proteins were separated from the haemolymph by use of PAGE.

Gooding and Huang (1969) studied Trypsin and Chymotrypsin from the beetle *Pterostichus melanrius*. Muraleedharan and Prabhu (1978) worked on food intake and midgut protease activity in the red cotton bug, *Dysdercus cingulatus fabr*. Martin *et al*. (1981) studied the digestive enzymes of detritus feeding stonefly nyphs. Moffat (1995) studied the synthesis and secretion of trypsin in the midgut of *Stomoxys calcitrans.*

However the information on female adult development of *Chilo partellus* is rather scanty. There exists a lacuna in the field of proteins and proteases activity during adult development of *Chilo partellus*.

Therefore present study attempts to provide information on proteins and proteases during female adult development of *Chilo partellus.*

The results are discussed in regards to changes undergone during female male adult development of *Chilo partellus*.

MATERIALS AND METHODS

The culture of *Chilo partellus* was maintained in the laboratory on natural food of cut pieces of fresh maize stems. Egg masses were put in tender maize shoots for hatching. The young larvae were trapped in tender maize shoots. The later instars were inserted in cut pieces of fresh maize stems.The pupae were collected and kept in glass jar lined with white paper at its inner side for emergence of moths. The pupal developmental period is of 10- days. Soon after the emergence of the moth was observed to be inactive for some time.

The female adult moth is medium sized yellowish brown insect measuring 25-30 mm across the wings. The straw coloured forewings have a double row of black spots along their outer margins. The hind wings in male moth are whitish. The tip of the abdomen in female is dilated and tufted with hairs. The adult life span is of 4-days.

The female moths were isolated, cleaned with distilled water weighed and homogenized in chilled buffer solution. The homogenates were diluted with chilled buffer solution so as to get various concentrations for electrophoresis and proteases activity.

The electrophoresis of proteins was carried out by method of Laemlli (1970) and the relative mobility (Rm) and molecular weights of separated proteins were calculated by method of Weber and Osborn (1969).

The biochemical assay of Cathepsin D and Cathepsin B like (acidic) proteases was carried out by the method of Mycek (1970).Biochemical assay of Neutral proteases was carried out by method of Wilkes and Prescott (1976).Biochemical assay of Chymotrypsin and Trypsin like (alkaline)proteases was carried out according to method of Rick (1965).

RESULTS

The electrophoretic protein patterns during female adult development were representedin PLATE–1.

In 1-day female adult 9 protein fractions, in 2-day 14 protein fractions, in 3- day 16 protein fractions and in 4- day 9 protein fractions were observed.

The gradual increase in number of protein fractions from 1 to 3-day adults and sharp decrease in

no. of protein fractions from 3 to 4-day adults were observed. Maximum no of protein fractions was observed in 3-day and minimum in 4- day adults.

The partial characterization and enzyme activity of acidic, neutral and alkaline proteases during female adult development was studied.Enzyme activity of acidic (Cathepsin D like and Cathepsin B like), neutral and alkaline (Chymotrypsin like and Trypsin like) protease during female adult development of Chilo partellus were represented in FIGURE- 1.

In female adult the maximum acidic, neutral and alkaline protease activity was noted in 1 day adult and steady decrease from 1 to 3- day adults was observed. Then it gradually decreases up to4-day adult. Minimum activity was noted in 4-day adults.

DISCUSSION

The metamorphic events culminate into formation of imago. The adult pterygot insect does not moult but it generally undergoes some further development after emergence. Most of the organs particularly reproductive ones attain their full functional status. Along with this certain age related physiological and biochemical changes also occur. The female adult involves predominantly yolk synthesis in relation to egg production. The major yolk proteins are synthesized in the fat body, secreted in the haemolymph and taken up by the oocytes.The synthesis in the fat body cells is under the control of hormones, which differ in different insects and may depend on series of internal and external factors (Kunkel & Nordin (1985). In *Drosophila melanogaster* it can be seen that for all amino acids the concentration appears consistently higher in females than in males (Chen, *et al*.1967).

At beginning of adult protease activity in the midgut increases much more rapidly in females than in males, obvious due to the need for large quantities of amino acids for vitellogenesis (Waldner-Stiefelmeier,1967).

Gravin & Williamson (1976) studied synthesis and deposition of yolk protein in adult *Drosophila melanogaster.* The newly enclosed *Drosophila melanogaster* females contain only previtellogenic oocytes. The development of protein containing yolk spheres in the ooplasm distinguishes the vitellogenic stages, which appear subsequent to eclosion as an exclusively adult event.

During vitellogenesis in the insect, the fat body initiates and maintains a massive synthesis of vitellogenin, a high molecular weight protein, which is released in the haemolymph (Engelmann, 1979; Hagedorn & Kunkel,1979).

An immunological probe was used to demonstrate the appearance and quantitative accumulation of female specific proteinin the haemolymph. Elliott and Gillott (1978) worked in the neuroendocrine control of protein metabolismin the migratory grasshopper, *Melanoplus sanguinipes*.

With regards to the proteins much attention has been paid to the haemolymph proteins since the haemplymph serves as an important internal environment for the metabolism the haemolymph proteins fluctuate much more widely than those of free amino acids. There is decrease in volume of haemolymph during adult development suggesting that the haemolymph proteins serve as an important source for protein synthesis of adult organ (Chen, 1966).

In the present work increase in number of protein fractions observed from 1- day to 3-day adults and maximum number of protein fractions in 3-day adults in *Chilo partellus*. This suggests that in adult female the major metabolic process is yolk synthesis and high protein content in female is related to egg production. This may indicates that in early days of vitellogenesis major yolk proteins are synthesized in fat body, released into haemolymph and taken up by developing oocytes. Sharp decrease in number of protein fractions from 3 to 4-day adults indicates completion of yolk protein synthesis and egg laying. Our results are in good agreement with Kunkel and Nordin (1985), Gravin and Williamson (1976); Engelmann(1979); Hagedorn & Kunkel, 1979; Elliott(1978).

During metamorphosis of an insect, processes like destruction of certain larval tissues and rejuvenation and remolding of various tissues into adult are take place. Since proteins are the first biological factors making their manifestation during development (Schmidt & Schwankl, 1975) number of plasma proteins increase during successive stages of development (Kanost,*et.al* 1990). In adult insects proteins are pre-requisite for egg maturation in females and proteases are responsible for degradation of proteins (Chen, 1978).

Muraleedharan & Prabhu (Muraleedharan, & Prabhu, 1978) studied the food intake and midgut protease activity in the red cotton bug, *Dysdercus cingulatus*.

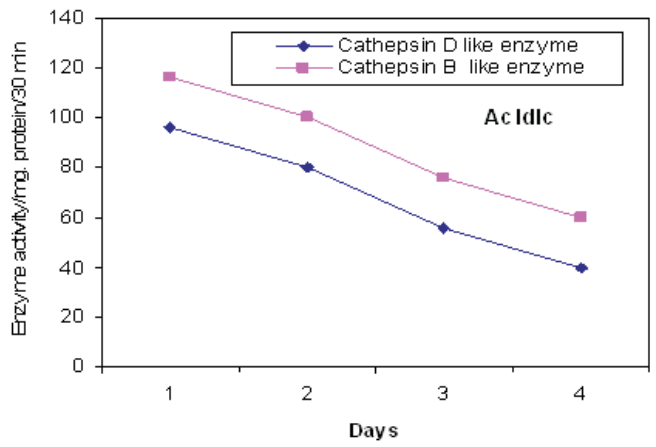
The pattern of midgut protease activity in *Dysdercus cingulatus* is comparable to vitellogenic activity. The daily increase in the total protein content of the ovary of *Dysdercus cingulatus* during first gonotrophic cycle has been demonstrated by Prabhu and Nayar (1971).Their observations show that a significant increase in protein content of ovary is noticed in3, 4, 5 and 6-day old insects This type of

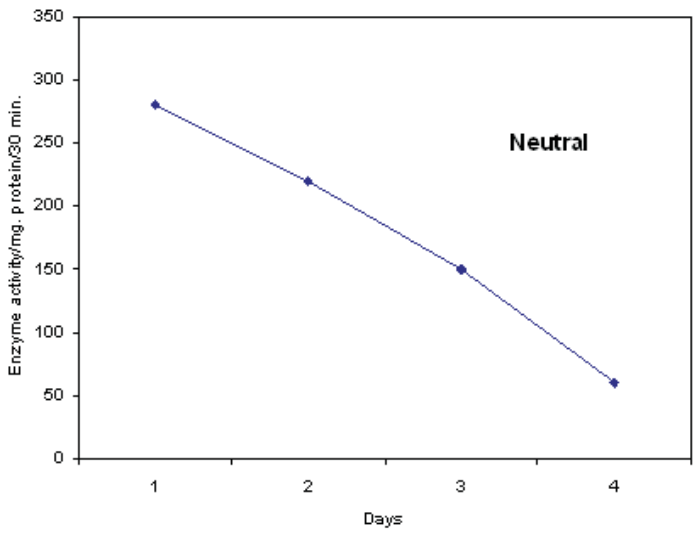
coordination between gonotropic cycles and the secretion of midgut digestive enzymes is noticed in many other insect species like *Aedes atropalpus*. Thus it becomes evident that there exists aclose relationship between oogenesis and midgut protease activity in *Dysdercus cingulatus*. A high level of protease acivityappearantly leads to greater protein digestion and an increased availability of precursors in the haemolymph for the synthesis of yolk proteins during vitellogenesis. Vitellogenesis starts in 3- day old insects and continues in 4 and 5-day and completed in 6-day old insects (Jalaja, & Prabhu, 1976) and this may be the reason why the 6- day insects show a steady decrease in the midgut protease activity.

Detinova (1962) Concluded that the completion of vitellogenesis slow down digestion in *Anopheles macculipnni.*

In the present investigations gradual decrease in enzyme activity of acidic, neutral and alkaline proteases from 1 to 4-day female adults of *Chilo partellus* was observed. This suggests gradual degradation of stored proteins for synthesis of yolk proteins in the fat body. Our results are in good agreement with Jalaja & Prabhu (1976), Detinova (1962), Prabhu and Nayar (1971) and Chen (1978).

FIGURE I
Proteases activity during female adult development of *Chilo partellus* (Swinhoe).





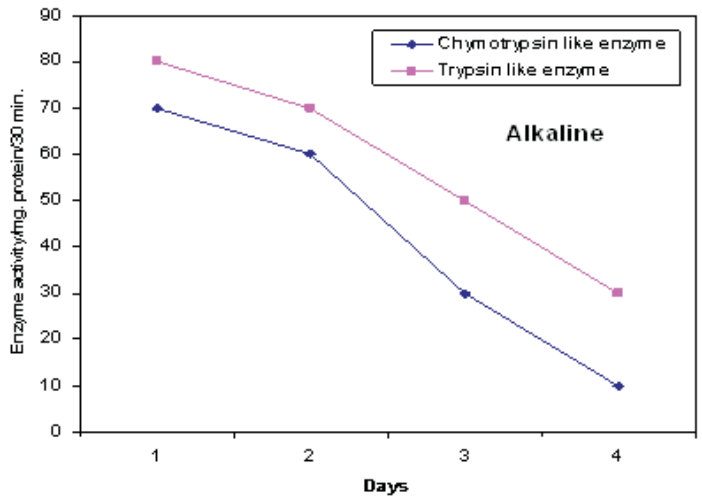
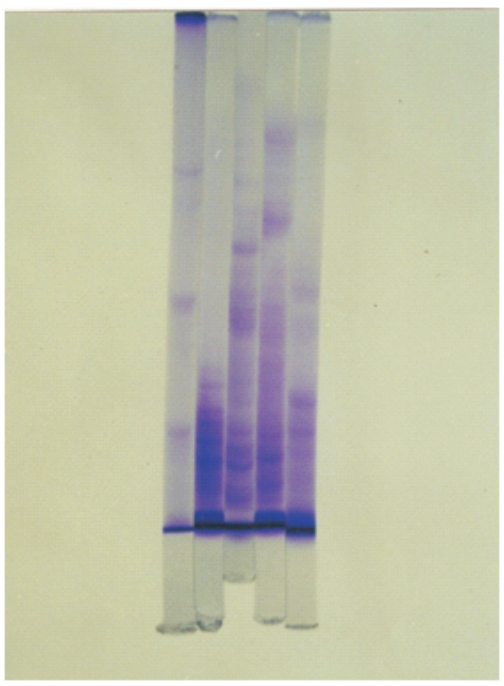


PLATE I
Electrophoretic protein patterns during female adult development of *Chilo partellus* (Swinhoe)



S 1 2 3 4
Female adult stage in days

REFERENCES

1.Chapman, R. F. (1969). In: "The Insects".(ed). The English Universities Press Ltd.,pp 70-106, 403-422, 675-691.
2.Chen,P.S. (1966). Amino acid and protein metabolism in insect development. In "Advances In
3.Chen, P. S., Honmann, F. & Briegel, H.(1967). Freieaminosaurenunde derivate in Eiern von Drosophila, Culexand Phormia. Rev. Suisse Zool. 74, 570-589.
4.Chen, P.S.(1978). Protein synthesis in relation to cellular activation and deactivation. In: "Biochemistry

of Insects". (Rockstein, M. ed.). Academic Press, New York and London. pp 145-203.
5.Detinova, T. D. (1962). Age grouping methods in Diptera of medical importance. World health organization, Monographs Series, No. 55.
6.Elliott,R. andGillott, C.(1978). An electrophoretic study of proteins of the ovary, fat body and haemolymph in the migratory grasshopper, Melanpplus sanguinipes. J.Insect Physiol. 25, 405.
7.Engelmann, F.(1979). Vitellogenin and vitellin in insects. Ann. Rev. Entomol., 24,475-505.
8.Gooding, R.H. and Huang, C.T. (1969). Trypsin and Chymotrypsin from the beetle, Pterosticus melanarius., 15, 339.
9.Gravin, J. A. & Williamson, J. H.(1976). Synthesis and deposition of yolk protein in adult Drosophila melanogaste, J. insect Physiol., 22, 1457-1976.
10.Hagedorn, H. H. and Kunkel,J. G.(1979).Vitellogenin and vitellin in insects., A Rev. Entomol., 24, 475-505.
11.Hayes, D. K.,Mensing, E.and Schechter,M. S.(1970). Electrophoretic patterns of proteins in haemolymph obtained from adult medeira cockroach, Leucophara maderae(I) during 24hr.period. Comp.Biochem.Physiol., 34,733.
12.House, H. L. (1962). Insect Nutrition. A.Rev. Biochem.., 31,653-672.
13.Jalaja, M. &Prabhu, V.K. K.(1976). Effect of the chemosterilantsapholate on the ovaries of the red cotton bug, Dysdercus cingulatusfabr.(Heteroptera, Pyralidae) Entomon, 1,43-53.
14.Kanost, M. R., Kawooga, J. K., Law, J. H., Ryan, R. O., Heusden, M.C.&Zerigler, R.(1990). Insect haemolymph proteins. In: Adv. Insect Physiol. Academic Press. 22, PP 299-397.
15.Kunkel, J. and Nordin (1985). In Comprehensive Insect Physiology Biochemistry and Pharmacology,(Kerkut, J. A.& Gilbert, L.I. ed.) Pergamon Press, New York. 10, PP 177-248.
16.Laemlli, U.K.(1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature, 227,681,685.
17.Martin, M.M., Martin, J. S. and Kukor, J.J.(1981). The digestive enzymes of detritus feeding stone fly nymphs (Plecoptera – Pteronarcyidae). Canadian J.Zool., 59, 1947-1951.
18.Moffatt, M.R. , Blackmore, D. and Lehane, M.J. (1995). Comp. Biochem. Physiol. 110B(2), 291-300.
19.Muraleedharan, D. and Prabhu, V.K.K. (1978). Food intake and migut protease activity in red cotton bug, Dysdercus cingulatusFabr. (Heteroptera- Pyrrhocoridae). Entomon., 3 (1), 11-17.
20.Mycek, M.J.(1970). "Proteolytic Enzymes". (Ed. By Gertrude, E.P. and Laszlo)London. 15, pp. 286.
21.Parker, R.(1971). Comparison of haemolymph proteins in two species of Leptinotarsa Beetles. J. Insect Physiol. , 17,55.
22.Prabhu, V.V.K. & Nayar, K. K. (1971). Protein and free amino acid concentration in the blood and total ovarian proteins in Dysdercus cingulatus fabr. (Hetereplere during reproduction. Comp. Biochem. and Physiol., 11, 961-971.
23.Rick, W. (1965). Chymotrypsin. In: "Methods in Enzymology". (Ed. By Colowick and Kaplan). 2, pp 800.
24.Schmidt, G. H.& Schwankl, W.(1975). Changes in haemolymph proteins during the metamorphosis of both series and castes of polygnous, Formica rufa. Comp. Biochem. Physiol., 52B, 365-380.
25.Waldner- Stiefelmeier, R. D.(1967). Untersuchungenuber die proteasen Im wild type und in den Letalmutariten(Ime and Itr) Van Drosophila melanogaster., Z. Vergl. Physiol., 56,268-289.
26.Weber, K. and Osborn, M.(1969).The reliability of molecular weight determinations by dodecyl sulphate polyacrylamide gel electrophoresis. J. Biol. Chem., 244(16), 4406-4412.
27.Wilkes, S. H. and Prescott, J.M.(1976). Ameromonas Natural Protease. In: "Methods in Enzymology". (Ed. By Colowick and Kaplan). 45, pp 405-415.