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Electrophoretic study on development profiles of proteins in hemolymph and salivary gland of the red cotton bug *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae)

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Abstract
SDS-Page of hemolymph and salivary gland protein of *Dysdercus koenigii* during larval-adult transformation (post embryonic development) find out the quantitative changes in protein patterns. Comparison of electropherograms of male and female revealed little difference and shows sexual dimorphism, the number of protein increased in all tissues till the third day of fifth instars (Synthetic phase). There after it decrease till the last day (consumption phase). Changes appeared in haemolymph protein during metamorphosis, the number of bands more in female. In adult number of proteins in haemolymph decreased suggesting their role in cuticle formation. By contrast salivary gland shows a sudden increase in number of protein band continuously from third instar to fifth instar and then there is a fall in number of protein band in adult. Some protein band are identify as major hemolymph protein (MHP) in both sexes.

Keywords: Protein pattern, SDS-Page, heteroptera, electrophoresis

Introduction
During metamorphosis of insect, processes like destruction of certain larval tissues and rejuvenation and remolding of various tissues in to adult one are bound to take place. There changes involve synthesis and consumption of the macromolecules as well. Since proteins are the first biological factors making their manifestation during development changes in haemolymph protein during metamorphosis (Schmidt and Schwanki, 1975) [1], studies on tissue specific proteins become morphologically of paramount significance.

The haemolymph, which is in direct contact with tissues, has specific metabolic function as transport, storage and regulation of enzymatic reactions. After preliminary study of haemolymph protein in *Hyalophora Ceoropia* by Telfer and Williams (1953) [3] demonstrated the presence of the female specific protein in the haemolymph of the silkworm. The salivary gland has also been shown to sequester some non-vitellogenic protein components from the haemolymph (Kumar 1979; Laufer and Nakase 1965) [8, 5]. Hitherto it was believed that the salivary lobules synthesize all the components of saliva plays important role in facilitation of blood meal, lubrication of mouth part and parasite transmission of same vector insect. Saliva composition changes during life time of insect and differences in saliva profile may influence its function. (Prates *et al*. 2014) [13]. Some of the salivary components are synthesized in the haemolymph and transported into the salivary glands (Miles 1972).

This study was undertaken on the premise to find out the qualitative changes in proteins of the haemolymph and salivary gland of hemimetabolous bug *Dysdercus koenigii* during larval-adult transformation. An attempt has also been made to unravel the possibility of the existence of any larval specific protein.

Materials and Methods
Experimental animal-*Dysdercus koenigii* were reared in the laboratory on soaked cotton seeds at 25-27 ºC under long day photoperiod (16L: 8D) as described (Venugopal *et al*. 1994) [7].

Sample Preparation
Haemolymph and salivary gland from 3rd instar to 5th instar larvae and adult, of both sexes were collected separately in chilled calibrated microcapillary tubes and stored at -20 ºC.

until used. Salivary gland of different stages were dissected out in insect ringer solution from insects, already used for haemolymph collection and washed thoroughly in the same. Wet weight of both tissue were taken after blotting and homogenized separately by hand drive glass micro homogenizer in 0.4 ml 62.5mM Tris Hcl buffer, pH 6.8 at 4 ^{o}C centrifuged at 10,000 rpm for 10 min. The supernatant was stored at -20 ^{o}C until used.

SDS- PAGE electrophoresis

SDS-PAGE (Slab gel 130:130:30 mm of 10% separating and 5% stacking gel) was performed (Laemmli 1970) [12] using 62.5 mm Tris buffer pH 8.6 containing 0.1% SDS at constant current of 10mA for stacking and 20mA for running gel for 3hrs. 4.5 ml supernatant of haemolymph and 20ul supernatant of salivary gland were mixed separately with 10µl each of these samples was loaded in the lanes of the gel. Following electrophoresis gel stained overnight in 0.25% commessive brilliant blue in 50% methanol and 10% acetic acid and destained in solution of 50% methanol 10% Acetic acid. Stained gels were stored in 7.5% Acetic acid at 4 ^{o}C.

Results

SDS-PAGE under denaturing condition was performed on male and female haemolymph and salivary gland of both the sexes from various developmental stages from 3rd instar to adult of *Dysdercus koenigii* to compare their protein during these developmental stages. A comparison of the electropherograms was made in order to know the developmental pattern of protein in two tissues with view to find out the presence of any stage specific protein during larval- adult transformation.

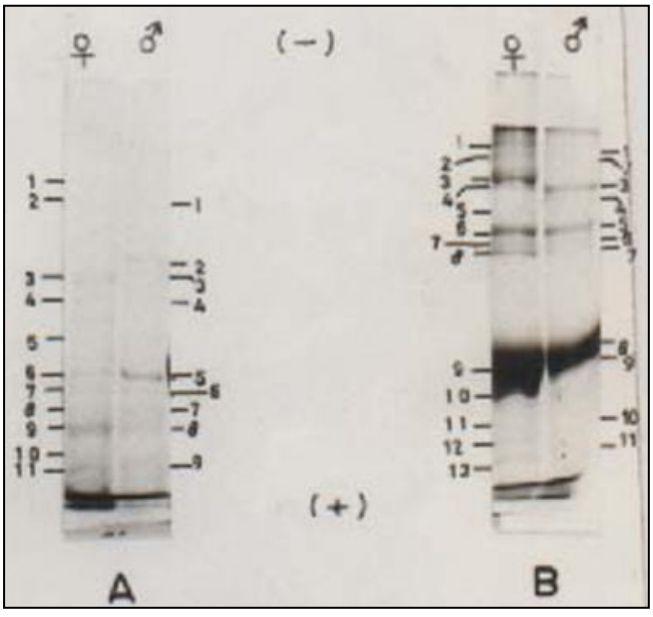

Fig 1: 3rd Instars: A-Salivary gland A-Salivary gland B- Haemolymph

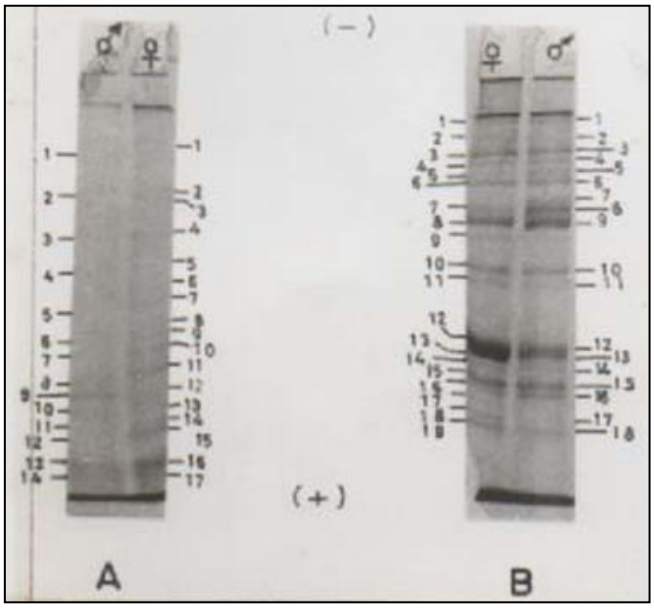

Fig 2: 4th Instars: A-Salivary gland B- Haemolymph

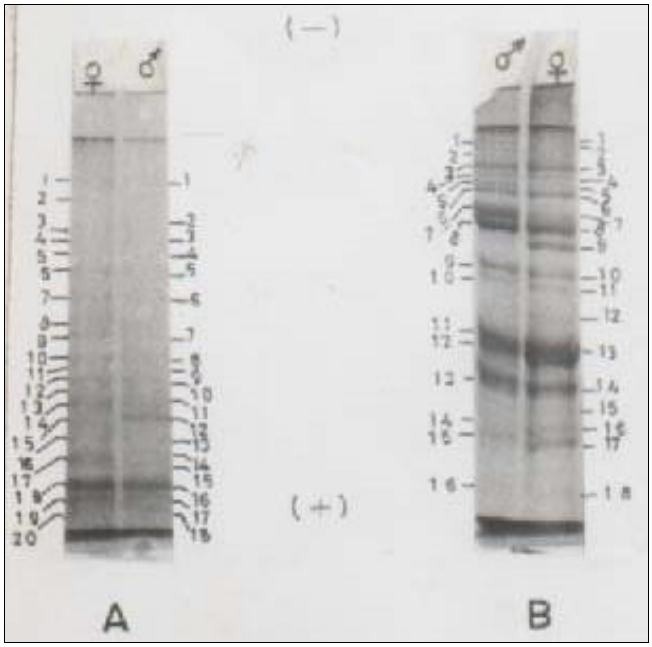

Fig 3: 1 5th Instar: A-Salivary gland B- Haemolymph

Fig 1: Illustrates the SDS-PAGE protein pattern of salivary gland and haemolymph of both the saxes in 3rd instars (3rd day) male haemolymph resulted in 9 band of varying intensities rf values as (0.28, 0.43, 0.45, 0.51, 0.70, 0.74, 0.80, 0.83 and 0.91) respectively out of the these band no.5 ins a major band and the rest are either thin or weakly stained and the salivary gland in 3rd instars having total eleven bands (rf values as 0.24, 0.29, 0.48, 0.53, 0.57, 0.80, 0.88 and 0.93 respectively out of these three bands no. 6, 9 and 11 of (rf value 0.70, 0.83 and 0.93) are the major band. It may be noted that band no.5 from male and band no.6 from female show similar mobility (the same rf values) the two extra protein band could be female or stage specific proteins.

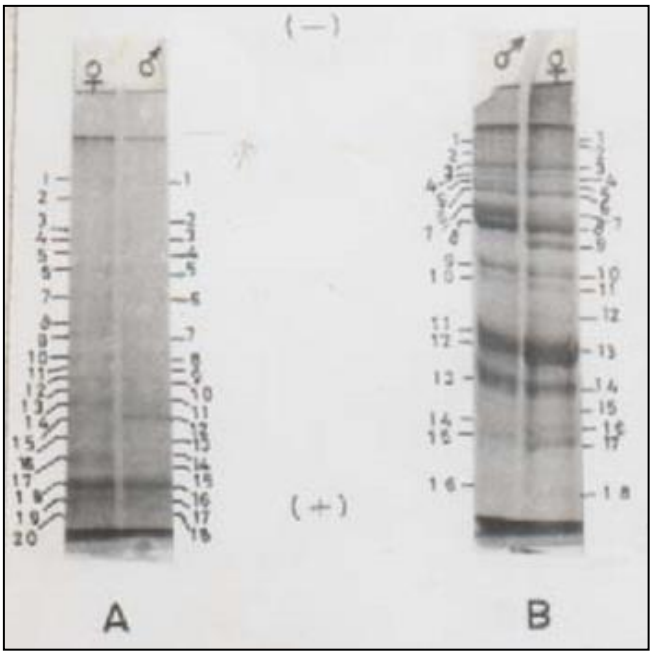

Fig 4: Adult: A-Salivary gland B- Haemolymph

SDS-PAGE of haemolymph results in 13 protein band in female and 11 band in male. Band no. 6 and 9 in female and 5 and 8 in male are major bands having the similar rf value of 0.38 and 0.70 respectively.

Protein zones 1 to 13 in female have rf values as 0.16, 0.19, 0.24, 0.32, 0.37, 0.40, 0.44, 0.70, 0.72, 0.83, 0.90 and 0.93 respectively. Band no 1 to 11 in male have rf values as 0.16, 0.19, 0.20, 0.29, 0.38, 0.41, 0.70, 0.72, 0.91 and 0.93 respectively. Band no. 8 and 12 in female haemolymph are the extra bands that may be female specific protein at this stage.

Fig 2: illustrates the SDS-PAGE pattern of proteins from salivary glands and haemolymph of both the sexes in 4th instars. In male there are 14 bands (rf values 0.20, 0.29, 0.40, 0.45, 0.63, 0.70, 0.75, 0.78, 0.81, 0.88, 0.95 and 0.98 respectively. Band no 13 and 14 (rf value 0.95 and 0.98) are the major band. In female shows total 17 bands (rf 0.16, 0.18, 0.19, 0.21, 0.30, 0.38, 0.40, 0.47, 0.57, 0.63, 0.65, 0.68, 0.73, 0.80, 0.93 and 0.98 respectively. Band no 15, 16, 17 (rf value 0.86, 0.95 and 0.98) are the major bands. Here in female there are three extra bands in comparison to male which could be female and stage specific. In haemolymph male have 18 bands (rf value 0.07, 0.09, 0.14, 0.16, 0.19, 0.24, 0.27, 0.31, 0.34, 0.45, 0.49, 0.63, 0.68, 0.72, 0.73, 0.80 and 0.83 respectively, four bands are major bands (rf 0.34, 0.63, 0.65 and 0.73) are the major bands and female presence of 19 bands having (rf value 0.07,0.09,0.13,0.18, 0.19,0.22,0.24, 0.31,0.32,0.37, 0.45, 0.63, 0.65,0.68,0.72,0.73,0.75,0.80 and 0.83 respectively. Out of these four bands are major bands having (rf value 0.31, 0.63, 0.65 and 0.72 are the major bands. It may be noted that in male and female major band no 9 and 8 have different rf value and rest have same value which extra and no 17 missing from male.

Fig 3: Salivary gland and haemolymph of both the sexes in 5th instars electropherogram reveals that there are 21 bands (rf, 0.19, 0.21, 0.29, 0.32, 0.36 0.41, 0.45, 0.50, 0.54, 0.60, 0.63, 0.69, 0.72, 0.74, 0.78, 0.81, 0.87, 0.90, 0.92 and 0.96) in male salivary gland, out of these four protein bands (rf 0.69, 0.74, 0.81 and 0.87) are major bands. The female gland shows a total of 24 protein bands having rf value as 0.19, 0.21, 0.29, 0.30, 0.34, 0.36, 0.38, 0.41, 0.42, 0.49, 0.52, 0.56, 0.60, 0.69, 0.72, 0.74, 0.76, 0.81, 0.85, 0.89, 0.90 and 0.96 respectively out of these band no. 16,18,19, 20 and 21 (rf value 0.69, 0.74,0. 76, 0.81 and 0.85) are the major bands. Out of these band there is one extra major band (19) in female and rest four are similar to that of male major bands. There are three extra bands in female salivary gland. The close examination of the electro programs of 4th and 5th instar reveals that 5th instar got more number of bands in both sexes.

SDS Page of 5th instar haemolymph result in 18 protein bands in male having rf value as 0.16, 0.20, 0.23, 0.25, 0.26, 0.28, 0.33, 0.36, 0.38, 0.40, 0.50, 0.63, 0.66, 0.71, 0.73, 0.80, 0.81 and 0.90 respectively. Four protein zones 7, 8, 12, 14 are the major bands. It may be noted that band is same as seen in 4th instar but the pattern in respect of mobility of major and minor band hare changed.

SDS-Page of haemolymph in 5th instar female shows 20 bands (rf 0.16, 0.18, 0.23, 0.25, 0.26, 0.28, 0.33, 0.35, 0.40, 0.43, 0.46, 0.51, 0.60, 0.63, 0.66, 0.70, 0.71, 0.76, 0.80 and 0.90 respectively. Out of these 5 bands are major bands (rf 0.28, 0.35, 0.60, 0.66 and 0.80) respectively. Rest 15 bands are minor bands. Further there in increase in total number of band in 5th instar in contrast to 4th in star and 3rd instar.

Fig 4: In adult salivary gland and haemolymph the gland in male reveals presence of total of 18 bands (rf 0.09, 0.27, 0.32, 0.35, 0.40, 0.46, 0.54, 0.59, 0.62, 0.66, 0.72, 0.77, 0.79, 0.80, 0.83, 0.88, 0.91 and 0.95) respectively, out of these two bands no. 12 and 16 (rf 0.77, 0.80) are major bands, and rest 16 protein bands are thin. It may be noted that there are protein zones are decreased from 21 (5th instar) to 18.

In female adult salivary gland shows the presence of total of 20 protein zone having (rf 0.09, 0.12, 0.18, 0.32, 0.35, 0.40, 0.45, 0.51, 0.54, 0.59, 0.61, 0.64, 0.67, 0.70, 0.72, 0.77, 0.82, 0.91 and 0.96 respectively. There are four major bands no. 14, 17, 18 and 19 (rf value 0.70, 0.82, 0.88, 0.91) and rest 16 are thin protein bands showing better sharpness that the male again observes a decreases in total band from 24 (in 5th instar) to20 in adult and also decrease in major bands. Haemolymph in adult presence of 16 bands in male and female respectively, in male 16 bands having rf value 0.12, 0.14, 0.17, 0.19, 0.22, 0.24, 0.29, 0.32, 0.40, 0.45, 0.51, 0.62, 0.67, 0.77, 0.80 and 0.91 respectively. Out of these 4 bands no. 7, 8, 11, 13 (rf value 0.29, 0.32, 0.51, 0.67) are major bands and the rest 12 protein zones are sharp and thin. In female rf value. 0.12, 0.14, 0.19, 0.20, 0.22, 0.24, 0.29, 0.32, 0.37, 0.43, 0.46, 0.53, 0.61, 0.69, 0.74, 0.77, 0.82 and 0.91 respectively here are 5 major bands no. 8,9,13,14 and 17 (rf 0.32,0.37,0.61,0.69 and 0.82) respectively. The extra band no. 9 and 12 could be the female specific protein.

It may be noted that there is a general decline in the number of protein bands in both the tissue when the 5th instars moults into adult. In case of haemolymph there is a loss of two bands each in male and female in the adult in both sexes. It is noted that the number of major bands remain constant in the 5th instars and adult. But in salivary gland there is no consistency in the number and pattern of bands in 5th instars and the adult.

Discussion

Results of the SDS-Page investigation revealed that the 5th instar is very crucial stage during larval-adult transformation. A lot of metabolic activities are recorded at this stage. Number of proteins in haemolymph and salivary gland

increased from 3rd instar to 5th instar larvae, and after that it decreased in the adult. Such qualitative changing profile of proteins observed during larval-adult transformation confirms that the same stage of the same insect have shown quantitative changes in protein (Venugopal *et al.* 1994, 1997) [7, 8].

Electropherograms revealed the presence of major haemolymph polypeptide bands. It was speculated that these bands in *Dysdercus Koenigii* might arise in last larval stages as true for hemimetabolous insect (Roberts and Brook, 1981). Nevertheless, the aforesaid proteins cannot be correlated with the larval protein of holometabolous insects as they are also detectable in the electropherograms of adult on the other hand, one can attribute a different function(s) of these proteins then the storage proteins. It is some new one appears, especially in the synthetic phase (Venugopal *et al*. 1994, 1997) [7, 8]. It is also observed that a few bands appears during consumption phase. This phenomenon of appearance and disappearance of protein in a phased manner implicate a genetic control mechanism. We strongly believe that at this development stage sexual difference is well established and hence the male and female haemolymph electropherograms must reveal the sexual dimorphism is being reflected the extra band could be specific protein because at this stage the sexes become organized into definite male and female organs. A close examination of the electropherograms of salivary gland and haemolymph reveal that there are many common bands in both organs. This reflects that from the early stage of development the gland sequester the protein from the haemolymph as was already demonstrated for the adult (Kumar, 1979) [8]. Such phenomenon is also known to be occurring in Drosophila (Pasteur and Kastritsis, 1971) [9] and in chironomus (Doyal and laufer, 1968) [10, 5]. A good understanding of the phenomenon occurring in individual organs or tissues of an organism can only be reached of these organs are considered not as independent entities, but rather as interdependent entities that could be affected by the function of each other. This necessitates the above comparison further so for the variation in the functions in form of protein quantity etc. of the salivary gland in various developmental stages reflect a precise developmental pattern, as it was observed for phynchoseiara (Winter *et al*. 1980) [11].

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