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METAMORPHIC CHANGES IN PROTEIN PROFILES AND PROTEASE ACTIVITY IN THE SILKWORM, *BOMBYX MORI* DURING PUPAL - ADULT TRANSFORMATION

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ABSTRACT

Metamorphic changes in protein profiles vis-a-vis protease activity have been studied in the fat body and haemolymph of the silkworm, *Bombyx mori* during pupal-adult transformation. The trends in these two biochemical constituents run counter parallel to each other and vary as a function of certain events such as the fat body remodeling, histolysis and histogenesis that characterize silkworm metamorphosis. Most of the proteins required for pupal-adult metamorphosis are either synthesized or accumulated in the fat body during the first half and they are shunted back to the needy tissues in the latter half of the pupal and adult stages. In this process, the haemolymph plays the role of transport medium for not only bringing tissue degraded proteins to the fat body, but also for taking away the newly synthesized or processed proteins to the growing tissues such as those in gonads and other organs. The trends in protease activity indicate that the silkworm fat body undergoes a dramatic remodeling process that involves an early phase of consolidation and a latter phase of disruption with regard to its biochemical composition. The findings were correlated with the reproductive role to be played by the silkworm in its adult life.

Keywords: *Bombyx mori, Fat body, Haemolymph, Proteins, Protease activity.*

Number of Tables: 3 **No. of Figures: 2** **Number of References: 36**

INTRODUCTION

Silkworm is a holometabolus insect and its metamorphosis involves histolysis, histogenesis and differentiation (Argell, 1953). These events are accompanied by physiologically conditioned metabolic events that are associated with the remodeling and disintegration of fat body and other tissues (Archana *et al.*, 2006). A recent report indicated the prevalence of a dynamic bio-chemical relationship between the disintegrating gut and haemolymph on one hand and between the haemolymph and fat body on the other (Sivaprasad and Sailaja, 2010). Such events, are obviously associated with the turnover of proteins, enzymes and many other biochemical constituents (Argell, 1953; Seong *et al.*, 2005; Yaginuma and Ushizima, 2005; Sarangi and Anitha, 2007). Most of the haemolymph proteins arise from the fat body at regular intervals during insect metamorphosis (Dean *et al.*, 1985 and Keely, 1985). They accumulate in the fat body in the form of dense granules during pupal stage but sharply decreased during the adult stage (Kim *et al.*, 2005). Storage proteins such as SP-1 and SP-2 are mobilized from haemolymph to fat body of insects during larval pupal transformation in a sex specific manner that differs in their levels in males and females (Tojo *et al.*, 1980).The metamorphic events of histolysis and histogenesis are accompanied by great turnover of proteins and assisted by a number of proteases during larval –pupal and pupal- adult transformations (Bharathi and Sucharitha, 2006; Wang *et al.*, 2008). Haemolymph proteins play a vital role in transport and metabolism in insect (Harliman and Chen, 1974). In this mechanism, the histolysis in one tissue is closely associated with histogenesis in another and that the products of histolysis are effectively used in histogenesis. Screening of available literature reveals that the biochemical correlation between histolytic and histogenetic tissues during insect metamorphosis has not been elucidated clearly. Hence, the present study attempts to examine such biochemical correlations among tissues in the metamorphosing silkworm *Bombyx mori* during pupal adult transformation by analyzing the levels of proteins and protease activity.

MATERIALS AND METHODS

Pure Mysore x CSR_2 hybrid strain of silkworm *Bombyx mori* was selected as test species for the present study. The silkworm larvae were reared under standard environmental conditions of 28^{o} C, 85% RH as per Krishnaswamy, 1986. After hatching the worms were fed with M_5 variety of mulberry leaves 5 times a day at 6 A.M, 10 A.M, 2 P.M, 6 P.M and 10 P.M throughout the larval period. The haemolymph was collected from silkworm by cutting their telson with fine scissors and the fat body was collected by mid–dorsal dissection of silkworm pupae in *Bombyx* Ringer (Yamaoka *et al.*, 1971). The protein levels (total, soluble, structural) were estimated by the method of Lowry *et al.*, (1951), in the 1% homogenate of fat body and 1:19 diluted haemolymph in distilled water. Protease activity was estimated by the method of Davis and Smith (1955) in 5% homogenate of fat body, 1:4 dilution of haemolymph.

Statistical analysis: The experimental data was statistically analyzed in terms of mean, standard deviation (SD), test of significance and per cent changes. While the mean and SD were computed using M.S Excel, the test of significance and percent changes were calculated online, using the Graph pad (www.graphpad.com/quick calcs/ index cfm/) and Percent Change (www.percent – change com/index php) softwares. In order to draw meaningful conclusions, the data were also interpreted in terms of an innovative parameter called compound periodical growth rate (CPGR), as given by Sivaprasad (2012).

RESULTS AND DISCUSSION

The biochemical changes in the silk worm, *Bombyx mori* during pupal adult metamorphosis, as reflected in the levels of total soluble and structural proteins and protease activity are presented in tables 1 to 3 and figures 1 and 2.

Changes in Protein profiles

Fat body: The insect fat body is a dynamic metabolic tissue analogous to the liver of higher vertebrates and performs multiple physiological functions in energy and intermediary metabolisms (Scott *et al*., 2004; Arrese and Soulages, 2010). In order to perform its functions the fat body in *B. mori* synthesizes and accumulates over 722 proteins, involved in larval growth and metamorphosis (Hou *et al*, 2007). As shown in Table 1, the total protein levels were elevated by ~76% on day 3 and by ~81% on day 5, and recorded a compound periodical growth rate (CPGR) of 15.99% during the first 5 days of the pupal development. But their levels started declining from day 7 onwards; showing ~37% decrease on day 7 and ~56% decrease on day 9, with a CPGR of 18.76%, during the last four days of the pupal development. On the other hand, when the pupa transformed to adult, the total protein levels showed contrasting trends in males and females. Their levels recorded a positive CPGR of 74.19% in males and a negative CPGR of 20.59% in females. By and large, the levels of soluble and structural proteins of fat body projected similar growth trends during pupal development. The soluble protein levels recorded an increase of ~76% on day 3 and ~70% on day 5 with a positive CPGR of 14.14% during the first half of pupal development. But their levels declined by ~52% on day 7 and ~67% on day 9, with a negative CPGR of 24.75% during the last 4days of pupal development. During pupal - adult transition, the soluble proteins showed a positive CPGR of 140.88% in males and a negative CPGR of 12.34% in the females (Table.1). Structural protein levels recorded an increase of ~78% on day 3 and ~103% on day 5 with a positive CPGR of 19.43% during the first half of pupal development. Subsequently in its second half their levels declined by ~ 13% on day 7 and ~37% on day 9, with a negative CPGR of 10.95% in four days. During pupal–adult transition their levels showed a positive CPGR of 17.09% in male flies and a negative CPGR of 48% in female flies (Table 1).

Clearly, the proteins levels follow a specific pattern of change in the fat body of *B. mori* during pupal – adult metamorphosis, as detailed below.An elevatory phase from day 1 of pupa through day 5, in which the total and structural proteins recorded an increase,

not withstanding a slight decline in the profiles of soluble proteins. A declining phase in all the types of proteins (total, soluble and structural) proteins from day 5 to day 9 of pupa.An elevation in the levels of all the three types of proteins in males and a decline in females during pupal – adult transition.

***Table 1**: Protein Profiles in the fat body of the silkworm, Bombyx mori, during pupal adult metamorphosis.*

Day	Statistical parameters	Total proteins	Soluble proteins	Structural proteins
Day 1	Mean S.D	39.89 ±0.59	26.65 ±0.69	13.25 ±0.43
Day 3	Mean P.C S.D CPGR	70.46 (+76.3) ±1.73* 32.9%	46.89 (+75.9) ±2.05* 32.65%	23.57 (+77.68) ±3.2* 142.46%
Day 5	Mean P.C S.D CPGR	72.19 (80.97) ±1.23** 15.99%	45.23 (69.72) ±1.84** 14.14%	26.96 (103.47) ±0.33* 19.43%
Day 7	Mean P.C S.D	45.14 (-37.47) ±2.01*	21.77 (-51.9) ±0.71*	23.25 (-13.39) ±1.59*
Day 9	Mean P.C S.D CPGR	31.46 (-56.42) ±0.82* -18.75%	14.5 (-67.96) ±1.54* -24.75%	16.96 (-37.09) ±1.66* -10.95%
Adult Male	Mean S.D CPGR	54.8 ±0.64* 74.19%	34.92 ±1.1* 140.83%	19.86 ±1.37* 17.1%
Adult Female	Mean S.D CPGR	24.98 ±0.97 -20.6%	16.29 ±0.7* 12.34%	8.7 ±0.2* -48.7%

*Statistically significant (P values: <0.001). **Statistically not significant.

Each value in the above table, expressed as mg protein / g wet weight of tissue, represents the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 larvae was pooled. The percent change (PC) for each day was calculated taking its previous value as the control. The compound periodical growth rates (CPGR) were computed for different periods on the basis of initial and final values, taken separately as day1-day 3, day1- day 5, day 5- day 9 and day 9 - adult stages respectively.

Table 2: *Protein Profiles in the haemolymph of the silkworm, Bombyx mori during pupal-adult metamorphosis.*

Day	Statistical parameters	Total proteins	Soluble proteins	Structural proteins
Day-1	Mean	16.59	12.12	5.46
	S.D	±0.49	±0.39	±0.89
Day-3	Mean	26.75	20.28	6.46
	P.C	(+61.24)	(+82.37)	(+18.31)
	S.D	±1.21*	±0.19*	±1.21**
	CPGR	26.98%	35.05%	8.77%
Day-5	Mean	16.18	12.39	3.78
	P.C	(-2.47)	(-11.42)	(-30.76)
	S.D	±0.33*	±0.81*	±0.0
Day-7	Mean	10.52	5.95	4.56
	P.C	(-34.98)	(-51.97)	(-20.63)
	S.D	±0.4*	±0.46*	±0.75**
Day-9	Mean	7.63	3.5	4.04
	P.C	(-52.89)	(-71.75)	(-6.88)
	S.D	±0.34*	±0.31*	±0.31**
	CPGR	-18.87%	-25.38%	-7.52%
Adult Male	Mean	15.96	7.3	8.63
	S.D	±0.97*	±0.90*	±1.3*
	CPGR	109.17%	108.57%	113.61%
Adult Female	Mean	4.51	1.9	2.59
	S.D	±0.48*	±0.31	±0.73*
	CPGR	-40.89%	-45.71%	-35.89%

*Statistically significant (P values: <0.001). **Statistically not significant.

Each value in the above table, expressed as mg protein / ml, represents the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 larvae was pooled. The percent change (PC) for each day was calculated taking its previous value as the control. The compound periodical growth rates (CPGR) were computed for different periods on the basis of initial and final values, taken separately as day1-day 3, day 3- day 9 and day 9 - adult stages respectively.

Table3. *Protease activity in the fat body and the haemolymph of the silkworm, Bombyx mori, during pupal-adult metamorphosis.*

Day	Statistical parameters	Fat body	Haemolymph
Day-1	Mean S.D	0.55 ±0.37	0.03 ±0.005
Day-3	Mean P.C S.D CPGR	0.105 (-80.0) ±0.02** -56.31%	0.01 (-66.7) ±0.004* -42.26%
Day-5	Mean P.C S.D	0.15 (+42.8) ±0.05**	0.02 (+100) ±0.0
Day-7	Mean P.C S.D CPGR	0.55 (+266.7) ±0.6* 51.28%	0.03 (+50) ±0.005* -
Day-9	Mean P.C S.D CPGR	0.35 (+36.4) ±0.04** -20.23%	0.03 (0.0) ±0.005 20.09%
Adult Male	Mean S.D CPGR	0.24 ±0.01 -31.43%	0.007 ±0.008 -76.67%
Adult Female	Mean S.D CPGR	0.65 ±0.03* 85.71%	0.06 0.004* 100%

*Statistically significant (P values: <0.001). **Statistically not significant.

Each value in the above table, expressed as μ moles of tyrosine/ mg protein/ hour, represents the mean ± standard deviation (SD) of four separate observations. For each observation tissue from 10 to 15 larvae was pooled. The percent change (PC) for each day was calculated taking its previous value as the control. The compound periodical growth rates (CPGR) were computed for different periods on the basis of initial and final values, taken separately as day1-day 3, day1- day 7, day 7- day 9 and day 9 - adult stages respectively.

Fig. 1: *Changes in protein profiles and protease activity in the Fat body of metamorphosing Silkworm Bombyx mori. The protein values expressed in mg proteins /g wet weight of tissue, and µ moles of tyrosine formed/ mg protein/ hr in case of protease activity.(P values: <0.001).*

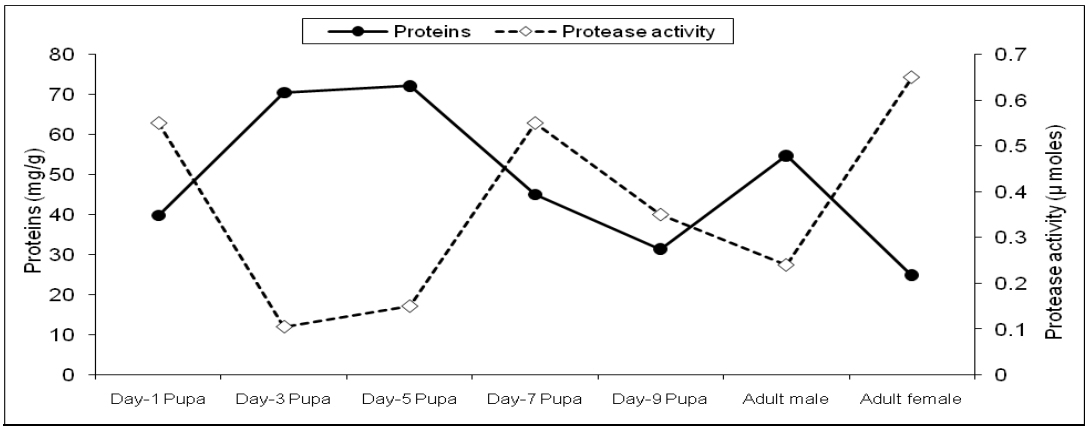
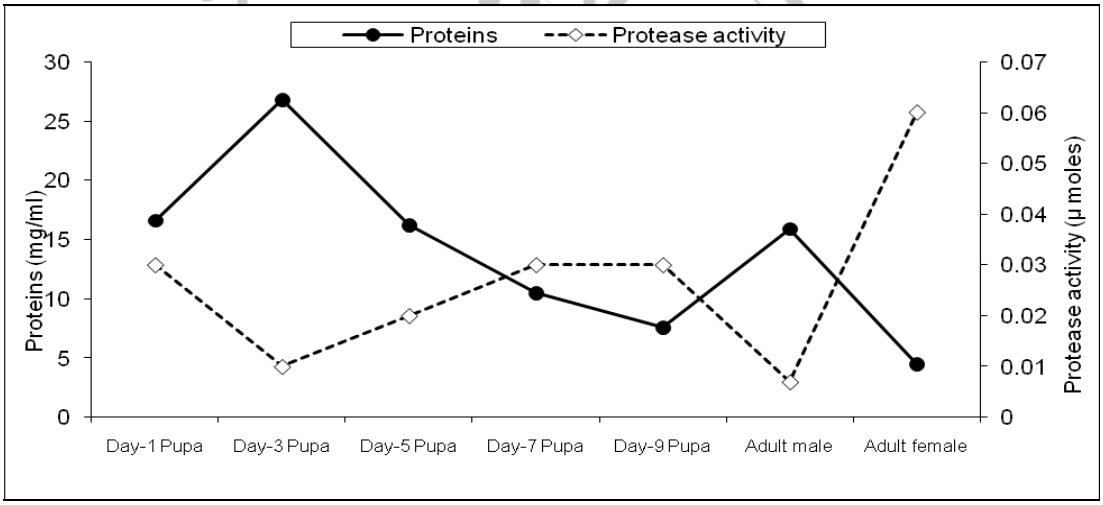


Fig. 2: *Changes in protein profiles and protease activity in the haemolymph of metamorphosing silkworm Bombyx mori. The protein values are expressed in mg proteins /ml of tissue, and µ moles of tyrosine formed/ mg protein/ hr in case of protease activity. (P values: <0.001).*



The fat body, being a major organ of metabolism in *B. mori*, is subjected to stresses and strains of metamorphosis. The metamorphic events exert pressure on the fat body and cause changes in its structure and biochemical composition. While, the structural changes involve, its remodeling through dissociation of fat body cells, (Archana *et al*., 2006), the biochemical manifestations are accompanied by heavy turnover of proteins, that involves their accumulation and mobilization depending on the stage of metamorphosis. Most probably, the protein accumulation is achieved, either by de novo synthesis or by uptake from other tissues and its mobilization by proteolysis or protein shift to other tissues (Haunerland *et al*., 1990; Hou *et al*.,2010; Pakkinathan *et al*.,2012).

Presumably, the protein accumulation in the fat body of *B. mori* is sustained during the first five days of pupal development, the rate of which varies significantly in respect of total, soluble and structural proteins. While, total proteins accumulate at a daily rate of about 16% (CPGR=15.99%), soluble and structural proteins accumulate at the rate of about 14% (CPGR: 14.14%) and 19% (CPGR: 19.43%) respectively (Table 1). Obviously, the higher accumulation rate of structural proteins in this tissue (CPGR: 19.43%) is indication of consolidation phase of proteins that characterize histogenesis in the fat body. At the same time the protein synthetic and uptake process proceeds slightly at lower rates as reflected in the levels of soluble proteins, (CPGR: 14.14%), a fact that supports the view that the protein synthesis and its uptake are limited to peripheral fat body cells (PPFs) and its storage or accumulation to peri-visceral fat body cells (PVFB) (Pakkianathan *et al*., 2012). Such regional disparity in protein profiles between peripheral and peri-visceral fat body cells is probably a metamorphic biochemical event that results from fat body remodeling in *B. mori*, a fact that requires further elucidation. The protein mobilization from fat body takes precedence in the latter half of the pupal development, and in most likeliness, it stems from increased disorganization of fat body in which some of its cells are either replaced or reshaped in the remodeling process (Haunerland, 1990). The rate of mobilization of total proteins during the latter half of the pupal development, appears to be faster (CPGR: 18.5%) than its rate of accumulation in its early stages (CPGR: 13.99%) of development (Table 1). Within the protein pool, the soluble fraction undergoes rapid mobilization (CPGR: 24.75%), compared to that of its structural counterpart (CPGR: 10.95%). Though the real cause of large-scale protein mobilization was not ascertained in the present study it may be attributed to the ongoing histolysis that characterizes the fat body remodeling. The histolysis is probably brought about by enhanced activity levels of protease, to be discussed later in this section. During the transformation process from pupa to adult, the fate of protein pool is different in males and females. While, the projected trends in protein profile indicate their secondary accumulation in the fat body of males, they tend to move away from the fat body in females. Obviously, the metamorphic event of pupal - adult transition stimulates protein accumulation in the fat body of pharate males and protein mobilization from it in their female counterparts. Interestingly the fat body cells show a tendency towards association in males and dissociation in females during its remodeling process, a suggestion that becomes a point for future investigations. Whatever be the fate of proteins in two sexes: the biochemical missionary ensures their complete utilization in all the metamorphic events culminating in successful reproduction. In the adult *B. mori* the accumulated fat body proteins are used up in energetically active processes such as vigorous wing- beating, a pre-mating courtship behavior and in copulation as reported earlier for other insects (Zara *et al*., 2003: Zara and Caetano 2004: Hou *et al*., 2010). Similarly in female, the low fat body protein profiles indicate that, most of the pupal proteins such as vitellogenin and asylophorin are mobilized to ovary as a source of key nutrients and used in egg development and

energy metabolism (Stopple *et al.*, 1981; Raikel and Lea , 1983; Haunerland *et al.*, 1990; Pascini *et al.*, 2011). The rate at which different proteins proliferate during the transition from pupa to adult stage assumes significance. In the male moth all the total protein levels were elevated significantly (CPGR: 74.19%), but there is a great upsurge in the levels of soluble proteins (CPGR: 140.83%), reflecting their role in energy production during pre mating and mating behavior of the male moth. On the other hand protein mobilization in the female moth appears to be different from that of the male. While all the three proteins (total, soluble and structural) recorded an increase in the male flies, only soluble proteins showed an increase of 12.34% in female flies, but the levels of total (CPGR: -20.6%) and structural (CPGR: -48.7%) proteins actually declined during the transition period (Table 1). Energetically, the female reproductive processes in *B.mori* appear to be more expensive than those of the male and that the egg production requires more proteins than the spermatogenesis in males. Nevertheless, the raise in the levels of soluble proteins in both males and females indicate that most of the energy needs of mating (copulation) are met from this pool in *B. mori*.

Haemolymph: In *B. mori* the haemolymph acts as a flowing reservoir for over 298 proteins that aid in heamocyte formation, ecdysis, eclosion, immunity, locomotion, cocoon spinning, reproduction , tissue degeneration and regeneration , heat shock control and in many more vital functions of metamorphosis (Li *et al.*, 2006: Kiuchi *et al*., 2008: Nakahara *et al.*, 2009 and Hou *et al*.,2010). Though there is a general agreement that most of these pupal proteins originate from the fat body (Keely 1985: Hou *et al*., 2007), they have their roots in the disintegrating tissues such as the gut, silk gland and muscle (Sivaprasad and Sailaja, 2010). Despite their multiple source of origin and disintegration haemolymph proteins of *B.mori* rise and fall in a stage – specific and time– specific manner during pupal–adult metamorphosis. Thus, the haemolymph proteins followed a phase of elevation from day 1 to day 3, a phase of retardation from day 3 to day 9 during pupal development. However, during pupal-adult transition, it recorded a secondary elevation in males, while continuously declined in females.

The total protein profiles of haemolymph recorded an elevatory trend from day 1 to day 3 and a declining trend thereafter during pupal development (Table 2, Fig 2). Their levels were elevated by ~ 61% on day 3, but declined marginally by 2% on day 5, moderately by 35% on day 7 and maximally by 53% on day 9. Their trends showed a positive CPGR of 26.98% during the first three days and negative CPGR of 18.87% during the last four days pupal development. During the transition period from pupa to adult, the total protein profiles of haemolymph recorded a positive CPGR 109% in the male moth and a negative CPGR of 41% in the female moth (Table 2). The growth trends in the profiles of soluble and structural proteins were similar to those of total proteins. The soluble protein levels recorded an increase of ~82% on day 3 with a positive CPGR of 35% during first three days of pupal development. However, they declined by ~11.42% on day 5 and ~52% on day 7 and ~72% on day 9, with a negative CPGR of 25.38% during the last 6 days. During

pupal adult transition the soluble proteins showed positive CPGR of 108% in the adult male and a negative CPGR of 46% in the adult female. The structural protein levels recorded an increase of ~18% on day 3 with a positive CPGR of 8.77% during first three days of pupal development. But thereafter their levels declined by ~ 31% on day 5 and 21% on day 7 and ~7% on day 9, showing a negative CPGR of 7.52% during the last 4 days of pupal development. During pupal –adult transition the soluble proteins showed positive CPGR of 113.6% in the adult male and negative CPGR of 35.89% in the adult female (Table 2). Compared to the levels of fat body proteins, which were elevated up to day 5 of pupal development, the haemolymph proteins rose only up to the day 3, showing a two – day difference in its protein turnover. Obviously a 2 –day early disappearance of haemolymph proteins, vis-a–vis the accumulation of fat body proteins up to day 5 of pupa, points to the fact that the haemolymph proteins are mobilized to the fat body during the first five days of pupal life. Probably this flow starts from the disintegrating tissues (larval gut, silk gland and muscle) and culminates in the fat body via the haemolymph. Given the fact that the fat body undergoes a consolidation phase in its remodeling process in the early pupa, it is presumed that the haemolymph proteins are most likely derived from the disintegrating gut and silk gland, during the first three days of pupal development and transported to the fat body. A high turnover of its soluble component in the haemolymph (CPGR: 35.05%) and a similar rise in the fat body (CPGR: 32.68%) during this period provides additional evidence in this regard.

Needless to say the haemolymph in *B.mori* acts as transient flowing reservoir of proteins during their mobilization from one tissue to the other during metamorphosis (Nagata and Kobayashi, 1990: Ravi Kumar and Sarangi, 2004). Further, the changes in the levels of haemolymph proteins were similar to those of fat body and substantiate their differential role in the male and female reproductive behaviors as discussed earlier.

Changes in protease activity

The protease activity projected interesting growth trends during pupal development and pupal-adult transition in both the fat body and haemolymph (Table 3).

Fat body: In the fat body the protease activity declined maximally by ~80% on day 3, representing a negative CPGR of 56.31% during the first three days of pupal life. Later from day 3 to day 5 (~43%) and from day 5 to day 7 (~267%) the enzyme activity registered increasing trends; While it was up by ~ 43% on day 5, but rose significantly by 267% on day 7. This represents a positive CPGR 51.28 during the mid pupal stage from day 3 to day 7 (4 days). But surprisingly the protease activity declined by ~36% and recorded a negative CPGR of 20.23 % during the last two days (day7 to day 9) of pupal development. Further during the pupal – adult transformation, the protease activity of fat body recorded opposing trends in males and females. It recorded a negative CPGR of 31.43% in male moths and a positive CPGR of 85.71% in female moths (Table: 3)

The trends in the activity levels of protease vis – a – vis those of proteins indicate that

the fat body remodeling involves both protein synthesis and proteolyses, a common feature of insect metamorphosis (Haunerland and Shrik, 1955: Sivaprasad and Sailaja, 2010). As shown earlier in this section, the protein biosynthesis and protein accumulation process are limited to first half of the pupal period, and those trends are associated with declining levels of protease activity, up to the third day of pupal development (Table 3: Fig.1). Thus, the early phase of pupal development involves consolidation of proteins and fat body cells, during which the protease activity was silenced at a rate of about 56% everyday, while protein levels were elevated by rates ranging from about 33% each in total and soluble proteins and maximally by about 142% in respect of structural proteins (Table 1). In the second half of the pupal stage, the decline in protein profiles was associated with raise in the protease activity, indicating the onset of proteolysis on day 5, which reaches its peak on day 7 by recording a nearly four-fold rise in its activity.

Obviously, the later pupal stage represents a disruptive phase in the fat body remodeling that involves protein degradation. However, the proteolytic activity subsided at the end of pupal stage by 20% on the day 9 of pupal stage (Table 3). Surprisingly along with protease activity the protein level also declined in the fat body, a fact that indicates that the protein disappearance from the fat body is caused more by its mobilization rather than degradation. The probable candidates that receive such proteins are the gonads, which reach functional maturity in the adult moth. Further the trends in the levels of proteins vis a–vis protease activity confirm that the metamorphic event of pupal – adult transition involves protein mobilization in males and protein degradation in females. The decline in the activity levels of adult males and their elevation in adult females bear strong testimony to this assumption. It is likely that the mobilized proteins cause the characteristic courtship and mating behavior in males, while the degraded proteins act as raw materials for the synthesis of egg proteins in the ovary. A thorough probe on these lines could provide vital biochemical clues on the reproductive behaviour of *B. mori*.

Haemolymph: Haemolymph protease activity recorded an initial declining trend from day 1 to day 3 followed by a continuous elevation throughout the pupal development (Table-3). The decrease in its activity was ~67% on day 3 and accounts for a negative CPGR of 42.26% during first three days of pupal development. Subsequent growth trends in its activity show 100% increase on day5, 50% on day 7 and day 9 with a positive CPGR of 20.09% during last 6 days of pupal development. On the other hand when the pupa transformed to adult, the protease activity showed contrasting trends in both the sexes of *Bombyx mori*, the enzyme recorded a negative CPGR of 76.67% in male and a positive CPGR of 100% in females. The trends in the protease activity levels of haemolymph, like those in the fat body were contrary to those of its own protein profiles. Only significant difference was that the enzyme activity of the fat body started declining from the day 5 during pupal life, while it declined from day 3 onwards, in case of haemolymph. Nevertheless, it reinforces the proteolytic activity initiated by the fat body on mutual exchange basis during pupal-adult

metamorphosis, thus reflecting the prevalence of a dynamic metabolic relationship that ensures mutual exchange of biochemical constituents between the two tissues, much like that of liver and plasma in vertebrates.

CONCLUSION

In *Bombyx mori*, the pupal and adult stages represent wonderful stages for the study of biochemical changes during insect metamorphosis. This is because, both pupal and adult stages represent non–feeding stages and that the nutrients required for metamorphic changes in form and function are to be supplied from within, rather than from outside. Obviously, the nutrients/metabolites or any biochemical constituents of one organ or tissue should be used in another organ or tissue. Clearly one organ or tissue grows at the expense of another organ. In other words, the construction (histolysis) in one part of the body is accompanied by destruction in the other part. The prolific changes in the profiles of proteins and protease activity in the two vital tissues (fat body and haemolymph) of the silkworm, *Bombyx mori* during pupal – adult transformation demonstrate that the metamorphic changes in this economically important insect are closely associated with biochemical changes at tissue level. Much like other insects, these changes are triggered by two metamorphic events namely, histogenesis and histolysis (Argell, 1953: Sivaprasad and Muralimohan, 1990; Bharathi and Miao, 2003; Sarangi and Anitha, 2007; Wang *et al*., 2008). In turn, histogenesis and histolysis are linked to tissue- specific metabolic events of proteins synthesis and proteolysis respectively (Sivaprasad and Sailaja 2010).

In the silkworm, histogenesis occurs during the development of gonads, antennae, compound eyes and wings while histolysis occurs during the disintegration of silk gland, gut and muscle (Sivaprasad and Sailaja, 2010). Needless to say two nutrients metabolites are mobilized from these disintegrating organs to gonads and other developing organs during pupal–adult metamorphosis. In this process haemolymph and fat body play vital, but functionally different roles. While, the haemolymph plays the role of a transient reservoir and transport medium, the fat body acts as an organ of metabolism like that of liver in vertebrates and either synthesizes organ–specific proteins or processes the proteins derived from the disintegrating tissues and pumps them to the needy tissues through the haemolymph. This, it does so, while itself undergoing a process of remodeling in a sex–specific manner. The findings of the present investigation on the profiles of proteins and protease activity (high proteins and low protease activity) denote that the fat body is subjected to a phase of metabolic consolidation during the first half of the pupal development and proceeds through a rigorous process of metabolic disruption as evidenced by the prevalence of low proteins and high protease activity during the latter part of pupal development. While, the disruptive process extends into adult females (with low proteins and high protease activity), the consolidation process is revived in adult males (with high proteins and low protease activity), probably in tune with the sex-dependent metabolic demands that ensures sex- different reproductive

behaviours in males and females. Though, the present study is not exhaustive enough to attribute any reason, nevertheless, it opens new vistas for further investigations in biochemical metamorphosis in insects.

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