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Weight and protein variations during the developmental stages of the red flour beetle *Tribolium castaneum*

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ABSTRACT

Tribolium castaneum, a red flour beetle is now-a-days used as a sophisticated model system for the development and evolutionary studies. As its mode of development is more insect typical than that of Drosophila, Tribolium has become a well-known subject for studies of the evolution of development while both are holometabolous. Insect life-histories show adaptations to withstand cold and dry conditions. The holometabolous group has distinct larval and pupal stages and undergoes some of the most complex transformations seen in animal kingdom.

Key words : *Tribolium castaneum*, weight, protein, variation

INTRODUCTION

Insects that encompass more than 70% of the entire animal kingdom are the most successful group of organisms living on earth. Insects are among the widely spread groups of animals on the planet, more than comprising of a million known species and including more than half of all known living organisms. Insects can able to exist in most of the environments, although very few species survive in the water, a group dominates habitually in the arthopods is the crustaceans (Chapman, 2006).

The holometabolous group has distinct larval and pupal stages and undergoes some of the most complex transformations seen in animal kingdom (Sehnal et al., 1996; Truman and Riddiford, 1999). The present study deals with this group of insect, the red flour beetle, *Tribolium castaneum* Herbst.

Before its rediscoverisation as a system in some biological phenomena such as emybronic development and others it was used by the geneticists over the decades. Tribolium widens its population with human agriculture as it is a major pest of stored grains and grain products

MATERIAL AND METHODS

Experimental insects:

Tribolium castaneum is commonly known as red flour beetle and belongs to the order coleopteran and family Tenebrionidae. It is a serious pest of bran, stored products flour, cereals and oil seeds in the tropical and subtropical regions of the world.

Collection of insects:

Insects used for this work were collected from the different stored product godowns in the Warangal district.

Stages of *Tribolium Castaneum* used for the study:

Stages of *Tribolium Castaneum* used were Early instar Larva (EL), Late instar Larva (LL), Pre-Pupa (PP), Male Pupa (MP), Female Pupa (FP), Male Adult (MA) and Female Adult (FA).

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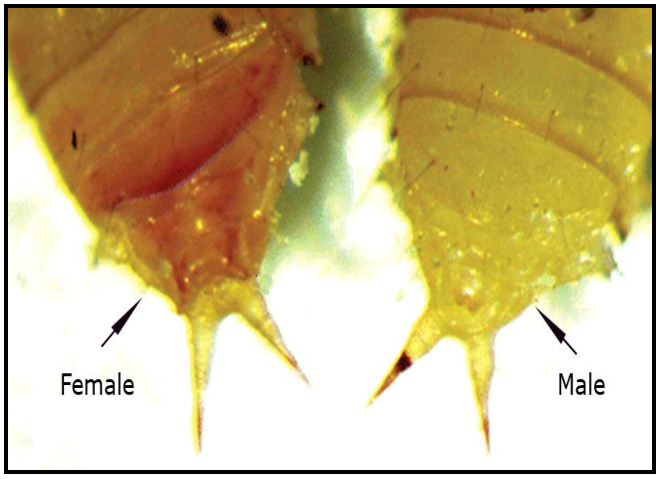
The larvae weighing 1.0 -1.5mg and small in size were categorized as early larvae (EL) and larvae weighing 3mg and large in size were categorized as late larvae (LL). Insects collected during non-feeding and wandering stage not as much as larvae were designated as prepupae (PP). Insects collected during immobile stage are recognized as pupae. During pupal stage insects were separated as male and female under microscope and reared in separate vessels. A few of the separated pupae were used for experiments. Separately reared pupae after becoming adults were collected and used for the study and some of the separated male and female adults were allowed to grow together in the different vessel to maintain culture.

Equal number of male and female adults which were separated at pupal stage and grown separately was introduced into the fresh vessel contained sufficient amounts of bran and allowed to breed under the above mentioned conditions. The larvae seem to be visible after 13±1 days. The larval development proceeds through five instars, divided depending upon the size and protein content and is completed in about 12-14 days from the day of appearance followed by the nonfeeding Pre-Pupal(PP) stage, a stage at which the larvae commits itself for metamorphosis to pupae. The Pre-Pupal stage extends over 1-2 days followed by the pupal stage which lasts for 4-5 days. Insects were separated as male female at pupal stage through microscope to study weight and protein contents of virgin insects.

Separation of male and female pupae:

To analyse genetically male and female need to be separated. sexing can be done at both the pupal and adult stages. if the study is on the virgin cross then is essential to sex the beetle at pupal stage to avoid the previous mating.. Sexing beetles at pupal stage is more easier than adult stage since pupae move very little compared to the adults, and do not need to be immobilized by cooling them on ice. sexing pupae can be done using a stereoscope.

Figure-.1 Separation of male and female *Tribolium* pupae.



The female pupae are distinguished from males by the presence of papillae, two finger-like structures, just anterior to the pointed urogomphi (paired "horns" at posterior tip of abdomen of larvae and pupae) which are much larger than those of the male (showed by arrow). The male papillae appear smaller and they just look like fingertips rather than fingers.

RESULTS

Variations in weight and quantification of protein during the developmental stages of Life cycle of the flour beetle *T. Castaneum*:

The red flour beetle, *Tribolium castaneum Herbst* is a tiny, commercially low-maintained beetle that has emerged as a new model system for the development and evolutionary studies. Its fast and vigorous growth providing and maintaining suitable laboratory conditions with a simple medium of wheat flour and yeast has become this species a popular choice as a model organism for studying the developmental genetics (Haas et al., 2001; Brown et al., 1994; Lorenzen et al., 2002; Sulston and Anderson, 1998; Shippy et al., 2000; Wade and Beeman, 1994).

Life cycle of *Tribolium Castaneum*:-

The details of the Tribolium castaneum life cycle are as follows:

Tribolium castaneum undergoes a complete metamorphosis which encompasses four stages of life cycle: egg, larva, pupa, and the adult (Fig-2 and 3). The eggs are colorless and microscopic.

Figure-2. Simplified representation of the life cycle, classification of different developmental stages of Red flour beetle, *Tribolium castaneum*. The fifth instar larval stage was predominantly used for most of the biochemical studies.

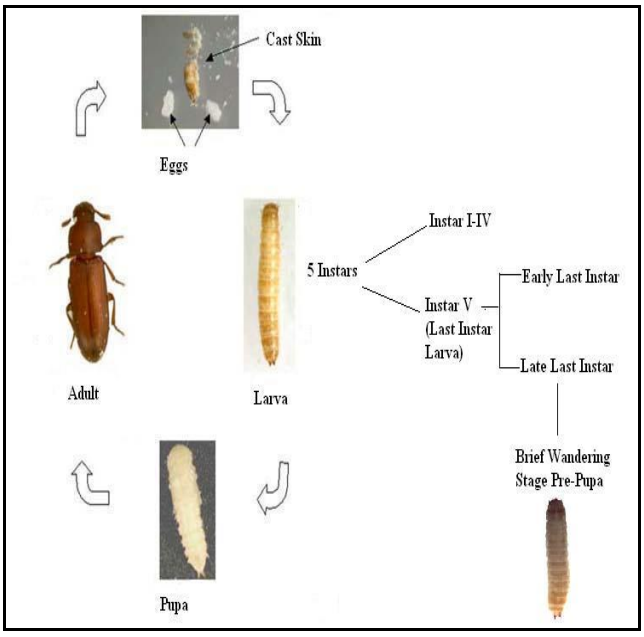
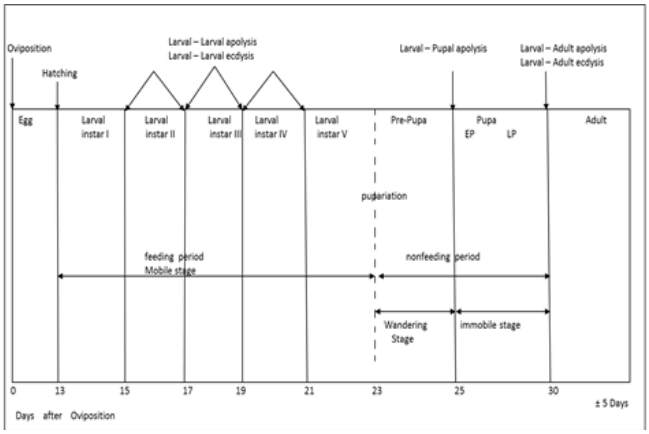


Figure-3. Schematic representation of the life cycle of red flour beetle, *Tribolium castaneum*



The egg's surface is sticky, they are mixed with the food particles and are invisible to the naked eye. Providing the optimal conditions after the male and female allowed to cross the larvae seems to appear within twelve to fourteen days (Table-1).

Table-1: Period of days, weight and Protein changes in all stages of *Tribolium castaneum*

	No. of days	Weight (mg) /Larva	Protein (µg) Content/ Larva
Emergence of larva after initiation of breeding	13±1	Mean ± SD	Mean±SD
Larva 1	2±0.5	0.128 ± 0.01	0.68±0.01
Larva 2	2±0.5	0.24 ± 0.02	1.27±0.03
Larva 3	2±0.5	0.598 ± 0.03	0.843 ± 0.02
Larva 4	2±0.5	1.279 ± 0.182	3.33±0.01
Larva 5	2±1	2.829 ± 0.04	6.25±0.02
Prepupa	<2	2.538 ± 0.07	5.26±0.02
Male Pupa	5±0.5	2.280 ± 0.37	6.25±0.09
Female Pupa	5±0.5	2.453 ± 0.25	7.27±.02
Male Adult		2.031 ± 0.04	7.27±0.01
Female Adult		2.028 ± 0.04	7.27±0.01

Column 1 represents the specific metamorphic stage
Column 2 represents the number/period of days that the specific stage exists
Column 3 represents the mean and (± SD) standard deviation values of weight at specific stages
Column 4 represents the mean and standard deviation values of protein at specific stages.

After hatching from each egg, a beetle larva emerges and appears to be peach or cream colour, slender and cylindrical having brown heads. Passing through the course of molting these insects generally grow by shedding its exoskeleton and emerging as a new one. During the larval stage, the beetle will undergo molting as many as 12 times rising up to a length of one-quarter inch. At this stage these larvae nourish hungrily and ultimately grow to the size of rice grain and lasts for a period over 15 to more days. Later a thin outer covering called pupal cuticle encloses the larva like a case. At this stage the beetle is inactive and will not eat as most of the energy is set into the process of metamorphosis. At this pupal stage, the beetle completely reorganizes itself to become an adult and this stage requires about five days. During this stage male and female beetles are separated easily and by placing separately breeding can be avoided to perform virgin cross tests. The new virgin adult seems flat, light brown and shiny with antennae and six legs after shedding its pupal case. The population of beetles, however, typically exhibits sustained oscillations.

Temperature also regulates the breeding rate and population size; in the present study at 32±2^{0}C the first instar larvae will appear within 13±1 days. As the temperature changes (increases or decreases) the breeding time increases to above 16±1days. The beetle appears to be in larval stage for about 10±3 days; exists in the prepupal stage <2days and pupal stage for 5±0.5 days (as shown in Table-1).

Weight variations:

The weight of larvae gradually increases from first instar larval stage (0.128 ± 0.01 mg per Larva) and reaches maximum at the fifth instar larval stage (2.829 ± 0.04 mg per Larva). The weight of the beetle appears to decline at prepupal stage to some extent (2.538 ± 0.07 mg per Larva) and remains constant as it grows to adult and throughout adulthood as shown in Table-1.

The weight variations at different stages of T. castaneum, male and female were shown graphically in Fig. 4 and 5 respectively. There was an exponential weight from first larval stage to adult stage.

Protein variations:

The assay of protein performed (Bradford method) showed variations in the protein content between different stages of *T. castaneum*. The total protein content appears to increase gradually from the first larval stage (0.68 ± 0.01 µg per Larva) to fifth larval stage (6.25 ± 0.02 µg per Larva), declines to 5.26 ± 0.02µg per pre-pupae in the pre-pupal stage and again gradually rises as the beetle grows to adult as shown in Table-1. The protein variations at different stages of T. castaneum, male and female were shown graphically in Fig 6 and 7 respectively.

Figure-4. Weight variations at the different developmental stages of *Tribolium castaneum* (Male)

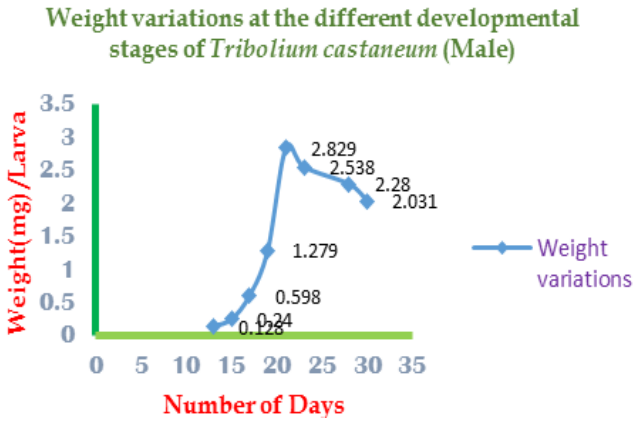


Figure-5. Weight variations at the different developmental stages of *Tribolium castaneum* (Female)

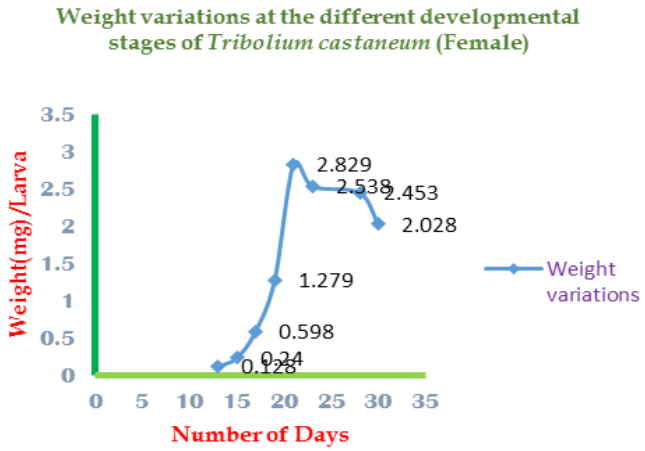


Figure-6. Protein variations at the different developmental stages of *Tribolium castaneum* (Male)

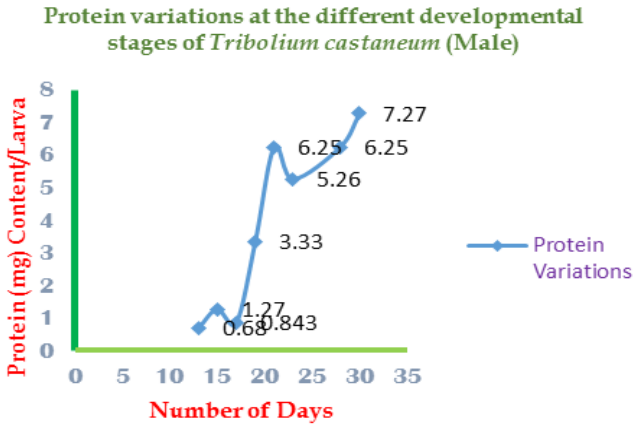
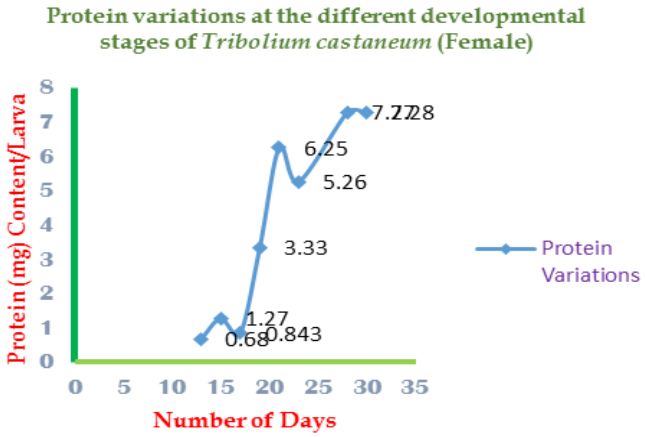


Figure-7. Protein variations at the different developmental stages of *Tribolium castaneum* (Female)



DISCUSSION

Various biochemical changes occur during the development, metamorphosis and reproduction of insects, (Lawrence, 2005). A wide range of biochemical variations are known to be found during the larval stages of development in the holometabolous insects including coleoptera (Majid Shafiei et al., 2001). It is well known that various hormones initiate the breakdown and reconstruction (turn over) of larval structures during metamorphosis; several mechanisms like autophagy, (Truman 1996, Lockshin and Beaulton 1974; Dean 1978; Sass and Kovacs 1975, 1977, 1980) and apoptosis are reported to be employed in the process (Abrams 1999). Steroid triggered metamorphosis by regulation of autophagy is well reported in Drosophila (Lee and Bachriecke 2001; Thumnel 2001). 20-hydroxyecdysone (20E) has been shown to elicit effects on autophagic process of the fat body by stimulating the activity of lysosomal enzymes, such as acid phosphatase (Verkuil 1979; 1980; Verkuil et al., 1979; Sass and Kovacs 1980; Ashok and Dutta-Gupta 1988; Sass et al., 1989).studies have also revealed that ecdysteriods stimulate the synthesis of various proteins in different tissues during the postembryonic development of lepidopteran insects (Ray etal., 1987a, b; Sridevi et al., 1988a, b, 1989; Ismail and Dutta- Gupta 1990a; Shanavas et al., 1996). Literature survey suggests that regulation of most of these actions by hormones is by modulation of transcription (Scheller and Karlson, 1977; Schenkel and Scheller 1986; Henrich et al., 1999; Riddiford etal., 2001). However, some of these actions have also been regulated at post-translational level (Verkull, 1979; Veno et al., 1983; Ueno and Natori 1984; Chunj et al., 1995; Tomaschko 1999; Burmester an Scheller 1997a, 1999).

CONCLUSION

The work presented in this paper catalogues the optimal conditions for rearing the red flour beetle as it a sophisticated model system for studying the evolution of development and also this paper presents the quantitative variations and comparison in weight and protein during the developmental stages of the beetle, *Tribolium castaneum* as they play a vital role in the metabolism.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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