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Research article

COMPLETE STUDY OF LIFE CYCLE OF TRIBOLIUM CASTANEUM AND ITS WEIGHT VARIATIONS IN THE DEVELOPING STAGES

Pranavi Sreeramoju[a*]. , Prasad M.S.K [a]. and Lakshmipathi . V [b]

[a]Department of Bio chemistry, Kakatiya University, Warangal – 506 009.
[b]Department of Zoology, Kakatiya University, Warangal – 506 009.

ABSTRACT: Tribolium has become a prominent subject for studies of the evolution of development (evo-devo) because of its mode of development is more insect typical than that of the classical system of Drosophila; 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, stage, variations.*

*Corresponding author: Pranavi Sreeramoju, [a]Department of Bio chemistry, Kakatiya University, Warangal – 506 009, India Email: pranisreeramoju@gmail.com

INTRODUCTION

Some temperate region insects are capable of activity during winter, while some others migrate to a warmer climate or go into a state of torpor [1]. Still other insects have evolved mechanisms of diapause that allow eggs or pupae to survive these conditions [2]. In the endopterygota there is a complete metamorphosis. In these insects the external (and internal) changes during the life history are the greatest. The eggs hatch into larvae which feed actively during the different instars. The larvae may or may not have legs. The development of wings is not visible during the larval stages. After several molts a pupa is formed. A pupa is an inactive stage, it does not feed and it does not move. Sometimes the pupa is protected by a cocoon of silk, or it is found in an earthen cell in the soil. During this pupal stage big changes take place internally. After the pupal stage, a highly active winged adult appears. Often, the larvae and the adults live in different types of habitat and use different types of food. The holometabolous group has distinct larval and pupal stages and undergoes some of the most complex transformations seen in animal kingdom [3,4]. The present study deals with this group of insect, the red flour beetle, Tribolium castaneum Herbst.

MATERIALS 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).

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 nonfeeding 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 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 or female at pupal stage through microscope to study weight contents of virgin insects.

Separation of male and female pupae

Separating the sexes is necessary in order to run a number of genetics tests. Both adults and pupae can be sexed. If the intended cross must be a virgin cross, it is necessary to sex the beetles as pupae to ensure no previous mating has taken place. Sexing beetles as pupae rather than as adults is easiest since pupae move very little compared to the adults, and do not need to be immobilized by cooling them on ice. A stereoscope is needed to sex the pupae. A small plate of a non-static generating material (approx. 3" x 4") is very handy for separating the sexes. A small natural bristle brush can be used to move the pupae on the "plate". During identification of sex, the two pointed structures at the very end of the pupa - which are the urogomphi, not the genital papillae should be ignored. The female papillae, which are much larger than those of the male, are two finger-like structures just anterior to the pointed urogomphi. The male papillae are enough smaller that they look like just fingertips rather than fingers as shown in Fig 1. Double check of each pupae should be done to verify its sex to get confirmation. Containers should be labeled and each sex was placed in separate containers with flour to allow them to enclose to adulthood. They can be used for crosses once the adults have darkened to brown. 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 are smaller and they look like just fingertips rather than fingers.

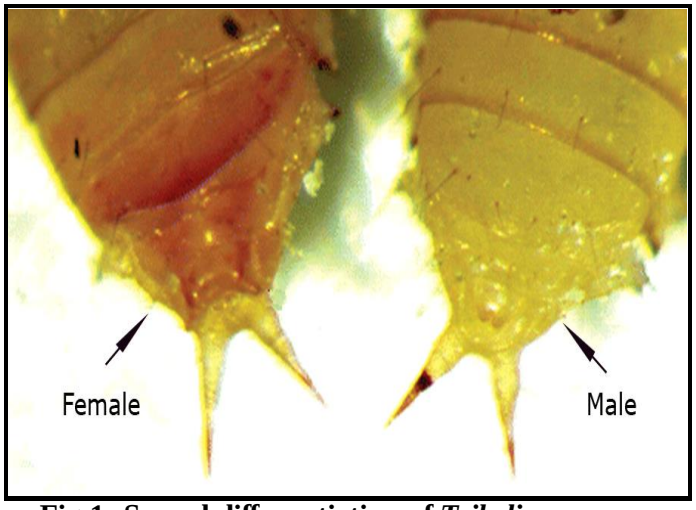

Fig 1: Sexual differentiation of *Tribolium* pupae.

RESULTS AND DISCUSSION

Tribolium castaneum is a small, low-maintenance beetle that has emerged as a sophisticated model system for studying the evolution of development. Its rapid life cycle and ease of maintaining laboratory cultures on a simple medium of wheat flour and yeast has made this species a popular choice as a model organism for studying the developmental genetics [5-10].

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. The egg's surface is sticky; dough and food particles can stick to its surface. The tiny eggs hatch in approximately four days under the laboratory conditions and cannot be seen by the unaided eye. After the female lays the eggs they will hatch within nine to twelve days (Table 1).

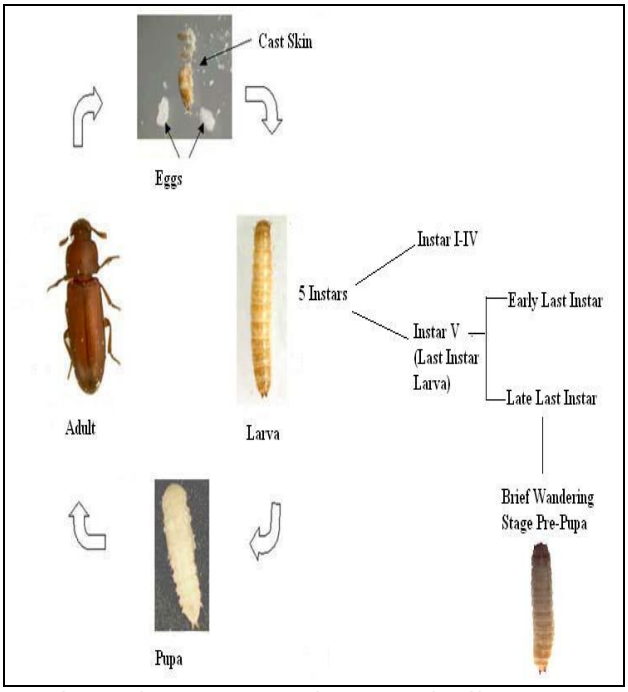

Fig 2: Simplified representation of the life cycle, classification of different developmental stages of Red flour beetle, Tribolium castaneum. The fifth instar larval stage was predominantly used for most of the biochemical studies.

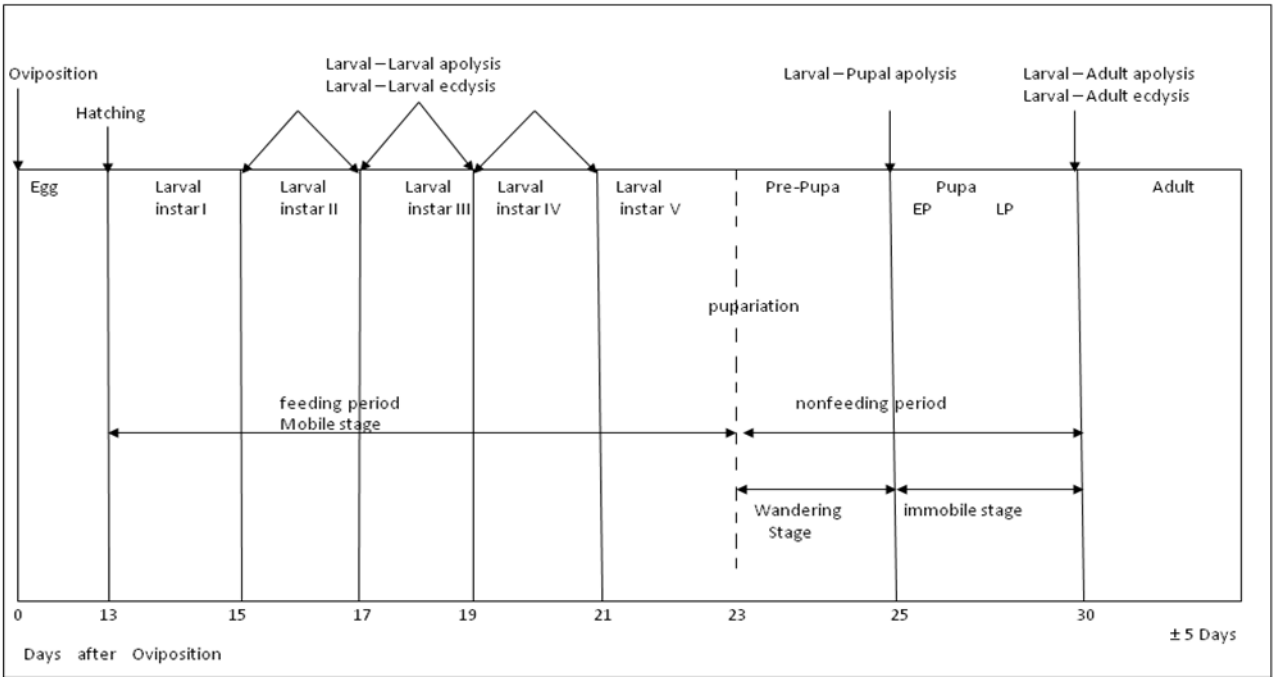

Fig 3: Schematic representation of the life cycle of red flour beetle, Tribolium castaneum

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

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

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

From each egg hatches a beetle larva; the larvae are slender, cylindrical and are a cream or yellow color with brown heads. Each larva has six legs with two forked projections at the rear. Insects generally grow through molting, a process where the insect sheds its exoskeleton and grows a new one. During this stage, the beetle will molt as many as 12 times growing up to a length of one-quarter inch. The larvae feed voraciously and eventually grow to the size of rice grains. The larval period lasts from 15 to more days. Each larva encloses itself in a pupal case which consists of a thin outer covering, the pupal cuticle. In this stage the beetle is dormant and not eating as all energy is put into the metamorphosis. The pupal stage lasts about five days. Sexing beetles as pupae is easier; the female papillae, which are much larger than those of the male, are two finger-like structures just anterior to the pointed urogomphi (paired "horns" at posterior tip of abdomen of larvae and pupae). The male papillae are smaller and they look like just fingertips rather than fingers as shown in Fig 1. During the pupal stage, the beetle completely reorganizes itself to become an adult. The new adult that emerges and sheds its pupal case is a flat, shiny, and brown with antennae and six legs; it emerges very hungry. An adult beetle can live up to three years and a female can lay 300 to 400 eggs in a lifetime. The population of beetles, however, typically exhibits sustained oscillations.

Effect of temperature

Temperature also regulates the breeding rate and population size; in the present study at 32 ± 2^0C 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.

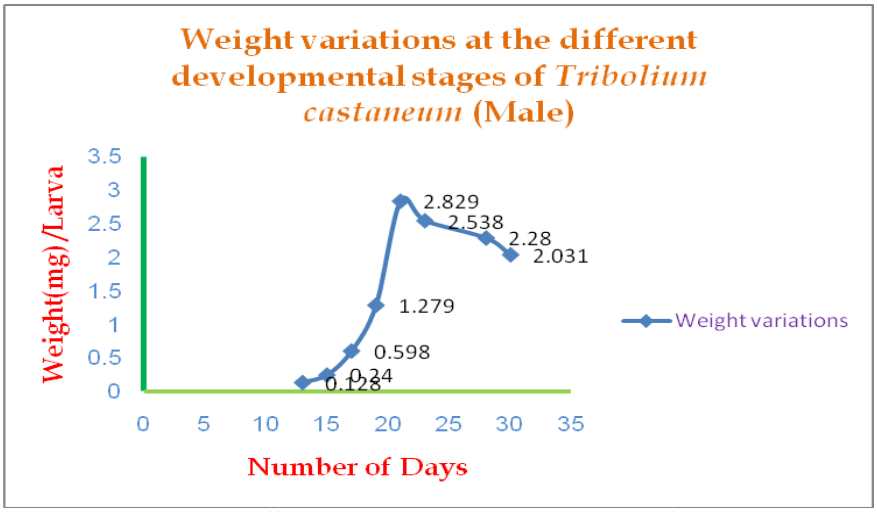

Fig 4: Weight variations at the different developmental stages of *Tribolium castaneum* (Male)

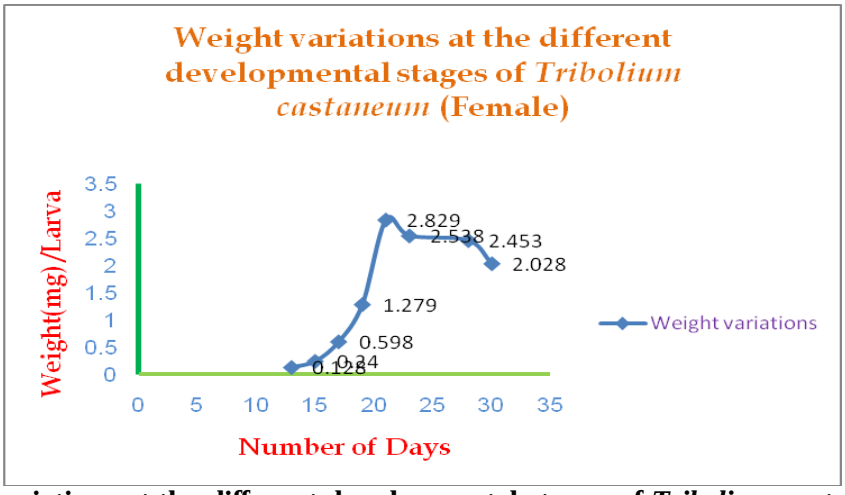

Fig 5: Weight variations at the different developmental stages of *Tribolium castaneum* (Female)

DISCUSSION

Although *Tribolium* and *Drosophila* are both holometabolous insects, they differ fundamentally in larval and adult morphology. Even generally conserved developmental features, such as body segmentation, are achieved by quite different means. Its rapid life cycle and ease of maintaining laboratory cultures on a simple medium of wheat flour and yeast has made this species a popular choice as a model organism for studying ecology [11, 12, 13, 14]. *Tribolium castaneum,* like other flour beetles undergoes a complete metamorphosis which encompasses four life cycle stages: egg, larva, pupa, and adult. The Tribolium egg is colorless and microscopic. Insects generally grow through molting, a process where the insect sheds its exoskeleton and grows a new one. During this stage the Tribolium beetle will molt as many as 12 times growing up to a length of one-quarter inch. The larvae feed voraciously and eventually grow to the size of rice grains. The populations of flour beetles typically exhibit sustained oscillations, which is attributed to the cannibalism prevalent in the species [15, 16, 17, 18]. In general, cannibalism is not a significant source of nutrition for these beetles and is not caused by limitations of food or space. Instead, the incidence of this behavior is believed to be under genetic control. Apparently, mobile cannibalistic animals tunnel through the flour and eat whatever they encounter, be it flour or immobile forms of their own species. Hence, the rate of cannibalism is inversely proportional to habitat volume. The speed of development and the developmental mortality of *Tribolium castaneum* were also reported to be influenced by temperature and humidity [19].

Various biochemical changes occur during the development, metamorphosis and reproduction of insects, [20]. A wide range of biochemical variations are known to be found during the larval stages of development in the holometabolous insects including coleoptera [21]. It is well known that various hormones initiate the breakdown and reconstruction (turn over) of larval structures during metamorphosis; several mechanisms like autophagy, [22, 23, 24, 25, 26, 27] and apoptosis are reported to be employed in the process [28].

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