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Developmental characteristics of *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) in different instars

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Abstract

Tenebrio molitor is a major pest of stored grain, although its larvae have potential for use as food. However, little is known about the characteristics of individual larval instars of this species, and the number of instars remains disputed. Therefore, we assessed *T. molitor* for the average number of instars and its characteristics at different larval stages. The focus of this study was to establish a foundation for further studies on the characteristics of each larval instar. All of the *T. molitor* larvae showed incubation periods of 7 to 8 d and a period of 3 to 4 d for the 1st instar. Beyond the 1st instar, there were relatively large variations in the number of days in each instar period. Before emergence, most of the larvae had typically gone through 15 to 17 instars. The highest rate of pupae formation, 28.32%, was observed in the 17th instars. The body length gradually increased with each successive instar, reaching its maximum at the 17th instar. Beyond the 17th instar, however, the body length decreased. The larvae were white in the first instar, and gradually turned brown after the 2nd instar.

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Introduction

Tenebrio molitor is a holometabolous insect that is considered to be a harmful pest of stored grain in South America (Schroeckenstein *et al*., 1990). However, its larvae, commonly referred to as mealworms, are used as pet food in many countries (Cotton, 1927). Because they are high in protein and fat and consume large amount of fiber, they represent a good food source for humans (Finke, 2002). Furthermore, they are high in oleic acid, which has the ability to decrease low-density lipoprotein (LDL) and increase high-density lipoprotein (HDL) levels in the blood (Yoo *et al*., 2013). In addition, they are inexpensive and easy to raise, with minimal harmful effects on the environment (Wang *et al*., 2012). For these reasons, many studies have been conducted on *T. molitor* to determine its potential uses.

The physiological features of *T. molitor* have been studied in some detail. Several studies on these aspects have found that the age of the parents influences the development of larvae; young parents are associated with the highest egg hatching rates (Ludwig and Fiore, 1960).

At the time of mating, the pheromone 4-methyl-1-nonanol is emitted by *T. molitor* females to attract males (Tanaka *et al*.,

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Table 1. Periods in each instar, number of pupae for each of the 14th to 21st instars, and body lengths of each larval stage in *Tenebrio molitor*.

larval period		number of pupae		body length
instar	mean (days)	number	mean (%)	(cm)
Incubation period	7.47±0.74	-	-	-
1	3.39±0.51	-	-	0.34±0.03
2	13.07±3.77	-	-	0.39±0.01
3	10.24±3.11	-	-	0.5±0.03
4	9.85±2.25	-	-	0.64±0.03
5	10.71±2.19	-	-	0.73±0.03
6	9.24±2.2	-	-	0.8±0.03
7	8.66±2.09	-	-	0.87±0.03
8	9.15±1.57	-	-	0.96±0.09
9	9.16±1.57	-	-	1.1±0.12
10	8.79±1.74	-	-	1.34±0.19
11	9.02±1.86	-	-	1.55±0.16
12	9.23±1.79	-	-	1.88±0.18
13	10.86±2.26	-	-	2.19±0.29
14	12±3.37	2±1.73	5.63±4.96	2.63±0.22
15	11.78±3.25	6.67±4.73	19.39±15.94	2.73±0.17
16	13.4±2.77	8.33±4.73	21.98±11.4	2.94±0.11
17	14.71±4.79	10.33±3.21	28.32±8.42	3.16±0.13
18	19.31±5.2	5.33±2.52	15.05±5.35	2.61±0.71
19	30.33±8.08	2.33±1.53	6.29±3.68	3.14±0.01
20	19±25.56	0.67±1.73	2.5±4.33	3.16±0

The values are indicated as mean±SD.

1986). This was confirmed by a study that indicated that males misperceived a glass rod that had been coated with 4-methyl-1-nonanol as a female, and consequently displayed mating behaviors (Hurd and Parry, 1991).

The optimal temperature for the reproduction of *T. molitor* is 25-27.5°C as shown by Hein and Tracey (Fiore, 1960), while the total developmental time is 80.0-83.7 d (Park *et al*., 2012). In addition, they rapidly hide in grain when they are exposed to light, because they are characteristically nocturnal (Balfour and Carmichael, 1928). A decrease in humidity was found to be inconsequential to adults, larvae, or pupae at a temperature of 25°C, but resulted in increased mortality at 10°C (Punzo and Mutchmor, 1980).

In the process of larval ecdysis, molting hormones play an important function to enable larvae to proceed to the next instar (Delbecque *et al*., 1978). Several studies have focused on changes in the number of instars in response to variation in oxygen density (Loudon, 1988), the change in the cuticle when *T. molitor* larvae molt into pupae and then adults, etc. (Delbecque *et al*., 1978). However, the characteristics of different larval stages remain unclear. In addition, the precise number of instars that *T. molitor* larvae go through at the optimal temperature of 25°C is debated. Therefore, this study was carried out to identify the characteristics of *T. molitor* larvae in different larval stages and to determine the average number of instars. The results of this study will help establish a foundation for investigating the various physiological characteristics of the different instars.

Materials & Methods

Breed condition

T. molitor adults (about 1,000) were raised in acrylic boxes (width: 48 cm, length: 49.5 cm, height: 10.5 cm) at 25°C. Bran was laid on the bottom of each box, and a cabbage leaf was placed atop the bran layer as a source of moisture. Once a mating couple was observed, they were moved to a petri dish (diameter: 10 cm, height: 1 cm) containing bran. Eggs with hardened shells were obtained after 3-4 d. Subsequently, 45 eggs were collected. To measure the number of larval stages, each egg was transferred to a petri dish (diameter: 5 cm, height: 1.5 cm) containing bran and cabbage (1 g). This experiment was repeated 3 times.

Measurement of body length of each instar

After hatching, larval exuviae were counted to determine the number of instars and subsequently removed from the petri dishes. When the larva pupated, instar counting was stopped.

Another set of experiments was performed to measure the body length of each instar. The body lengths of 10 larvae were measured using Vernier Caliper each time an exuvium was observed. Generally, the width of the head capsule was measured because it exhibits distinct variation between larval stages (Hsia and Kao, 1987). In this experiment, however, the body lengths of larvae were measured owing to the small head capsule size during the early larval stages. Finally, photographs of each instar were taken using a DSLR camera.

Results & Discussion

As shown in Table 1, the incubation period was 7-8 d and the duration of the 1st instar was 3-4 d, on average. Interestingly, the larvae were not significantly different in terms of the incubation period or the duration of the 1st instar. Between the 2nd and 20th instars, the duration of each instar was not uniform among larvae. Very few larvae were detected in the 19th -20th instar. The lack of uniformity among larvae between the 2nd and 20th instars may be attributed to malnutrition. An experiment on *Manduca sexta* by Nijhout (1975) revealed that the number of instars increased when the larvae had a poor nutritional status. However, it remains to be verified if this phenomenon occurs in *T. molitor*. Furthermore, pathogens may also cause *T. molitor* larvae to exhibit symptoms of malnutrition. It was also revealed that a gregarious nature enhanced the ability of *T. molitor* to resist pathogens (Barnes and Siva-Jothy MT, 2000). Therefore, further studies need to be conducted to investigate whether the duration of each instar is influenced by poor nutritional status, pathogen activity, or larval behavior.

Pupation occurred after the 14th instar. Approximately 69.69% of total pupation was observed between the 15th and 17th instars. The largest proportion of pupae (28.32%) was observed in the 17th instar. In other words, most of the larvae used in this experiment exhibited 15 to 17 instars in their life cycle. Before the 19th instar, most of larvae pupated. Based on a study by Ludwig (1956), the average number of instars 25°C ranged from 11 to 15 (Fiore, 1960), which is smaller than what we found. This discrepancy may be explained by many factors, such as the nutritional status of the larvae and parents (Ludwig and Fiore, 1960). Repeating this study over a wider range of temperature may explain how ambient temperature influences the number of larval stages.

The body length of *T. molitor* instars increased gradually, reaching a maximum in the 17th instar, and decreasing thereafter. This is because larvae with more than 17 instars were underdeveloped and therefore shorter than those in the 17th instar. In addition, several pupae and dead larvae were detected between the 18th and 20th instars. Moreover, only 1 larva was observed in the 20th instar. Owing to the massive occurrence of either pupation or mortality between the 17th and 20th instars, there was an insufficient number of larvae to accurately assess body length after the 17th instar. Therefore, any study investigating this aspect must use a larger population of larvae.

Although the 1st instar was white, the larvae gradually turned brown from and after the 2nd instar. Except for the change in color, no significant differences in the morphological characteristics of the larvae were detected (Fig. 1)

Through this experiment, we confirmed the incubation period, the duration of the 1st instar, and the average number of instars in *T. molitor*. These results may be useful as preliminary data for further studies on the characteristics of each instar period.

Fig. 1. Photographs of each larval instar. Photographs were taken using a DSLR camera after the exuvium was observed.

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