·论　著·

Development of *Dermestes Maculatus* at a Constant Temperature and Its Larval Instar Determination

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Abstract: **Objective** To establish the basic data for estimating minimum postmortem interval（PMI_{min}）of heavily decayed and skeletonized remains by studying the development of *Dermestes maculatus* DeGeer（Coleoptera: Dermestidae）. **Methods** The developmental stages of *Dermestes maculatus* were observed at four constant temperatures of 20 ℃, 24 ℃, 28 ℃ and 32 ℃, and the changes in body length were also examined as the biological indicator to estimate larval day-age and instar. **Results** The total developmental time from egg to adult at 20 ℃, 24 ℃, 28 ℃ and 32 ℃ were（126.7±10.6）d,（69.4±8.2）d,（50.4±8.4）d and（49.6±6.5）d, respectively. The body length increased gradually, but changed irregularly as a whole. **Conclusion** The study provides basic data on the development and growth of *Dermestes maculatus*, especially on its developmental duration as a significant value for estimating PMI_{min} of heavily decayed and skeletonized remains. Nevertheless, the change of body length is not found to be the best biological indicator for instar determination.

Keywords: forensic entomology; skeletonized remains; minimum postmortem interval; instar determination; *Dermestes maculatus*

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恒温下白腹皮蠹的生长发育规律及幼虫的龄期推断

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摘　要：**目的** 研究白腹皮蠹(双翅目:皮蠹科)的生长发育规律,为严重腐败或白骨化尸体最短死亡时间(minimum postmortem interval，PMI_{min})的推断提供基本数据。 **方法** 在20 ℃、24 ℃、28 ℃和32 ℃ 4个恒温条件下,研究白腹皮蠹的发育历期,并统计体长的变化,作为用于推断其幼虫日龄和龄期的生物学指标。 **结果** 20 ℃、24 ℃、28 ℃ 和32 ℃ 4个恒温条件下白腹皮蠹的发育历期分别为(126.7±10.6)、(69.4±8.2)、(50.4±8.4)和(49.6±6.5) d,体长开始逐渐增长但整体呈非规律性变化。 **结论** 本研究提供了白腹皮蠹发育规律的基础数据,发育历期对推断严重腐败或白骨化尸体的最短死亡时间具有重要意义,体长变化规律并非白腹皮蠹龄期推断的最佳生物学指标。

关键词：法医昆虫学;白骨化尸体;最短死亡时间;龄期判定;白腹皮蠹

Introduction

Dermestes maculatus DeGeer（Coleoptera, Dermestidae; herein after referred to as *D. maculatus*）, also known as the hide beetle, is a carrion beetle and cosmopolitan pest that damages stored products, as they prefer dry and protein-rich organic matter[1-4]. It occasionally acts as intermediate hosts of parasites or as vectors of disease, causing allergic reactions[5], and even attacks and eats live turkeys[6]. De-

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spite its causing harms, *D. maculatus* is the species typically used by universities and museums to remove flesh from bone in skeleton preparation[7-9]. *D. maculatus* is also a forensically important species to be found in insect succession studies and case reports[10-15], often found during the dry stage of decomposition where large numbers of larvae and adults develop when the corpse has only skin, hair and bone left[15-17]. Though the most common application of forensic entomology is based on the development of the earliest arriving species of blowflies (family: Calliphoridae), the minimum postmortem interval (PMI_{min}) estimation is less inaccurate once blowfly adults emerge and leave the corpse. Therefore, the development of this species is of great importance to the estimation of PMI_{min} of skeletonized and mummified remains having a long PMI.

The larval instar of Diptera can be easily determined by checking the number of openings in the posterior spiracles, and PMI_{min} can be accurately estimated for the species with larval instar or body lengths measurements[18]. However, most forensically significant beetle larvae lack morphological features which are specific to developmental stages or particular instars, making the larval instar determination of Coleoptera, including *D. maculatus*, difficult to differentiate[19]. Resent studies on *D. maculatus* mainly center on biological characteristics, such as behavior[20], reproduction[21-23], and effect of diet and refugia on development[24]. Though the developmental stages of *D. maculatus* have been well studied[5, 25-28], developmental data are needed to accurately determine the larval stages, such as instar classifiers and changes in larval body length.

In this study, we observed the developmental stages of *D. maculatus* at four constant temperatures of 20 ℃, 24 ℃, 28 ℃ and 32 ℃, and also measured the changes of body lengths. Our research aimed to investigate the developmental duration of *D. maculatus*, examining whether the body length could be used to determine day-age or instar for PMI_{min} estimation of corpses in the late stages of decomposition.

Materials and methods

Colony establishment

Forty-five *D. maculatus* adults were collected from pig carcasses in a field-based insect succession study in Panyu, Guangzhou, China (22°45' N, 113°14' E) during late spring of 2010. The adults were placed in a rearing box (323 mm×220 mm×100 mm) with a window (30 mm×50 mm) made of nylon mesh in the center of the lid for ventilation. A Zeiss stemi 2000-C stereo stereomicroscope (Carl Zeiss AG, Germany) was used to visually verify species characteristics based on the identification keys of ZHANG *et al*.[29] The collected insects were all identified as *D. maculatus* DeGeer. The pork sausage, prepared using pure pork from the market, were sliced 6 cm in diameter and 2 mm thick to be placed in the rearing box for the adults and oviposition media. Before feeding, the pork sausage was exposed under the sun, with the additional grease wiped off to avoid death of younger larvae from being glued and restrained by the grease. The eggs oviposited on the sausage were picked out carefully using a soft brush before placed into a new rearing box. When larvae hatched, sufficient food was provided until pupation. Newly emerged adults were removed to be placed in new insect-rearing boxes to protect the developing pupae. The entire process was repeated to establish a colony containing over 100 adults.

Animal care approval was received from the Ethics Committee of Soochow University (ECSU-20190000109).

Investigation of developmental stages

The eggs laid within 2 hours (n=1-57) were picked out and placed into a culture dish containing food. The dish was placed into a rearing box, and then cultured in a microenvironment incubator at 4 constant temperatures of 20 ℃, 24 ℃, 28 ℃ and 32 ℃, with 50%-60% humidity and a light-dark cycle of 12 h light and 12 h dark. A piece of thick black paper was placed on the top of the box, creating a comparatively dark environment for the beetles. The eggs were observed every 2 hours, and the larvae and pupae, twice a day. The pork sausage slices were replenished as needed. The time points of hatching, ecdysis, pupation, and adult emergence were recorded during the experiment. The ecdysis was determined by checking the number of exuviate in the rearing boxes. Each experiment was repeated 4, 5, 3 and 4 times under 20 ℃, 24 ℃, 28 ℃ and 32 ℃, respectively. Two different incubators LHP-300H (Yingmin Co. Ltd, Suzhou, China) were applied to each experimental temperature.

Larval sampling and morphometric indicator measurement

The eggs of *D. maculatus* were collected and reared as above. After hatching, 2-3 larvae were sampled daily until pupation, and larval instar was recorded. If there were insufficient number of larvae for sampling prior to pupation, multiple clones were combined for sampling. Sampled larvae were preserved in 75% alcohol.

Larval body length was measured using a digital vernier caliper (Sangon Biotech, Shanghai, Co., Ltd.) at a precision of 0.01 mm (Fig. 1).

Fig. 1　The criteria of the body length measurement

Statistical analysis

The duration of various developmental stages was analyzed by one-way analysis of variance and least significant difference (LSD) test using SPSS 14.0 (IBM, USA), and α=0.05 was taken as the standard of test.

Results

Developmental duration of *D. maculatus*

The developmental rate of *D. maculatus* increased at higher temperatures. The overall developmental duration decreased from (126.7±10.6) d at 20 ℃ to (49.6±6.5) d at 32 ℃; there was a significant effect of temperature on the developmental rate ($P<0.05$), as indicated by the results of the multiple comparisons in Tab. 1. However, no significant difference was observed between 28 ℃ and 32 ℃ on the day of adult emergence according to the LSD test ($P=0.871$). The egg stage was found to be the shortest stage, lasting 6.55%-7.54% of the total duration of development, while the feeding larval stage, the longest which comprised 45.97%-53.12% of the total duration of development (Tab. 2).

Intriguingly, the number of ecdysis was different even under the same temperature, with 6-9th, 5-7th, 6-7th and 7-8th ecdysis observed under 20 ℃, 24 ℃, 28 ℃ and 32 ℃, respectively.

Tab. 1　Duration for each developmental event of *D. maculatus* at 4 constant temperatures

($\bar{x}\pm s$, d)

Developmental event	20 ℃		24 ℃		28 ℃		32 ℃	
	n	Duration	*n*	Duration	*n*	Duration	*n*	Duration
Egg hatching	30	8.3±0.8	72	5.1±3.5	35	3.8±0.2	28	3.5±0.8
1st ecdysis	27	15.6±1.6	68	9.1±2.8	30	7.6±0.8	25	6.6±0.9
2nd ecdysis	25	22.7±1.2	64	13.0±2.6	26	11.2±0.5	17	10.1±2.1
3rd ecdysis	25	30.0±1.3	62	17.3±3.6	24	14.7±2.7	15	12.9±2.1
4th ecdysis	22	37.7±1.6	56	21.9±2.9	20	17.2±2.8	13	15.7±2.9
5th ecdysis	20	45.4±2.1	50	26.5±3.2	18	20.0±2.8	12	19.1±3.6
6th ecdysis	13	54.8±2.1	15	32.0±1.2	9	24.2±1.3	6	22.8±4.0
7th ecdysis	8	62.1±0.7	–	–	–	–	2	25.6±5.4
8th ecdysis	1	69.6±0.0	–	–	–	–	–	–
Post-feeding	13	72.1±4.0	37	36.7±3.0	16	28.2±4.6	6	27.7±5.9
Pupation	11	113.2±2.8	35	57.8±4.1	15	43.7±5.1	5	43.9±2.0
Adult emergence	10	126.7±10.6	32	69.4±8.2[1)]	15	50.4±8.4[1)2)]	5	49.6±6.5[1)2)]

Note: The initial numbers of eggs, observed at 20 ℃, 24 ℃, 28 ℃ and 32 ℃, were 71, 152, 87 and 50, respectively. '–', no data. 1) Compared to adult emergence day under 20 ℃ with LSD test, $P<0.05$; 2) Compared to adult emergence day under 24 ℃ with LSD test, $P<0.05$.

Tab. 2　Duration of each developmental stage as a percentage of the total time from oviposition to adult emergence in *D. maculatus* at 4 constant temperatures

(%)

Development stage	20 ℃	24 ℃	28 ℃	32 ℃
Oviposition -- Egg hatching	6.55	7.35	7.54	7.06
Egg hatching -- Post-feeding	53.12	47.84	46.63	45.97
Post-feeding -- Pupation	29.68	28.10	32.54	35.48
Pupation -- Adult emergence	10.65	16.71	13.29	11.49

The data of body length change

The longest body length at 20 ℃, 24 ℃, 28 ℃ and 32 ℃ was (13.08±0.37), (14.18±0.02), (13.69±0.62) and (12.28±0.23) mm, respectively. The overall trend of the larval body length before reaching the post-feeding larval stage produced a gradual increase, but changed irregularly. After entering into the post-feeding stage, the change of body length fluctuated without any specific pattern (Tab. 1 in appendix).

Discussion

Our findings, which corroborated the previously published results[5, 25-28], showed that the overall developmental stages of *D. maculatus* from egg to adult were longer than those of carrion flies, which are often used to estimate PMI_{min}. The larval stage constitutes over 70% of the *D. maculatus* immature stages[5], and the precise instar determination of larval *D. maculatus* is valuable for estimating PMI_{min} of remains with longer PMI.

In forensic entomology, different measuring methods are usually used to evaluate the changes in larval body length. As to the reliability of this practice, BUGELLI *et al*.[30] conducted a comparative study to examine the influence of different measuring methods on the accuracy of larval age estimation, the results showing that there were no significant differences in the measurement of larval body length; this supports the view that a simple tool like a geometrical micrometer can produce reliable results in forensic entomology. In the current study, we used a more reliable digital vernier caliper at a precision of 0.01mm to measure the larval body length of *D. maculatus*, which consequently confirmed the reliability of our data. The body length began to increase gradually, but as a whole changed in an irregular way. Thus the change of body length cannot be the useful biological indicator for instar determination, as in the case of the species of *Oxelytrum discicolle* (Brullé)[31], *Necrodes surinamensis* (Fabricius), *Oiceoptoma inaequale* (Fabricius)[32]. Moreover, FRĄTCZAK *et al*.[19] found that the most useful features for instar determination of *Necrodes littoralis* and *Creophilus maxillosus* did not include body length.

The length of development in *D. maculatus* from egg to adult was longer in our study than in other previously reported ones[5, 22-24], which may have been caused by the absence of suitable pupation sites for the post-feeding larvae, such as corks. *D. maculatus* post-feeding larvae may have had a significantly longer post-feeding period and longer developmental stage while searching for a pupation site. MARTÍN-VEGA *et al*.[5] showed that the post-feeding larval stage of *D. maculatus* comprised 10% of total development time. In contrast, we found that the post-feeding stage represented approximately 30% of total development time. Our findings, however, were similar to those which had been reported by the study[5] on all other developmental stages.

Not in the case of other sarcosaphagous beetles larvae, such as rove beetles (Staphylinidae) and carrion beetles (Silphidae), we found that the ecdysis frequency of *D. maculatus* larvae was inconsistent (over 6 times) and varied significantly; this variation in instars was consistent with the results from ZANETTI *et al*.[25] and MARTÍN-VEGA *et al*.[5] Previous studies have found that the frequency of ecdysis could be influenced by temperature, humidity, light and food[33]. However, ecdysis frequency can still differ among different individuals, even under rigorously controlled conditions, which suggests that ecdysis frequency could be genetically determined[25]. Future studies are needed to focus on larvae separation by groups at a high and low ecdysis frequency to conduce a genomic scan for genes that may underlie this trait, which would further clarify whether genes frequently regulate ecdysis.

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Appendix Tab. 1　Body length of *D. maculatus* at 4 constant temperatures　($\bar{x}\pm s$, mm)

Day	Body length							
	n	20 ℃	*n*	24 ℃	*n*	28 ℃	*n*	32 ℃
1	6	2.56±0.52	6	2.23±0.32	9	2.51±0.58	9	2.62±0.96
2	6	2.45±0.30	6	2.77±0.23	9	2.56±0.25	9	2.00±0.01
3	6	2.69±0.11	6	2.69±0.09	9	3.52±0.29	9	2.99±0.02
4	6	2.78±0.64	6	3.12±0.23	9	3.10±1.15	9	2.31±1.00
5	6	2.95±0.74	6	2.83±0.40	9	4.13±0.06	9	3.80±0.23
6	6	3.01±0.47	6	3.10±0.55	9	4.02±0.04	9	3.32±0.48
7	6	3.46±0.06	6	3.93±0.03	9	6.08±0.04	9	3.07±0.18
8	6	2.93±0.34	6	5.04±1.00	9	6.49±0.42	9	3.54±0.24
9	6	3.34±0.33	6	5.50±0.37	9	7.55±1.32	9	5.15±0.19
10	6	3.11±0.04	6	6.11±0.51	9	7.50±0.54	9	5.09±0.31
11	6	4.09±0.62	6	6.02±0.35	9	7.07±0.54	9	9.10±0.24
12	6	4.31±0.13	6	5.32±0.75	9	7.28±1.15	9	9.30±1.70
13	6	4.36±0.11	6	6.67±0.16	9	8.09±0.71	9	6.48±0.71
14	6	3.03±0.09	6	5.68±0.86	9	9.17±0.04	9	10.48±1.80
15	6	4.21±0.41	6	4.52±1.39	9	8.07±0.10	9	10.38±0.41
16	6	4.31±0.06	6	10.19±0.56	9	8.08±1.28	9	9.38±0.65
17	6	4.58±0.11	6	8.35±0.04	9	7.80±1.24	9	10.74±0.64
18	6	3.78±0.43	6	12.28±1.07	9	5.42±0.99	9	9.62±0.53
19	6	4.19±0.02	6	11.62±1.03	9	7.01±0.04	9	8.91±0.93
20	6	3.58±0.08	6	7.70±1.33	9	13.68±0.48	9	12.28±0.23
21	6	4.75±0.57	6	5.54±0.16	9	13.45±0.17	9	10.58±1.23
22	6	5.61±0.66	6	9.02±0.37	9	13.01±0.65	9	10.75±0.49
23	6	6.86±1.26	6	8.00±1.61	9	13.08±0.47	9	10.65±1.27
24	6	5.61±0.09	6	7.93±2.43	9	13.69±0.62	9	11.46±0.05
25	6	6.13±0.51	6	9.09±0.04	9	11.56±0.44	9	10.68±0.23
26	6	6.38±0.54	6	11.46±0.11	9	11.22±1.10	9	10.32±1.07
27	6	6.57±0.40	6	10.38±0.39	9	11.05±0.86	9	9.22±0.80
28	6	5.59±0.08	6	11.46±1.70	9	10.66±1.92	9	9.80±1.26
29	6	5.28±0.03	6	10.85±0.92	9	9.57±0.04	9	7.66±0.13
30	6	6.07±0.66	6	10.44±0.91	9	10.87±0.72	9	8.56±0.20
31	6	4.95±0.33	6	10.09±0.89	9	8.77±0.21	9	7.71±1.73
32	6	4.73±0.39	6	5.95±0.84	9	9.60±0.81	9	7.32±2.77
33	6	5.51±0.08	6	14.04±0.27	9	10.12±1.10	9	10.25±0.36
34	6	4.59±1.36	6	13.44±0.60	9	5.17±0.26	9	5.75±0.71
35	6	4.69±1.26	6	11.73±0.41	9	5.57±1.24	9	9.51±2.92
36	6	4.23±0.31	6	12.98±0.13	9	11.99±1.35	9	6.86±2.43
37	6	11.63±1.26	6	14.18±0.02	9	9.33±3.08	9	7.13±0.88
38	6	9.49±0.02	6	13.25±1.14	9	11.44±2.77	9	4.79±0.78
39	6	9.31±0.59	6	12.43±1.15	9	6.97±0.91	9	4.40±0.54
40	6	10.41±0.71	6	13.70±0.74	9	7.79±2.55	9	11.65±0.54
41	6	9.41±0.81	6	12.57±0.45	9	10.33±3.15	9	7.07±1.06
42	6	9.28±0.02	6	13.07±0.26	9	8.61±3.02	9	9.93±0.29
43	6	8.43±0.17	6	14.04±0.71	9	4.94±0.79	9	3.98±1.67
44	6	7.79±0.25	6	12.96±0.06	9	11.62±0.66	9	7.72±2.23
45	6	8.53±0.42	6	11.90±0.02	9	12.37±0.13	9	5.47±1.94
46	6	6.84±1.87	6	12.09±0.65	9	9.77±0.73	9	5.90±0.49
47	6	8.07±0.65	6	10.57±2.73	9	9.54±1.03	9	4.13±0.45
48	6	7.65±0.62	6	10.29±0.71	9	8.06±2.26		
49	6	11.51±0.07	6	11.88±1.17	9	7.79±0.83		
50	6	10.14±1.87	6	10.67±0.17	9	7.11±1.21		
51	6	10.68±0.01	6	10.69±0.26	9	5.61±0.17		
52	6	10.43±0.12	6	9.38±1.52	9	5.58±1.99		
53	6	10.76±0.44	6	13.01±0.28				
54	6	9.80±0.88	6	12.78±0.23				
55	6	10.74±0.10	6	12.83±1.23				
56	6	10.12±0.47	6	12.81±1.46				
57	6	9.76±0.15	6	12.57±0.81				
58	6	9.48±1.17						
59	6	10.11±0.08						
60	6	10.64±0.64						
61	6	11.38±0.11						
62	6	11.29±0.73						
63	6	8.90±1.55						
64	6	9.65±1.02						
65	6	9.59±0.37						
66	6	9.63±0.40						
67	6	9.99±0.27						
68	6	7.66±0.06						
69	6	8.00±0.57						
70	6	7.89±0.27						
71	6	7.95±0.09						
72	6	7.08±2.75						
73	6	7.81±0.99						
74	6	8.66±0.43						
75	6	7.20±2.72						
76	6	8.87±1.55						
77	6	13.08±0.37						
78	6	12.17±0.04						