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Observations on the Biology and Larval Instars Discrimination of Wax Moth *Achroia grisella* F. (Pyralidae: Lepidoptera)

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ABSTRACT

The lesser wax moth (*Achroia grisella*), is a noxious pest of honey bee hives. The biology of this deleterious species was studied under laboratory condition at 31±2°C and of 66.28% RH. This study was conducted to provide an exhaustive considerate of the life cycle and larval growth of this insect under laboratory conditions. The average duration of egg, larval and pupal stages were 3.62±0.11, 30.72±0.21 and 7.65±0.083 days, respectively. Based on width and girth of head capsule, five larval instars were distinguished and further confirmed by Dyar's rule. The larval period varied significantly between male and female with the average being 29.84±0.27 and 31.42±0.33 days, respectively. The average adult lifespan varied significantly between males and females. The males lived almost twice as long as the females with the average days of 13.03±0.51 and 7.46±0.29, respectively. The coupling time between males and females range between 9-18 min with the average of 12.20±0.33 min. Developmental duration stages, adults' life span and larval instars were determined in the laboratory.

Key words: Wax moth, instars, head capsule girth, head capsule width, dyar's rule

INTRODUCTION

Malaysia being located in tropical region and has an abundance of natural resource to be used for honey production and bee keeping in most parts of the country. A species of pests has been discovered that is considered a noxious pest to bee hives (Nomura *et al.*, 2006; Emiru and Namusana, 2010). Among these pests the lesser wax moth *Achroia grisella* (Fabricius, 1754) (Lepidoptera, Pyralidae) seriously damages bee hives and threatens the well-being of the honey bee population.

Achroia grisella is common in the tropical, subtropical and temperate regions and more widely distributed than the greater wax moth *Galleria mellonella* (Gulati and Kaushik, 2004). The perilous threat of this pest on the stored or unprotected combs in the weak honey bee hives (Chhuneja and Sunita, 2009). The infestation and feeding of *A. grisella* was reported to be most active in the monsoon season (Sharma *et al.*, 2011). The adults could not consume wax or drink until they die after mating and female laying their eggs (Strauss and Reinhold, 2010). The developmental stages of this insect live inside honey bee wax combs. The females normally lay their eggs in the cracks inside the honey beehive. The developing larvae normally create feed tunnels in the area around the midrib of the wax comb. The larva feed on wax and other materials stored in the comb cells, making the observation of the larval instars of the lesser wax moth inside the beehive difficult to discriminate at this stage in the field conditions.

The moulting process takes place between these stages. This process is controlled by the endocrine system and secretion of hormones. The interaction between several hormone actions, such as prothoracicotropic (PTTH), juvenile and ecdysone, lead to differentiation between the developmental stages of the insect. This differentiation requires changing the old skin for the new skin, such as in the larval stage. Cuticle is an essential component of insect skin, while the head capsule contains thicker cuticle materials to protect the head of the larvae. The larval skin cuticle is more flexible than the head capsule cuticle (Rudall, 1963). A new capsule will be produced with each new moulting instar. The capsule size can grow tenfold during the period of development from the first instar to the last (Delbac *et al*., 2010). Measurement of the sclerotized head capsule to differentiate between the larval instar is common practice (Mohammadi *et al*., 2010). Larval length and width have also been used to support the differentiation between larval instars observed during the larval stage development. Although the morphometric characters are commonly used to discriminate between different larval instars in insect species (Chen and Seybold, 2013), there are many mathematical procedures that can be used to differentiate between larval instars in insect species.

This study was aimed to determine the life cycle of the lesser wax moth *A. grisella* feeding on honey bee wax under laboratory conditions to clarify some features of the biology of the insect including the number of larval instars based on larval head capsule morphometric measurements. This information will help to develop strategies for control system of this pest.

MATERIALS AND METHODS

Insect collection and rearing: *Achroia grisella* samples were collected from local honey bee apiary located at Batu Pahat in Johor, Malaysia. The infested honey bee wax combs contained all stages of insect development and were used to establish the laboratory stock culture for further studies. Natural honey bee combs were pre-treated by freezing them in -20°C conditions for 2 h to disinfest them from other moths. The stock culture was started by introducing thirty pairs of adult moth. The moths were placed and allowed to reproduce in the laboratory with a temperature of 31±1°C, 66.28% RH and 12L: 12D photoperiod (These were the average conditions for all farther experiments) and placed in a closed aquarium tank (9.2×16×9.2 cm), covered with muslin cloth for good aeration to study the biology and life cycle.

Life cycle of *A. grisella*: Adult pairs of *A. grisella* were collected from rearing cage to mate in small plastic jars covered with tissue papers to stimulate eggs lying. Eggs were then collected to start the life cycle. The larvae hatched from these eggs were fed separately on two grams of sterilized natural honey bee wax in 9 cm petri dishes until pupation. The pupation occurs after the prepupa spins the cocoon from silk, Frass and wax impurities. The remaining bee wax was removed after pupation to facilitate adult eclosion observation. All observations related to stages duration were recorded.

Larval instars discrimination: A parallel experiment was carried out in the same generation to determine the number of different larval instars of *A. grisella*. This was conducted by measuring the head capsule width and girth for ten larvae each day until the pupation occurred after the 34th day (Fig. 1) by QuickphotoMicro2.3 (Hajek and Fikacek, 2008). The statistical percentile frequency distribution was used to differentiate between instars (Delbac *et al*., 2010).

Fig. 1: Measurements of head capsule of *Achroia grisella* larva

The results obtained by percentile distribution of the head capsule width and head capsule girth measurements were confirmed using Dyar's rule (Dyar, 1890), developed for Lepidoptera larvae. The rule assumes that the growth rate of the head capsule in successive instars follows the geometric development $y = a\ b^x$. According to procedure carried by Hsia and Kao (1987) Dyer's ratio and the expected instar can be calculated the through the following equation:

$$\text{Dyar's ratio} = \frac{II}{I}, \frac{III}{II}, \frac{IV}{III}, \frac{V}{IV} = \text{constant} \qquad (1)$$

$$\text{Dyar's ratio average} = \frac{II}{I} + \frac{III}{II} + \frac{IV}{III} + \frac{V}{IV} /N \qquad (2)$$

$$\text{Expected head growth rate} = \frac{I}{Dr^*} = \text{IIexp}, \ldots, \frac{IV}{Dr} \qquad (3)$$

where, I, II, III, IV, V are instar head capsule for both width and girth, *Dr is the dyar's ratio average.

Data analysis: All the data acquired from the biological and fecundity parameters was analysed using the t-test to compare between periods for all developmental stages. The percentile frequency distribution was used to differentiate between larval instars. Dyar's rule was applied on the larval measurement and the ratio of instar groups were confirmed using the t-test. Statistical significance of differences between instar groups was determined using the Analysis of Variance (ANOVA) and comparison was done by Tukey Kramer test. All data was analysed using the JMP v.9 statistical tool.

RESULTS

Life cycle of lesser wax moth *A. grisella*: The life cycle of lesser wax moth *Achroia grisella* consist of four stages to complete the cycle. Among these stages, the only feeding stage is the larval stage. The description and duration of each stage is detailed as follow.

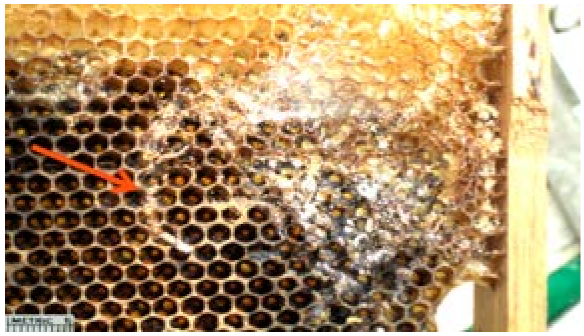

Fig. 2: Feeding tunnels inside bee wax comb created by larvae of *Achroia grisella*

Table 1: Rang and average duration of developmental stages of lesser wax moth *Achroia grisella*

Developmental stages	Range duration (days)	Average duration (days)
Egg	3-4	3.63±0.11
Larvae	27-37	30.72±0.21
Pupa	6-10	7.65±0.08
Adult male	5-21	13.03±0.5
Adult female	5-12	7.46±0.29

Egg stage: The female of *A. grisella* deposited their eggs individually or in small patches in the walls of bee hives or inside the wax combs. Similar behaviour was observed in laboratory which they deposited their eggs at the cage wall or in the tissue paper used to cover the cage. The newly eggs deposited were near white to creamy colour but gradually turned darker over time. The eggs of the lesser wax moth were hatched in the range of 3-4 days after being deposited by the female with an average of 3.62±0.11 days (Table 1). The important factors affecting the longevity of egg hatching were temperature and relative humidity. The embryonic growth of larva can be observed at the third day as a reddish circle behind the egg chorion. In this stage, the larvae were ready to hatch and come out from the egg. After hatching the larvae gradually released themselves from the eggs and ate the remains of the egg chorion.

Larval stage: The grown larvae appeared behind the egg chorion as a brown circle. The neonate larvae of *A. grisella* were tiny with a creamy to whitish creamy body colour with a noticeable light brown head. The body was cylindrical and had 11 segments. The new larvae burrowed directly to search and find their food source in the bee combs Fig. 2. The observed larvae built a feeding tunnel during feeding in the midrib of the bee wax comb. The occurrence of cannibalism was observed between the neonate larvae when there was no food available for these larvae.

The larval stage duration was observed in laboratory conditions and results reveal that the larval stage duration ranged between 27.5 and 37.5 days with an average of 30.72±0.21 days (Fig. 3a). The results (Table 2) show that the average stage duration of male larvae is significantly ($p<0.001$) different from the average stage duration of the female larvae, 29.8±0.27 and 31.4±0.33 days, respectively. The results also shows that the female larvae spend a significantly ($p<0.001$) longer time to reach pupal stage compared to the male larvae.

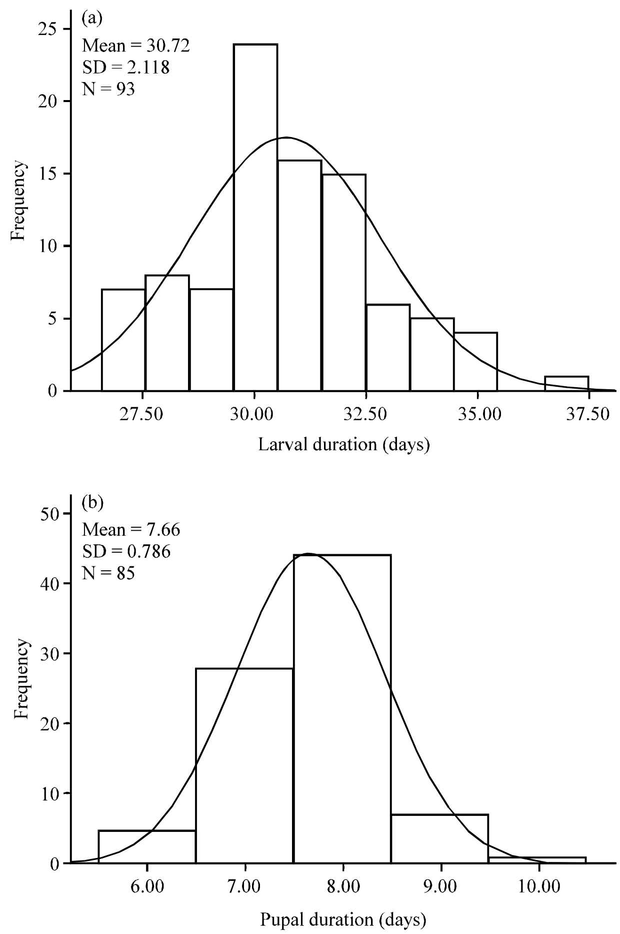

Fig. 3(a-b): Frequency distribution of *Achroia grisella*, (a) Larval and (b) Pupal stage duration

Table 2: Average duration according to sex of larvae and pupae of *Achroia grisella*

Stage	N	Mean±SD	t-value
Larva			
Male	38	29.8±0.3	3.671**
Female	48	31.4±0.3	
Pupa			
Male	38	7.8±0.1	1.133ns
Female	47	7.6±0.1	

**Results are significantly different between male and female, ns: Not significant

Pupal stage: The full gown larvae start to search for a suitable place for pupation. The last instar larvae dig and create boat shaped holes in the wooden parts of the honey bee hive walls and bottom. The Prepupa continued to spin silk threads to build a white cocoon. In addition to the silk, they tend to attach in between the spin silk frass drops and other debris to offer more strength to the cocoon and more protection for the developing pupa. The results showed that the pupal stage period ranged from six to ten days, with an average of 7.65±0.08 days (Fig. 3b). The observation presented in Table 2 confirmed that there was no significant difference between the male and female pupal duration time.

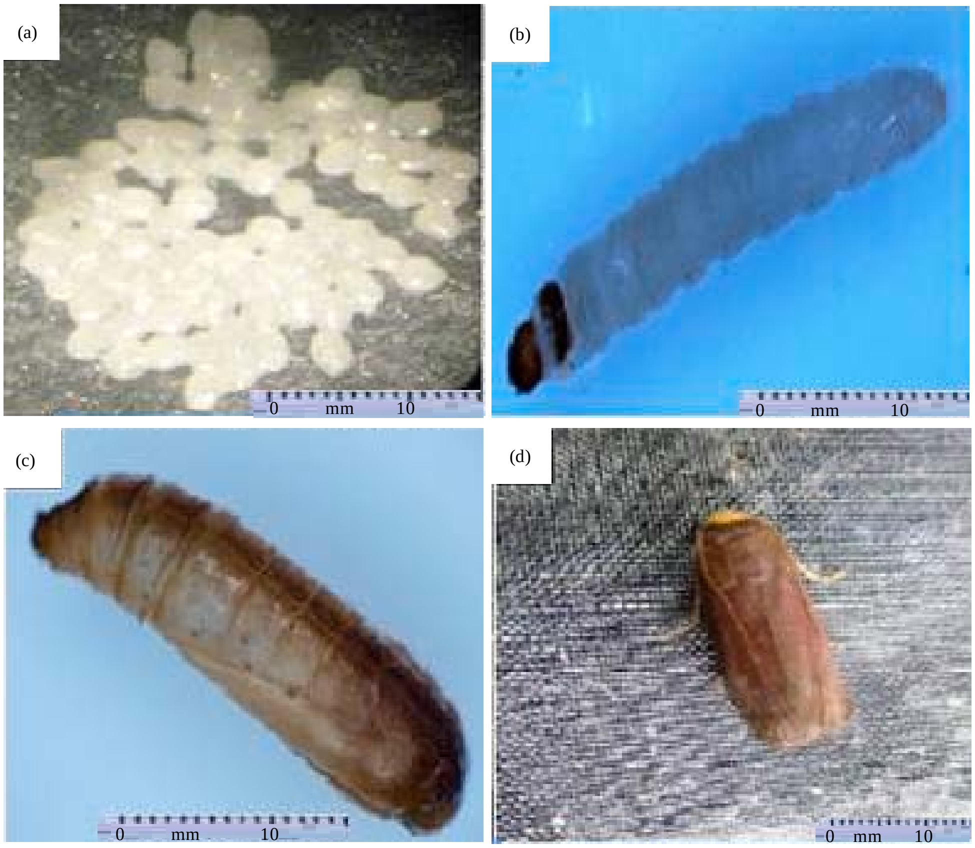

Fig. 4(a-d): Developmental stages of *A. grisella*, (a) Eggs, (b) Larva, (c) Pupa and (d) Adult

Adult stage: The adults of the lesser wax moth have grey to silver wings with no marks on them. The measurements of the adult's length showed that the male's length was shorter than the females, being 13 and 19 mm, respectively. The wingspan of the males was also shorter than the females which was measured at 22 and 16 mm, respectively.

The average adult duration of the male was 13.03±0.5, while the adult females lived an average of 7.46±0.29 days; the overall average for the duration of the adults was 9.92±0.41 days (Fig. 4).

Discrimination of *A. grisella* larval instars: The measurement of the head capsule width, girth and body dimensions for 34-day *A. grisella* larvae shows that the head capsule width ranged from 0.17-0.99 mm and that the head girth ranged from 0.52-3.27 mm during the larval stage development.

The data for the head capsule width and head capsule girth (Table 3) shows that the average measurements of the first instar head capsule width and girth were 0.17±0.12 and 0.52±0.05 mm, respectively. The developmental period of this instar was 6 days. The second larval instar revealed an increase in the head capsule width which was measured at 0.29±0.03 mm with an average head

Table 3: Measurements of head capsule of different larval instars of *Achroia grisella*

Instars	Range (mm)	Mean±SE (mm)	Growth rate
Head capsule width measurements			
I	0.13-0.19	0.17±0.12	-
II	0.21-2.99	0.29±0.03	1.72
III	0.35-0.62	0.52±0.13	1.74
IV	0.72-0.88	0.79±0.11	1.51
V	0.95-1.23	0.99±0.07	1.30
Head capsule girth measurements			
I	0.48-0.66	0.52±0.05	-
II	0.68-0.87	0.73±0.11	1.40
III	0.91-2.37	1.64±0.42	1.65
IV	2.52-2.80	2.71±0.36	1.56
V	2.95-3.72	3.27±0.31	1.21

Table 4: Conformity of lesser wax moth *Achroia grisella* head capsule with Dyar's rule

	Head capsule measurement (mm)	
Instars	Observed	Calculated
Head capsule width		
I	0.17	0.17
II	0.29	0.26
III	0.52	0.40
IV	0.79	0.62
V	0.99	0.96
Head capsule girth		
I	0.52	0.52
II	0.73	0.79
III	1.03	1.20
IV	2.71	1.82
V	3.27	2.77

girth of 0.73±0.11 mm. The developmental period of the second instar was also 6 days. For the third instar the head capsule width was 0.52±0.13 mm and the head capsule girth was 1.64±0.42 mm. The developmental period was 8 days. However, the fourth larval instar had a developmental period of 8 days. The head capsule width measured 0.79±0.11 mm and capsule girth of 2.71±0.36 mm. Finally, the fifth larval instar had a head capsule width and capsule girth of 0.99±0.07 and 3.27±0.31 mm, respectively. Many mathematical models and procedures can be used to determine the larval instars of insect species. Most of these procedures use the measurement of the sclerotized body parts.

The findings were obtained using the percentile frequency distribution. It was observed that the head capsule width and girth increased with each successive new instar. The average growth rates were 1.55 and 1.52 for the head capsule width and girth, respectively. There were significant ($p<0.05$) differences among the larval instar groups. Similarly, the measurement of the head capsule width and girth falls within the range of each group (Table 3) at a confidence level of more than 95%.

The results presented in Table 4 show the conformity of the larval instar head capsule measurements with Dyar's rule. This rule was applied successfully on the lesser wax moth larval

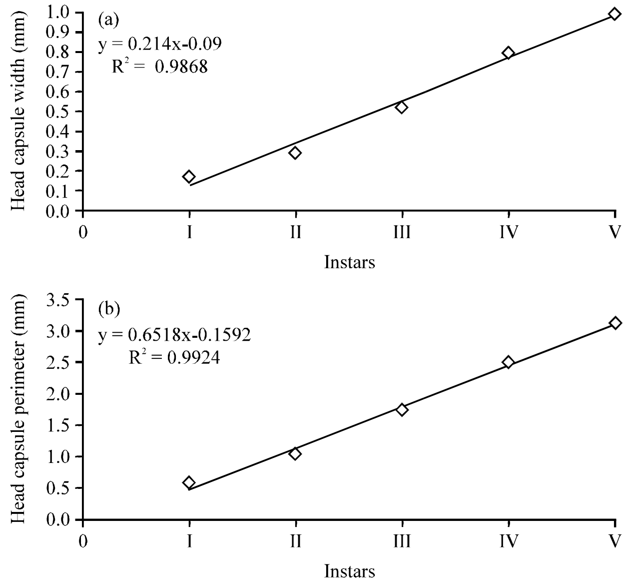

Fig. 5(a-b): Head capsule (a) Width and (b) Girth and corresponding larval instars of *Achroia grisella*

instars head capsule width and girth. The growth rate of both head capsule measurements is considered as Dyar's ratio averages were laid within the limits of the Dayr's rule. Furthermore, the observed measurements (Eq. 1, 2) of larval head capsules (Eq. 3) for the five instars of *A. grisella* were not significantly different from the expected head capsule for each instar using Dyar's ratio.

According to the assumption generated by Gains and Campbell in 1935 and recently reported by Panzavolta (2007) "An impeccable geometrical growth of head capsule width can be displayed by straight line, if the all successive instars head capsule width were plotted against the number of instars". The results of this study applied this assumption successfully on *A. grisella* head capsule width measurements which the linear regression (Fig. 5a) show a highly significant equation $R^2 = 0.9868$. In addition, this rule was applied on the head capsule girth of the lesser wax moth. The equation of linear regression was highly significant $R^2 = 0.9924$ (Fig. 5b). It appears there is an excellent fit to the linear regression model for two measurements (i.e., head capsule width and head capsule girth). This could be reliable confirmation that in both cases no instar was overlooked among the measurements. As an indication of these results, in addition to the head capsule width, the capsule girth seems to be a suitable measure for discrimination of the larval instars although the capsule width is the most commonly used measure. Furthermore, the fit of the head girth show better fit to the model compared to the capsule width.

DISCUSSION

The observation on the incubation period of the eggs of *A. grisella* was similar to that observed by Chandel *et al.* (2003) who recorded a 5-10 day incubation period for the eggs. However, the observed results were not in agreement with Ellis *et al.* (2013) who recorded that the egg hatching period ranged from 7-22 days in which the longevity of this stage was dependent on the climatic factors. However, their observation was taken in a low temperature zone compared to this study in which the observations were taken in different conditions. Nevertheless, the results were the same concerning the observed colouration of the eggs; the change happened gradually over time from white to a light brown colour.

The larval stage is the only stage where they can consume bee wax among all the developmental stages of wax moth including the adults. That makes this stage the most destructive and harmful stage of this insect (Sharma *et al*., 2011). Studies on the life cycle of insects confirmed that the larval stage duration longevity depends on the environmental conditions in addition to the availability of food (Mohammadi *et al*., 2010).

The observation on the pupal stage of *A. grisella* was recorded by Chandel *et al*. (2003) showed similar period with ranging between 5 and 7 days under normal conditions. They also mentioned that the pupation of this pest could take up to 143 days for winter generations. However, the results of our work are not in consonance with this finding because this study was carried out in an ambient controlled condition. In the tropical and subtropical regions, continuous generation of pests exists throughout the year. This increases the ability of the insects to produce a larger population and become more harmful to the honey beehives and stored wax combs.

A similar observation on the adult wing span and length was recorded by Chandel *et al*. (2003). The study results showed that the adult male life span was significantly different from the female which agrees with the results observed by Akratanakul (1987) and Chandel *et al*. (2003). Analysis confirmed that the life span was significantly different between the male and female. The male duration was almost twice the life span of females (Table 1). Males of *A. grisella* start to produce courtship signaling by wing-fanning shortly after emergence. This wing signal attracts the females to start the mating process. The average coupling time ranged between 9-18 min, with an average of 12.20±0.3 min. These results were in agreement with the observation of Greenfield and Coffelt (1983).

Head capsule measurements method was previously reported by Delbac *et al*. (2010) and Onekutu *et al*. (2013). However, they reported their method of discrimination based on measurements carried on larval head capsule to determine the instars. While, this study reported the measurements of larval head capsule and head girth to distinguish between the five instars within *A. grisella* larval stage.

CONCLUSION

The record of the life cycle study of *A. grisella* showed that the eggs of the lesser wax moth hatched in an average duration of 3.62±0.11 days. The important factors affecting the longevity of egg hatching duration were temperature and relative humidity. The record of the larval stage duration revealed an average duration of 30.72±0.21 days, while the pupal stage period ranged from six to ten days, with an average of 7.65±0.08 days. The adult average duration of the male was 13.03±0.5 while the adult females lived an average of 7.46±0.29; the overall adult's duration average was 9.92±0.41 days. An observation has shown that the life span was significantly different between the male and female. The determination of the number of larval instar of *A. grisella* was from the daily records of the developmental larvae over a period of 34 days. These measurements resulted in five successive larval instars of this moth in laboratory conditions. This result was confirmed using Dyar's rule. The information provided in this study will help to develop some strategies for control programs of this honey bee pest.

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