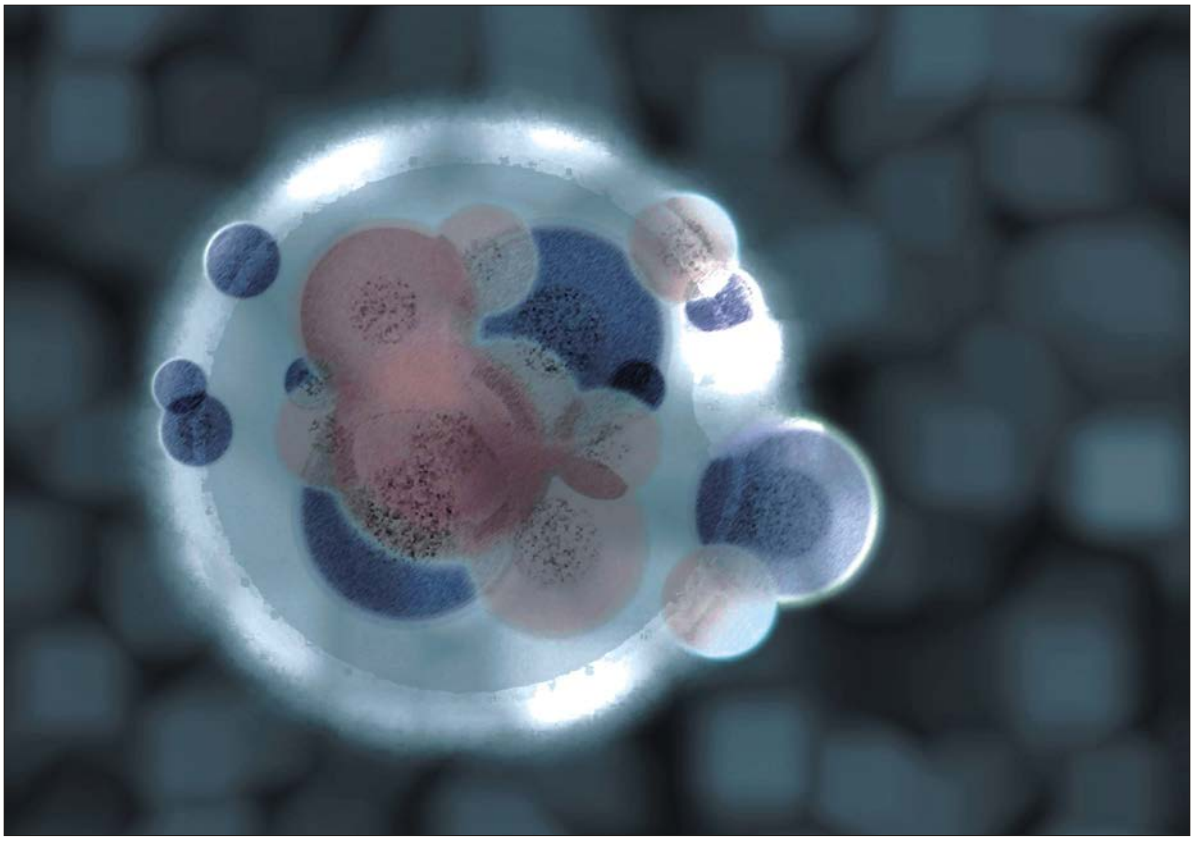

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Developmental Biology of the Tiger Moth, *Atteva sciodoxa* Meyrick (Lepidoptera: Yponomeutidae) under Laboratory Conditions

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Abstract: The tiger moth, *Atteva sciodoxa* is a serious pest of tongkat Ali, *Eurycoma longifolia*. The morphology, development times and fecundity aspects were studied at 27±2°C, 80±5% relative humidity and 12 h photoperiod. The eggs were yellow and ovoid in shape with a mean length and width of 1.19±0.02 and 0.86±0.02 mm, respectively. Width measurements of larval head capsules showed that *A. sciodoxa* undergoes five larval instar stages. The mean head capsule widths of the first to fifth instar larvae were 0.55±0.01, 0.89±0.01, 1.23±0.02, 1.52±0.01 and 2.11±0.02 mm, while the body lengths were 4.71±0.1, 8.63±0.1, 12.87±0.1, 16.29±0.1 and 21.74±0.2 mm, respectively. The mean male and female pupal body lengths were 10.36±0.1 and 11.26±0.2 mm, respectively. The mean male and female wing span were 21.63±0.2 and 24.28±0.2 mm, respectively. The mean pre-oviposition and oviposition periods were 6.2±0.23 and 8.5±0.28 days, respectively. A single female laid on average 106.1±4.85 eggs with maximum production between days 8-15 of adult emergence. The maximum number of eggs laid per female per day was 20.1±0.5. The mean hatching time was 5.7±0.1 days with a mean hatchability of 81.1±0.6%. The mean larval, pupal and adult periods were 20.7±0.2, 6.2±0.8 and 13.2±0.5 days, respectively. The female pupal period and adult lifespan were significantly longer than the male. *Atteva sciodoxa* completed its life cycle in 46.28±0.49 days.

Key words: *Atteva sciodoxa*, development, *Eurycoma longifolia*, tongkat Ali

INTRODUCTION

Eurycoma longifolia Jack (Simaroubaceae), commonly known in Malaysia as tongkat Ali, grows wild in rainforests of South East Asia (Osman *et al*., 2003; Hussein *et al*., 2005; Musa *et al*., 2005). Although, *E. longifolia* roots have been used in traditional medicine and as a health supplement for decades, scientific evidence of its potential use as alternative medicine is fairly recent. *E. longifolia* has been reported to have a range of medicinal properties including anti-malarial (Ang *et al*., 1995; Chan *et al*., 2004), anti-ulcer (Tada *et al*., 1991) and anti-pyretic (Chan *et al*., 1995) properties. It has also been reported to show certain cytotoxic effects on human cancer cells (Kuo-Ping *et al*., 2003) and aphrodisiac properties (Ang and Ngai, 2001; Ang and Lee, 2002). These fairly recent awareness of the multitude of medicinal benefits of *E. longifolia* resulted in extensive extraction of *E. longifolia* roots from the natural forest and the species has been threatened with extinction due to indiscriminate collection and over exploitation (Hasnida *et al*., 2001).

Atteva sciodoxa (Lepidoptera: Yponomeutidae) is a common pest of *E. longifolia* in Brunei Darussalam, Indonesia, Malaysia and Thailand. In Malaysia, the pest is found both in lowland and montane areas (Robinson *et al*., 1994). The recent onset of tongkat Ali plantations has led to widespread outbreaks of *A. sciodoxa* (Musa *et al*., 2005). This study reports on the morphology, development times and ovipositional aspects of different metamorphic stages of *A. sciodoxa*.

MATERIALS AND METHODS

This study was conducted at University Putra Malaysia, Serdang-Malaysia in 2007-08. Fourth instar larvae of *A. sciodoxa* were collected from seedlings in the nursery and reared to adult stage in wooden cages (34×34×60 cm) with wire mesh (1.5 mm^2). They were fed on *E. longifolia* leaves and maintained in the laboratory at 27±2°C, 80±5% relative humidity with 12 h photoperiod. Pupae were collected and transferred to cylindrical containers (40×30 mm diameter) until adult emergence.

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Four pairs of newly emerged adults were released on each seedling in a wooden rearing cage containing 10% honey placed on a cotton swab. The honey was replenished on alternate days. The leaves were checked daily for eggs, which were then transferred in cylindrical plastic containers (40×30 mm diameter) until hatching.

Upon hatching, each first instar larva was carefully transferred onto tender leaves of *E. longifolia* placed upright in cylindrical plastic containers (40×30 mm diameter) containing 10 mm of moistened sterilized sand which was then covered with Whatman® filter paper and capped with a lid. This set up maintained a relative humidity of 80±5% besides keeping the leaflets fresh for 24 h. The first and second instars were fed on young leaves, while the 3rd to 5th instars on semi-mature leaves. The containers were cleaned and leaves replaced daily. For the 3rd instar larvae onwards, medium sized cylindrical containers (66×40 mm diameter) were used. Pupae were separated according to sex using a stereomicroscope and kept singly in plastic containers (40×30 mm diameter).

For adult rearing and recording of oviposition parameters, cages were set up for single pairs. Sterilized moistened sand was levelled to a depth of 10 mm in a 10 cm petri dish and covered with Whatman® filter paper. Fresh *E. longifolia* shoot with 4-5 leaflets was inserted upright through the centre of each set up. These leaflets were then placed in cylindrical containers (8×12 cm diameter). Three drops of 10% honey were placed on the inside base of the container and then inverted. Single pairs of virgin adults were introduced in each container and two drops of honey were added on alternate days. The adult moths were transferred to different containers whilst cleaning the experimental containers and adding drops of honey. Observations were made on the time as well as duration of copulation, number of eggs laid per day and also longevity of the adult moths. Leaves bearing eggs were transferred to new containers (66×40 mm diameter) to record hatchability.

The morphological measurements of the egg, larval stages, pupa and adult moth were recorded using a stereomicroscope equipped with an image analyzer. Larval head capsules were collected following every moult to determine the head capsule width. To measure the larval body length, larvae were immobilized by subjecting to low temperature of 10°C for10 min. Calculated head capsule width and ratio of increment was calculated as described by Dyar (1890). The difference between observed and calculated head capsule width, development periods and morphological measurements between males and females were compared using the unpaired two tailed t-test.

RESULTS AND DISCUSSION

Adult morphology: *Atleve sciodoxa* adults are small golden brown moths bearing white spots of variable size on the tergum and fore-wings (Fig. 1a). The white spots are bigger towards the anal margin and the vertex region is white. The fore-wings are elongate and slightly triangular at the apex. The antennae are long and filiform. The hind-wings are broad, dark grey with short, light brown fringe. The dorsal part of the abdomen is orange, while the ventral light yellow with white transverse bands. Adults are feeble fliers. The sexes can be differentiated based on the abdominal tip and genital orifice. The male abdomen is long and pointed distally with a narrow slit while, the female abdomen is blunt with a prominent genital orifice bordered by white scales (Fig. 1b, c).

The mean wing span of the adult is 22.97±0.2 mm, where that of the female is 24.28±0.2 mm and the male 21.63±0.2 mm. The results indicate a significant difference ($p<0.01$) between the wing span of the two sexes (Table 1).

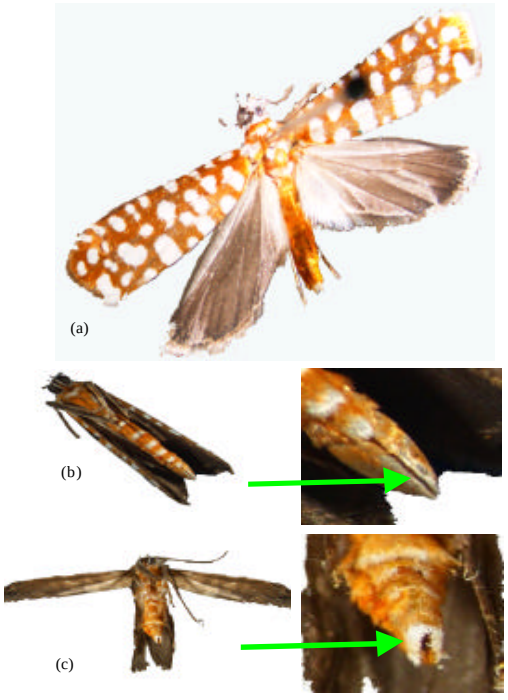

Fig. 1: Adult moths of *A. sciodoxa*, (a) dorsal view of female moth (0.65x), (b) ventral view with male genital orifice (20x) and (c) ventral view with female genital orifice (20x)

Table 1: Wing span of adult *A. sciodoxa* under laboratory conditions

Adult	Mean±SE (mm)	Minimum	Maximum
Male	21.63±0.2	19.34	23.26
Female	24.28±0.2	22.03	26.42
Overall	22.97±0.2	19.34	26.42

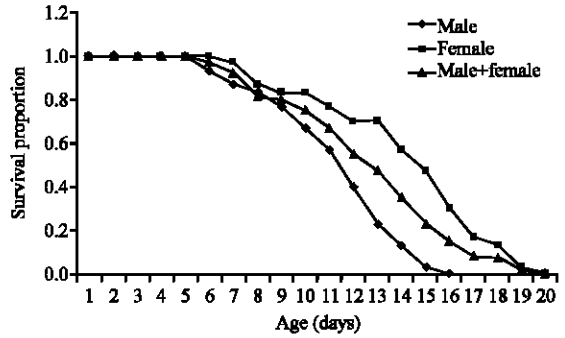

Fig. 2: Survivorship curves of adult *A. sciodoxa* at 27±2°C and 80±5% relative humidity with 12 h photoperiod

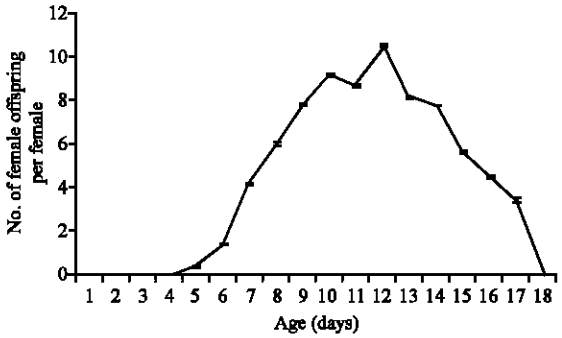

Fig. 3: Mean number of female offsprings per female (±SE) of *A. sciodoxa* at 27±2°C and 80±5% relative humidity with 12 h photoperiod

Table 2: Reproductive parameters of *A. sciodoxa* at 27±2°C and 80±5% relative humidity with 12 h photoperiod

Parameters	Mean±SE	Minimum	Maximum
Pre-oviposition period (days)	6.2±0.2	4	9
Eggs per female per lifespan	106.1±4.9	50	171
Oviposition period (days)	8.5±0.3	6	11
Incubation period (days)	5.7±0.1	5	7
Hatchability (%)	81.1±0.6	75	87
Maximum eggs per female per day	20.1±0.5	14	26
Minimum eggs per female per day	5.9±0.6	1	13
Mean No. of eggs per female	12.6±0.5	8.3	17.6

n = 30 females

Adult lifespan: The female lifespan ranged between 6-19 days with a mean of 14.8±0.6 days while that of the adult male ranged between 7-16 days with a mean of 11.6±0.6 days. In terms of longevity, the female adult lived significantly longer than the male ($p<0.01$). The survivorship curve for the male, female and combined population is shown in Fig. 2. The difference in mortality rate between male and female populations was more pronounced after day 7 of emergence. The cumulative mortality over time for *A. sciodoxa* depicts a type II survivorship curve as described by Southwood (1978).

Oviposition aspects: Mating activity usually commenced at dawn and may occur more than once. Initial copulation took place after 3-4 days of adult emergence and lasted for 2-3 h, while the second copulation, generally lasted for 10-12 h. Mating time varied with age. There was a 1:1 sex ratio of male to female. Pre-oviposition period varied between 4 to 9 days with a mean of 6.20±0.2 days.

On average, a single female laid 106.1±4.9 eggs during its lifespan. The minimum number of eggs laid per female was 50, while the maximum was 171. The maximum number of eggs laid per female per day was 20.1±0.5 (Table 2) and the mean number of eggs per female per day during the oviposition span was 12.6±0.5. The fecundity curve showed that the most productive period was from day 8-15 of post emergence with a peak on day 12 (Fig. 3).

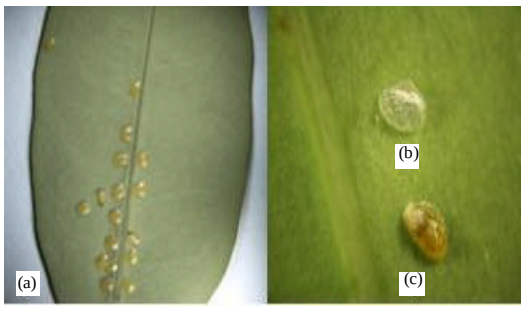

Fig. 4: Eggs of *A. sciodoxa*, (a) typical distribution of freshly laid eggs, (b) chorion and (c) mature egg, 24 h prior to hatching

Table 3: Egg and pupal dimensions of *A. sciodoxa* under laboratory conditions

	Length (mm)			Width (mm)		
Stage	Mean±SE	Min.	Max.	Mean±SE	Min.	Max.
Egg	1.19±0.02 (n = 25)	1.08	1.39	0.86±0.02 (n = 25)	0.72	1.00
Pupa						
Male	10.36±0.1 (n = 21)	9.46	11.38	2.17±0.1 (n = 21)	1.86	2.87
Female	11.26±0.2 (n = 21)	10.18	12.72	2.72±0.1 (n = 21)	2.00	3.26
Overall pupa	10.80±0.1 (n = 42)	9.46	12.72	2.42±0.1 (n = 42)	1.86	3.26

Egg: *Atteva sciodoxa* egg is ovoid and yellow in colour when freshly laid. The mean length and width of the egg was found to be 1.19±0.02 and 0.86±0.02 mm, respectively (Table 3). Eggs were laid singly and preferentially on young leaves, along the mid rib and also towards the tip of leaflets (Fig. 4). Fertile eggs were also observed on the surface of rearing containers. The eggs turned grey and eventually darkened near hatching time. The incubation period was 5.70±0.1 days with a hatchability of 81.1±0.6% (Table 2).

Larva: Newly hatched larvae were dark grey in colour with black hairs on the dorso-lateral positions of the thorax and

Table 4: Dimensions of larval instars of *A. sciodoxa* and application of Dyar's Rule on head capsule

	Head capsule width (mm)			Body length (mm)		
Instar	Mean*±SE	Calc**	Ratio	Mean±SE	Calc**	Ratio
1st	0.55±0.01 (0.40-0.68)	0.54	0.62	4.71±0.1	3.06	5.97
2nd	0.89±0.01 (0.71-0.99)	0.87	0.73	8.63±0.1	7.03	9.92
3rd	1.23±0.02 (1.0-1.40)	1.20	0.77	12.87±0.1	11.19	14.82
4th	1.52±0.01 (1.41-1.79)	1.55	0.74	16.29±0.1	15.06	17.96
5th	2.11±0.02 (1.92-2.31)	2.10		21.74±0.2	20.09	24.18
Mean: 0.72						

*Observed mean head capsule and **Calculated head capsule

Table 5: Development periods of different metamorphic stages of *A. sciodoxa* at 27±2°C and 80±5% relative humidity with 12 h photoperiod

		Period (days)			
Stages	Instar/sex	Mean±SE	Min.	Max.	No.
Larva	1st	4.0±0.1	3	5	48
	2nd	3.1±0.1	3	4	46
	3rd	3.8±0.1	3	4	46
	4th	4.1±0.1	3	5	45
	5th	5.7±0.1	5	6	44
Overall larval duration		20.7±0.2	19	22	44
Pupa	Male	5.5±0.1	5	6	30
	Female	6.5±0.1	5	7	30
Overall pupal duration		6.2±0.1	5	7	52
Adult	Male	11.6±0.6	7	16	30
	Female	14.8±0.6	8	19	30
Overall adult lifespan		13.2±0.5	7	19	60
Life cycle	Male	43.6±0.7	34	50	30
	Female	47.8±0.7	38	52	30
Overall life cycle		45.7±0.6	34	52	60

abdomen. The head was reddish-brown in colour. The body colour turned yellowish-green in 2-3 days. Prior to the first moult, the thoracic and last three abdominal segments turned to a creamy colour. The mean head capsule width and body length were 0.55±0.1 and 4.71±0.7 mm, respectively (Table 4). The calculated head capsule width was 0.54 mm. There was a 1.8% difference between observed and calculated head capsule widths. The ratio of increment of head capsule was 0.62. The lifespan of the 1st instar ranged between 3-5 days with a mean of 4.00±0.1 days (Table 5). The first moult took place between 24-36 h.

For the second instar, the mean head capsule width and body length was 0.89±0.01 and 8.64±0.12 mm, respectively, while the calculated head capsule width was 0.87 mm (Table 4). The difference between observed and calculated head capsule width was 2.2% and the ratio of increment of head capsule was 0.73. The head of the second instar was also brown in colour. The body turned greenish-yellow, particularly at the terminal segments of the abdomen whilst the dorsal region of the abdomen was dark green. One pair of small black dots appeared on the dorso-lateral position of each thoracic and abdominal segment. The mean duration of the 2nd instar was 3.10±0.1 days. Prior to moulting, the entire body turned yellowish-orange in colour. The second moult took place between 24-30 h.

Fig. 5: Full grown 5th instar larva of *A. sciodoxa* (0.65x)

The mean head capsule width and body length of the third instar was 1.23±0.02 and 12.87±0.1 mm, respectively, while the calculated head capsule was 1.2 mm. There was a 2.4% difference between observed and calculated head capsule width. The ratio of increment of head capsule was 0.77. The head was reddish brown. The dorso-lateral black dots increased in size and the body turned green. The mean duration of the 3rd instar was 3.80±0.1 days. Prior to moulting, the body turned orange in colour. The 3rd moult took place between 20-24 h.

The mean head capsule width and body length of the 4th instar was1.53±0.02 and 16.29±0.1 mm, respectively. The 4th instar was similar to the 3rd instar in appearance except increased body size and the black spots on the dorso-lateral margin of the tergites increased in size. The difference between observed and calculated head capsule width was 2.0% and the ratio of increment of head capsule was 0.74. The 4th instar period lasted between 4-5 days (mean 4.1±0.1 days).

The mean head capsule width of the 5th instar was 1.9-2.3 mm while the body length of the full grown 5th instar was 20.1-24.2 mm. There was a 0.5% difference between observed and calculated head capsule widths. The 5th instar larvae were dark yellowish-green. The black spots coalesced on the tergites to form conspicuous black bands (Fig. 5).

The mean 5th instar duration was 5.7±0.1 days. Prior to pre-pupation, the larvae turned bright orange in colour. The larvae ceased feeding and attach themselves to silken webs spun around leaves or other objects in the vicinity. There was a distinct quiescent pre-pupation stage which lasted for 24-36 h.

Fig. 6: Pupa of *A. sciodoxa* (a) dorsal view of pupa; (b) male g enital orifice and (c) female genital orifice

The overall mean ratio of increment of head capsule for the five larval stages was 0.72. The mean overall larval period of *A. sciodoxa* was 20.7±0.2 days, ranging between 19-22 days. The shortest larval period was of the 2nd instar (3.1 days), while the longest was of the 5th instar (5.7 days). The difference in developmental periods between the 1st and 4th instar as well as between the 2nd and 3rd instars were marginal (0.1 and 0.7 days, respectively).

Pupa: Upon pupation, *A. sciodoxa* appeared orange in colour and turned reddish brown prior to adult emergence. Paired black dots were present dorsally on each thoracic and abdominal segment. There were paired black dots aligning both sides of the ventro-lateral margins of the 5th, 6th and 7th sternites. The male genital orifice was apparent on the middle of the ninth abdominal sternite, bordered by elevations. In the female pupae, the genital orifice was located ventro-anteriorly of the eighth abdominal sternite (Fig. 6a, b). Similar external differences in the location of the genital orifice have been documented in other lepidopterous pests such as *Paliga* (syn *Pyrausta/Eutectona*) *machaeralis* (Misra, 1975) and *P. damastesalis* (Intachat, 1999). The mean pupal body length and width was 10.80±0.1 and 2.42±0.1 mm, respectively. The female pupal body was 11.26±0.2 mm long and 2.72±0.1 mm broad while that of the male was 10.36±0.1 and 2.17±0.1 mm, respectively. There was a significant difference between the female and male body length and width ($p<0.01$). The duration of the pupal stage ranged between 5-7 days with a mean of 6.2±0.1 days. The female pupa survived significantly longer ($p<0.01$). than the male (6.5±0.1 and 5.5±0.1 days, respectively.) (Table 5).

Atteva sciodoxa completed its life cycle between 34-52 days with a mean of 45.68±0.6 days. There was a significant difference ($p<0.01$) in the developmental period and life cycle between males and females. The mean male life cycle was 43.6±0.7 days ranging between 34-50 days while that of the female was 47.8±0.7 days, with a range between 38 to 52 days.

CONCLUSION

Atteva sciodoxa are small golden brown moths with a mean wing span of 22.97 mm. The fore-wings and tergum has white spots. The adult lifespan ranged between 5-19 days. The eggs are ovoid, yellow and laid singly. The larval period ranges between 19-22 days with five instar stages. A pair of black dots aligns each dorsal thoracic and abdominal segments of the 2nd to 4th instar larvae. The 5th instar larvae are yellowish-green with black spots on tergites coalesced to form conspicuous black bands. The larval period ranges between 19-22 days. The pupa is orange with black dots and turns reddish-brown prior to adult emergence. The mean body length is 10.80 mm. The pupal stage ranged between 5 to 7 days. The oviposition period is 8.5 days, during which a female lays on average 106 eggs. *Atteva sciodoxa* completes its life cycle between 34 to 52 days.

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