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Biology studies of *Melanitis leda* (Linnaeus, 1758) using Dyar's law

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Abstract
The larvae of *Melanitis leda* were reared under laboratory conditions during September 2016. The study is mainly focussed on the application of Dyar's law in the Lepidopteran pest and to check the geometric progression of the *M. leda*. The eggs were collected and reared in Guinea grass *Megathyrsus maximus* after consequent hatching. The head capsule measurements (widest head width) were taken in the moulted head capsules. The common evening brown undergoes 5 instars with a maximum and minimum head width of 1.123 and 2.78 mm respectively. The mean geometric progression in the growth of *M. leda* was observed to be 1.21.

Keywords: Biology, Butterfly, Dyar's law, Head capsule, Nymphalidae

1. Introduction

Melanitis leda feeds on the different members of the family Poaceae. It is a pest of important crops such as *Oryza indica* and *Zea mays* and it also feeds on different host plants such as *Axonopus compressus, Bambusa arundinacea, Brachiaria mutica, Cyanodon dactylon, Eleusine indica, Paspalidium grminatum, Pennisetum setaceum* and *Sorghum halepensis* [1].

To understand about insects, firsthand knowledge of living, how insects feed, grow, behave, disperse and reproduce should be thoroughly understood. Arthropods are covered by a cuticle that mainly functions as an exoskeleton [2]. In most arthropod species at least some parts of this exoskeleton are strongly sclerotized and unable to expand [3, 4, 5]. The rigidity prevents continuous growth, and such structures, therefore, grow in a stepwise manner with the aid of moulting. Growth of sclerotized parts can be analyzed by assessing their size in successive instars. The stepwise growth results from moulting, i.e. the replacement of the old cuticle by a new one which was formed during the intermoult cycle prior to ecdysis [5]. Growth of sclerotized parts can easily be analysed by assessing their size in successive instars. Many authors studied growth of immature insects [4, 5 6, 7, 8]. Head capsule width has been used frequently for instar determination in species of Lepidoptera [8, 31, 32, 33]. Measurements of head capsule width give basic information for the development of morphometric and ecological studies addressing pest management [34].

Several mathematical models have been used to describe linear measurements of sclerotized parts in successive instars. Linear progression, $y = a+bx$, and the geometric progression $y=a\ bx$ which terms Dyar's rule were often used by entomologists to ascertain the actual number of instars [9]. According to this law, the head capsule of caterpillars grows in a geometric progression, increasing in width at each moult by a constant ratio for a given species. This rule is also applied to many other parts of the body, such as body weight, larval length, length of posterior segment of prothorax, diameter of eye, etc [6]. Another method is frequency, which is a simple and easy method for field population. Instars are indirectly determined through a plot of the number of individuals per size class, where each distinct peak in the plot infers one instar. Rearing insects in controlled conditions that adjusts for the nearly best condition for insect's development and monitoring developmental stages for larval moulting is the best way for studying the life cycle of insects [10]. The number of instars and other information concerning an insect's biology help in the development of phenology models, or in the refinement of existing models. Larval head capsule widths were often used to determine the age of various lepidopteran pests [29, 31, 35, 36]

Dyar's Law assumes that each individual undergoes a geometric progression of head width growth, with constant ratios between post- and pre-moult sizes [6]. Dyar's law will be useful in determining the instars, constant development of the lepidopteran pests'. *M. leda* is an

important pest of rice and maize, biology studies and geometric progression in the growth will hugely help in management strategies, which will help in case of any secondary pest outbreak.

2. Materials and Methods:

2.1 Collection of Adult butterflies

The study was conducted in the Biosystematics laboratory; Tamil Nadu Agricultural university, Coimbatore during September 2016. The adult butterflies were trapped using sleeve traps with fermented banana as the bait [Fig 2-A]. The collected butterflies were released in rearing cages with Rice seedlings, Guinea grass and left for oviposition [Fig 2-B]. The female brown's oviposited on the leaves of the grasses and also in the walls of the rearing cages.

2.2 Biology studies

The eggs were collected after using camel brushes. Single eggs were placed in plastic containers with Guinea grass and labelled properly. The larvae were fed with fresh tender grasses daily and the excreta were removed regularly. The plastic containers were observed daily for the exuvia. The exuvia of all the larvae were collected and stored in Cryo tubes. The head capsule (Exuvial- maximum head width) photographs for all the individuals were taken using Leica stereo zoom microscope (Model: S8APO) and the head capsule widths were measured using LAS4.0. Head capsule measurement data were analyzed using a method presented by McClellan and Logan (1994) [33], in which analysis of a plot of mean instar sizes against a presumed instar number. Summary statistics and linear regression was calculated using Microsoft Excel 2007.

3. Results and Discussion

The total life cycle lasts for nearly 26.50 – 39.50 days and five instars were observed. The egg period was about 2 – 4 days, larval period 13.50 – 21.50 days, pupal period 5 – 7 days and adult 6 – 7 days respectively [Table 2]. The average head capsule width of the 1st, 2nd, 3rd, 4th, and 5th instars were 1.216, 1.58, 1.88, 2.22, and 2.601 mm respectively [Table 1]. The mean geometric progression of the *M. leda* was 1.21. The egg width was observed as 1 -1.3mm. The 1st, 2nd, 3rd, 4th, and 5th instars larval height was observed as 5 – 6.5; 10.4 – 11; 18 – 19.5; 31 – 32.5; 45 – 50 mm and pupal length was 21 – 22 mm respectively. The larvae were pale green in colour with a black head. The larvae have a pair of horn and
tail-like processes in the posterior end. Numerous setae were distributed all over the head capsule. The larva attached itself to the leaf with its tail and then started pupating (pre-pupal stage Fig 3-E). The pupae were also green in colour and it becomes darker later. The adults were dark brown in colour with two prominent eyespots in the apical region of forewings. The upperside of the hind wings with three eyespots in the tornal region. The Underside of the wings pale brown in colour with five small eyespots in the marginal region of forewing. The underside of the hindwing having six distinct eyespots with white pupil surrounded by black and golden colour rings the marginal region (Wet season forms) [Fig 2-G].

Linear regression analysis showed significant relationship between larval instars and head capsule width (R^2 =0.998) (Fig. 1). Thus the head capsule width could be used for the estimation of larval instar in laboratory populations of *M. leda*. Dyar's ratios for the subsequent instars were instars were 1.30; 1.19; 1.17; 1.17 for first to last larval instars.

Our studies indicated that *M. leda* had five larval instars and showed that the head capsule width (exuvia) is useful in separating the larval instars. Observations on the increase in the head width in the successive instars indicate that the dyar's law can be used as a unit of growth in the lepidopterous pest.

Similar results were reported in earlier studies on various insects [5, 11-14, 23, 24] and also in other arthropods such as spiders [15], centipedes [16], and crustaceans [17]. Many authors studied growth of immature insects [5, 6, 7, 8] especially in lepidopterous pest such as *Lymantria obfuscata* [19, 28], *Spalgis epius* [20], *Helicoverpa armigera* [21] *Streblote panda* [25], *Lymantria dispar* [26, 29], *Acrobasis vaccinii* [27], *Cydia pomonella* [30] *Phoenicoprocta capistrata* [31]. The study concludes that the Dyar's law can be used to calculate the number of ecdyses, larval instars' (growth measurement) of different lepidopteran pest which will aid in tackling these pests.

Hutchinson & Tongring (1984) argued that Dyar's rule might result from a maximization of growth efficiency, assuming that the size of the first instar, the number of instars and the arithmetic mean of growth ratios are predetermined [18]. Several factors such as parasitism [35] temperature, food availability [37] locality, and rearing regimes may affect growth rates and morphometrics, either between populations or between individuals of the same population [8]. However, the approximate constancy of growth ratios can as well be seen as resulting from the physiological base of moulting [4, 5].

Dyar's hypothesis (1890) indicates that mean head capsule widths follow a geometrical succession in lepidopteran larval development [6]. Dyar's theory may have more notoriety than utility [38], as it applies in some cases [36, 39] but not in others [35]. A constant geometric relation, in the strict sense, is not a fundamental feature of insect development in general, because growth rates can deviate from linearity when temperatures approach maximum and minimum tolerable ranges [40].Although Dyar's rule is strongly debated in lepidopteran head capsule analysis, the theory as well supports the five instar model for the Common evening Brown's.

Table 1: Geometric growth ratio of *M. leda* larvae using head capsule width as a parameter

Instars	Mean ±SE	Min	Max	SD	Average Instars width	Geometric progression (Dyar's Ratio)
I	1.216 ± 0.02	1.123	1.36	0.086	1.2167	1.300
II	1.58 ± 0.03	1.436	1.745	0.102	1.5818	1.194
III	1.888 ± 0.013	1.803	1.954	0.041	1.8889	1.176
IV	2.22 ±0.02	2.124	2.39	0.07	2.2226	1.170
V	2.601 ± 0.03	2.45	2.78	0.103	2.6018	
				Mean geometric progression	1.21	

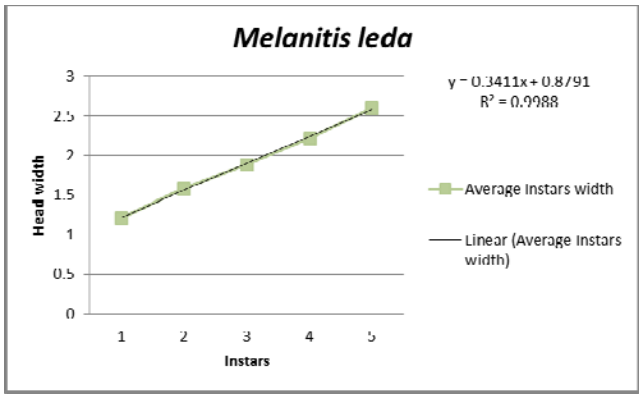

Fig 1: Linear regression

Table 2: Life cycle of *M. leda*

Stages	Duration (Days)	Egg width/ Larval / Pupal Height (mm)
Egg	2 - 4 ± 0.25	1 – 1.3
1st instar	2.5 - 4 ± 0.18	5 – 6.5
2nd instar	2 - 3 ± 0.14	10.4 -11
3rd Instar	2 - 3 ± 0.14	18 – 19.5
4th Instar	3 - 5.5 ± 0.27	31 – 32.5
5th Instar	4 -6 ± 0.24	45 – 50
Pupa	5 - 7 ± 0.19	21- 22

Fig 2: A. Trapping of *M. leda* with sleeve trap; B. Butterfly rearing cage used for mating and oviposition; C, D, E. Egg stage of *M. leda*; F. 1st instar larva.

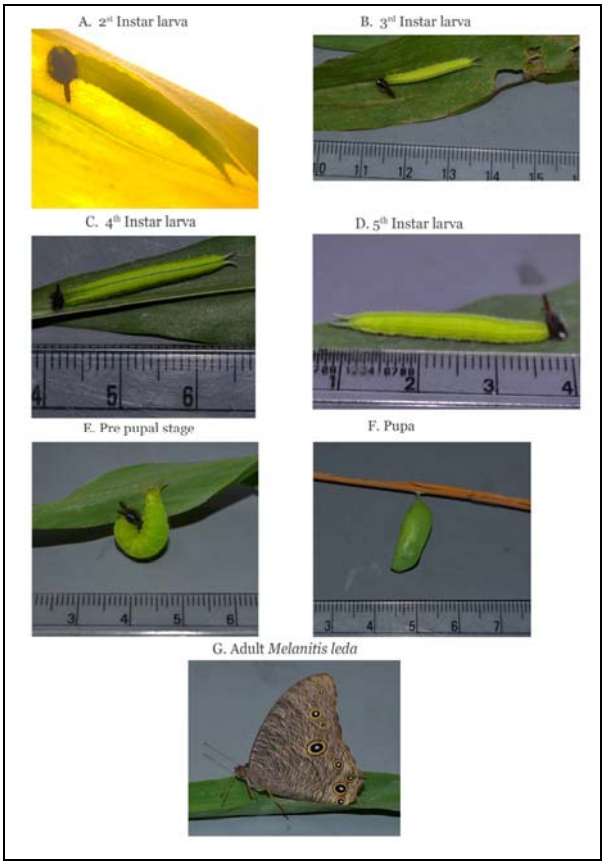

Fig 3: A, B, C & D. Larval stages of *M. leda*; E. Pre pupal stage; F. Pupa; G. Adult butterfly.

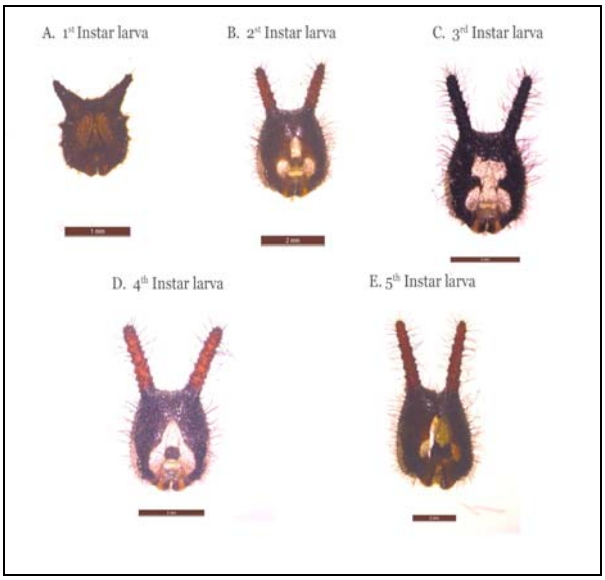

Fig 4: Head capsules (from exuviae) of different instars of *M. leda*

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