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The study of head capsule width of different larval instars of Indian Gypsy Moth *Lymantria obfuscata* Walker in Himachal Pradesh (India)

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Abstract
The mean width (n=5) of the cephalic capsule instar of *Lymantria obfuscata* (Indian Gypsy Moth) was determined. During the course of investigation, average temperature and relative humidity were 19.98±4.53 ºC and 58.46±16.62%, respectively. The results showed that the life cycle of Indian Gypsy Moth has six larval stages. The head capsule width of the 1st instar larvae measured was 0.75±0.03 (Mean ± SD) and the last instar was 4.28±0.04 (Mean ± SD). The growth rate as determined by Dyar's rule, was 1.43. The data recorded may be helpful for further studies on phenology and the control of *L. obfuscata* in the forest ecosystem.

Keywords: Head capsule width, *Lymantria obfuscata*, Dyar (1890).

1. Introduction
Morphometrics is the measurement and analysis of anatomical structures. Early biometricians recognized that insects are advantageous subject for studies of variation [1]. An insect's mode of life is very much reflected in the dimensions and shapes of its exoskeleton. The exoskeleton is easily measured and largely free of the physical distortions suffered by the soft bodies of many other animals [2]. The sclerotized structure of an individual insect is usually assumed to remain constant in size during any given instar [3]. Knowledge of the number of the larval instars and the biological cycle is necessary to understand their population dynamics, determination of community structure [4], and in control programs of pest species where population management targets the larval stage [5].
During June 1976, an outbreak of *Lymantria obfuscata* Walker Indian Gypsy Moth (IGM) was noticed on *Quercus dilatata* Lindl. and *Q. leucotrichophora* Roxb. in Haripurdhar Forest, Rajgarh Forest Division, in Himachal Pradesh. This pest has gained economic importance in Kashmir valley by defoliating trees particularly of poplar, willow, apple and walnut [6]. Another outbreak was reported by Singh *et al.*, in 2005 from Sarahan and Narag in Sirmour district (H.P.), where massive defoliation of oak trees took place [7]. Kumar *et al.*, made investigations on major pest species which caused damage to poplar and willow in cold arid region of Ladakh (J&K) and reported gypsy moth (*Lymantria* spp.) as the major pest [8].
Though, some preliminary observations were made by earlier biologists on the biology [9, 10, 11, 12] and on the head capsule [13] of *L. obfuscata*, but the study on the head capsule width of this pest has not been conducted in detail on ban oak in Himachal Pradesh. So, the present study mainly pertains to the morphometrics of the head capsule width of different larval stages of this pest and to draw a relation with respect to its number of instars.

2. Materials and Methods
The present study on the biology of *L. obfuscata*, was conducted in the laboratory of Forest Protection Division, Himalayan Forest Research Institute (HFRI), Shimla (H.P.), during 2006-2009. The egg-masses collected from the field (Sarahan, Himachal Pradesh), were placed in the laboratory for over-winter storage. As the temperature rose above 20 ºC, the hatching of eggs took place, and the 1st instar neonate larvae were fed on fresh tender leaves of *Q. leucotrichophora* (ban oak) with their petiole wrapped in moist cotton swab placed in a conical flask. The average temperature and relative humidity, during the course of the investigations were 19.98±4.53 ºC and 58.46±16.62%, respectively [Thermo-Hygro Clock M288CTH, Mextech]. The larvae were reared individually so as to determine the respective larval stage.

2.1 Preparation of permanent microscopic slides

During the period of investigation, larvae of different instars were preserved in 70% ethanol in specimen tubes. Their head capsules were warmed in 10% KOH solution, till the inner tissues of the sample were completely dissolved and hence appeared clearer. Afterwards dehydration of the sample was done with ascending grades of 70%, 90% and 100% ethanol for 10-15 minutes each, and finally mounted in Bersale's mounting media. Microscopic observations were made under the Radical stereo zoom microscope [Model RSM-9] with USB Digital Scale 1.1E software in a digital microscopic workstation, and the photography was done with Nikon D80 SLR camera, in order to study the morphometrics of the head capsule of *L. obfuscata*.

The data obtained for each larval instar regarding the average head capsule width (five individuals for five larval instars and two for last larval instar), was further utilized for the determination of growth ratio of respective larval stage and analysis by linear regression.

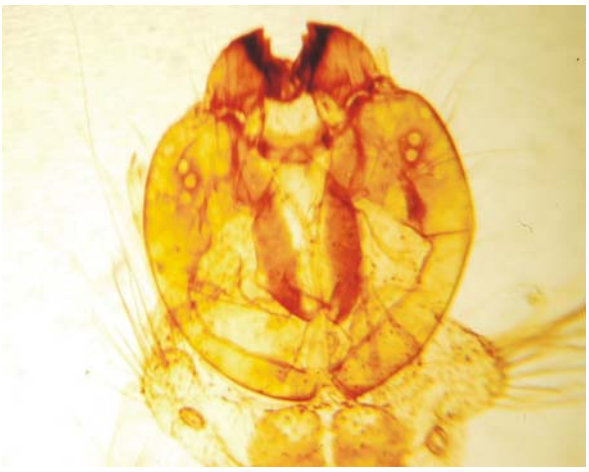
3. Results and Discussion

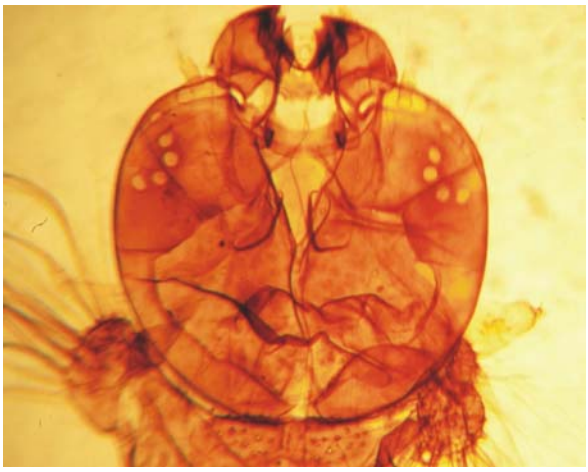
In the present investigation, individual rearing of freshly hatched larva showed that *L. obfuscata* has six larval instars separated by five moulting stages. It was observed that the external morphological characters of each larval instar remained the same but the size and the body colour differed according to the age of the larva. The colour of the head capsule changed from black in neonate larvae to reddish-brown in mature larvae.

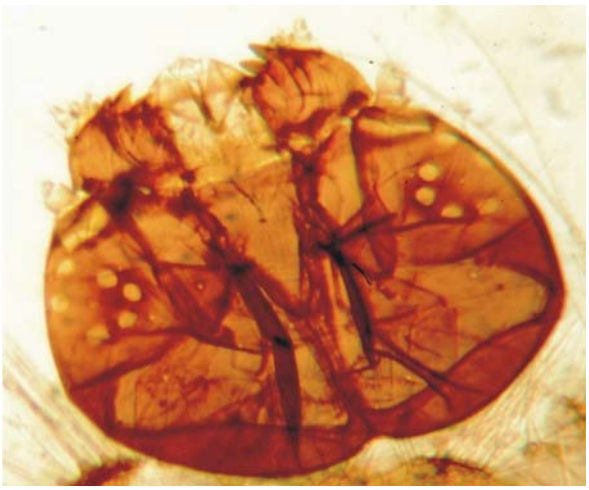
The mean head capsule length and width of 1st, 2nd, 3rd, 4th, 5th and 6th larval instars were recorded as 0.78±0.04 mm and 0.75±0.03 mm; 1.20±0.30 mm and 1.19±0.20 mm; 1.71±0.36 mm and 1.78±0.36 mm; 3.10±0.36 mm and 3.05±0.26 mm; 3.09±0.44 mm and 3.26±0.28 mm; and 4.12±0.42 mm and 4.28±0.04 mm, respectively (Table 1; Figs. 1-6).

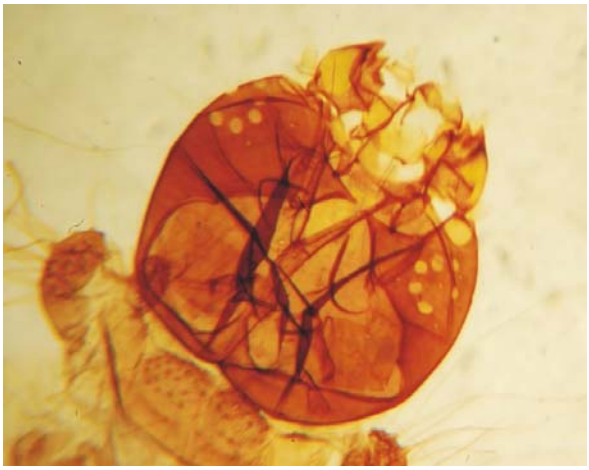
Table 1: Head capsule length and width of different larval instars of *L. obfuscata*

Larval Instar	Head Length (mm)		Head Width (mm)	
	RV	Mean ± SD	RV	Mean ± SD
1st	0.72-0.83	0.78±0.04	0.69-0.79	0.75±0.03
2nd	0.70-1.51	1.20±0.30	0.83-1.32	1.19±0.20
3rd	1.27-2.04	1.71±0.36	1.34-2.04	1.78±0.36
4th	2.74-3.68	3.10±0.36	2.77-3.45	3.05±0.26
5th	2.64-3.65	3.09±0.44	2.96-3.56	3.26±0.28
6th	3.82-4.42	4.12±0.42	4.24-4.31	4.28±0.04









Figs. 1-6 Head capsule of 1st (×45), 2nd (×30), 3rd (×20), 4th (×15), 5th (×10), and 6th (×10) instar larvae of *L. obfuscata.*

Growth ratio of head width in successive instars was determined by dividing the mean head capsule width of the respective instar stage with that of the previous instar stage. The exact number of larval instars was confirmed by measuring the width of head capsule of each larval instar and applying Dyar's law. The head capsule width of larval instars showed successive increase in a specific ratio at each moult. Therefore, the ratio between 1st and 2nd instar was 1.58, 2nd and 3rd was 1.49, 3rd and 4th was 1.71, 4th and 5th was 1.06 and 5th and 6th obtained was 1.31 (Table 2). Hence, the average ratio of increase in head capsule width in each instar was calculated as 1.43, which was further used for the calculation of head capsule width of each larval instar. It showed that the consecutive larval instars followed a regular geometrical progression of 1.43 and the difference between the observed and calculated head capsule width rule out any possibility of missing of any larval stage (Table 2). Further according to Craig, Crosby growth rule can be used to verify the accuracy in the grouping process of the larval instars [14].

Table 2: Average width of head capsule of *L. obfuscata* larvae and growth rate during larval development

Larval Instar	Width of Head Capsule (mm) Mean ± SD	Variation Coefficient (VC)	Growth Ratio	Crosby Ratio (%)
1st	0.75±0.03	0.05	1.58	-5.36
2nd	1.19±0.20	0.17	1.49	14.29
3rd	1.78±0.36	0.20	1.71	-37.55
4th	3.05±0.26	0.08	1.06	22.68
5th	3.26±0.28	0.08	1.31	-
6th	4.28±0.04	0.01	-	-
Mean of growth rate			1.43	

Dyar proposed that the width of the head capsule of a larva follows a regular geometrical progression and any deviation from this calculated progression may be due to manual error or abnormal larval behavior [3]. Therefore, if two sets of observations show a different number of stages for the same insect but each follows its own progression, it should be concluded that this variation is actual. He also recommended that the width of head capsule should be given for each larval stage while studying the insect biology. It predicts that a linear measure of size increases by a constant factor from one instar to the next. When insect growth follows Dyar's rule, growth ratios are commonly of the order of 1.4. A strong linear relationship is predicted between the log of a linear measurement of size and instar number. Previous studies have investigated the use of head capsule widths from various insect species to describe the distribution of each instar. Mcclellan and Logan presented a unique method to classify instars of the gypsy moth *L. dispar* (L.), based on the head capsule width [15].

The equation of a straight line for linear regression, done between the average of head capsule measurements of five individuals for five larval instars and two for last (6th) larval instar, was y = -0.128+0.718x, and $r^2 = 0.97$ (Fig. 1). Pearson's correlation between the mean capsule widths and the larval instars was $r^2 = 0.98$.

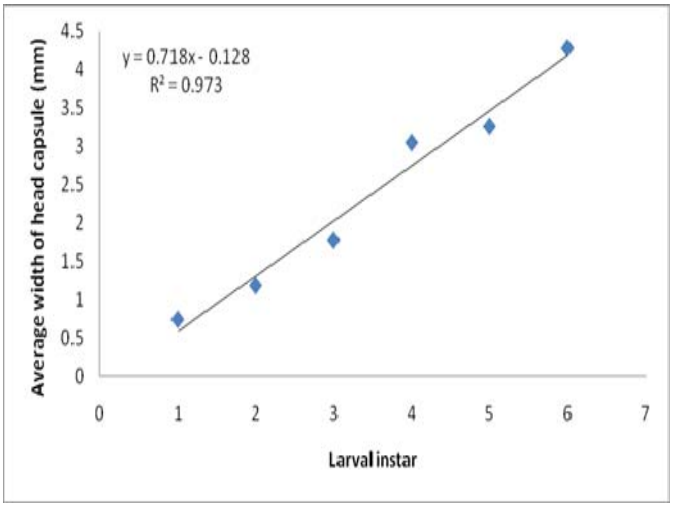


Fig 1: Regression between the average width of head capsule and larval instar of *L. obfuscata*

Adhikari made detailed investigations on the head capsule of the mature larva of *Lymantria obfuscata*, from individuals which were collected from trees growing at an altitude of 5000 ft above sea level [13]. Francisco and Prado conducted the studies with the objective of verifying the average size of each larval instar of *Alphitobius diaperinus* (Panzer) and thus to contribute toward future population surveys of this plague beetle [16].

The average number of moults is four in Lepidoptera, but the number varies considerably, the greatest number was found to occur in *Phyrrarctia isabella* which according to Dyar, moulted ten times [3]. The number of moults is climate dependent, such as insects growing faster in warmer climates consequently often shed their skins, for example, the same species may moult once in the southern than in northern States, as in the case of *Callosamia promethea*, which in West Virginia is double-brooded. Hibernating larvae moult more than those of the summer brood [17]. Dimmock made studies on *Platysamia cecropia*, and concluded that certain species of Lepidoptera can have many or fewer stages according to the conditions or according to the way in which they were reared [18].

Alvan-Aguilar and Hamada studied the number of larval instars in *Simulium rubrithorax* and verified the differences in

the size of last-instar larvae of this species from two different populations (Northern and Central regions of Brazil) [19]. According to Crosskey, the larval development of the black flies is related to environmental conditions [20]. Many factors affect the growth and development of insects, especially temperature, which can influence the development time, the number of larval instars and the larval size at maturity [21]. Holland worked on the life cycle and growth of senita moth, *Upiga virescens* Hulst, and concluded that might be due to selection pressure, nutritional quality and quantity, there was a loss of an instar of this moth and therefore only three larval instars were reported [22].

For holometabolous insects, occurrence of metamorphosis is not determined through the number of instars. Rather, a threshold size determines that a particular instar is the last [23]; and within this instar, a critical weight decides when metamorphosis will occur [24]. The number of instars required to reach this critical weight can vary with the nutritional quality and quantity of food resources [25]. For example, individuals feeding on a nutritionally poor food, such as low nitrogen, may require more instars to reach their critical weight than the individuals feeding on a much higher nitrogen-rich diet [26, 27, 28]. Moreover, the constancy in the quality and quantity of larval food resources may explain why there is a strikingly little variation in growth size (HCW) of larvae within instars and among cohorts in senita moth, *Upiga virescens* [22].

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