

## HEAD CAPSULE GROWTH AND EARLY SEXUAL DIMORPHISM IN *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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**ABSTRACT.** The rate of growth of the head capsule widths in *Culex quinquefasciatus* Say has been ascertained in larvae reared individually in the laboratory. The mean and range of the head capsule widths have been determined for each larval instar. There is no overlap between successive instars, making it possible to distinguish accurately among them.

### INTRODUCTION

Studies of mosquito larval development indicate that larvae originating from a common egg batch grow at different rates under uniform conditions, as observed for *Anopheles quadrimaculatus* Say (Jones 1953). A similar situation was encountered with *Culex quinquefasciatus* Say larvae. A morphometric parameter was measured as a simple and precise method for determining larval instars. Head capsule width was chosen as the variable having the most constant relationship with larval stage. Dyar (1890), by measuring head capsules of caterpillars, showed that the relation between measures of any 2 successive instars was constant, and therefore that the growth of this variable followed a geometric progression. The method has been used since for other insect orders, especially in studies on Hymenoptera (Miles 1931, Taylor 1931, Ghent 1956, de Oliveira 1972). However, few head capsule growth data are available for mosquitoes. In some cases studies are incomplete for such an analysis, lacking precision and sufficient data (Abdel-Malek and Goulding Jr. 1948, Sen and Das Gupta 1958, Danks and Corbet 1973). In other cases data are sufficient but no mathematical description of growth is given (Jones 1953, McDonald et al. 1977).

A complete study was done recently on

A sexual dimorphism appears at the 3rd instar, head capsules being significantly larger in females. The rate of growth of the head capsule with larval instar follows closely Dyar's rule. The exponential function  $Y = 0.200 \cdot 1.563^x$  accords with the data better than a linear function.

*Culex territans* Walker (de Oliveira and Durand 1978) and gave evidence for an exponential growth curve for this species. However, that study dealt with a natural population and measures were made on dead material. For this reason it was impossible to determine whether sexual dimorphism existed at the larval stage.

### MATERIALS AND METHODS

Immediately upon hatching, larvae were isolated in plastic cells, 30 mm. in diameter and 10 mm. deep (tissue culture plates). Rearing was at 30°C with a 14-hour photoperiod. Because food requirements for each larval instar were not known, a fixed quantity of a suspension containing crushed guinea pig granules and alfalfa was added daily to each cell, so that food was always in excess.

Each day, larval molts were collected and placed in 70% ethanol. Head capsules were measured in water, using a microscope equipped with a micrometer standard, at 100X for L<sub>1</sub> and L<sub>2</sub> instars (1 unit = 0.0067 mm) and at 40X for L<sub>3</sub> and L<sub>4</sub> (1 unit = 0.0168 mm). This measurement was taken on the dorsal aspect of the larvae and at the point of maximum head width.

As the larvae pupated, they were isolated in order that we could determine the sex of each adult which emerged.

## RESULTS AND DISCUSSION

The graph of frequency distribution (Fig. 1) shows the 311 measurements taken. Values obtained for each instar are distinct, as shown by the total absence of overlap between them. Ranges observed are: L<sub>1</sub>, 0.282 - 0.342 mm; L<sub>2</sub>, 0.429 - 0.530 mm; L<sub>3</sub>, 0.654 - 0.906 mm; L<sub>4</sub>, 1.091 - 1.292 mm.

Frequency peaks representing the 4 larval instars are quite narrow, indicating little variation of this parameter within each instar. This is confirmed by the small variation coefficients (Table 1). However, measures of L<sub>3</sub> and L<sub>4</sub> are more heterogeneous. Since this could indicate an early manifestation of sexual dimorphism, as postulated for *Cx. territans* by de Oliveira and Durand (1978), we also analysed head capsule width data according to sex.

Results from the Student test (Table 1) show that in L<sub>3</sub> and L<sub>4</sub> instars, the difference between the sexes is highly significant ( $p < 0.01$ ), although in the first 2 instars it is not ( $p > 0.05$ ). The appropriateness of this test is confirmed by the Fisher test, which shows that the variances for the 2 sexes are equal.

Because considerable data were available, an analysis of head capsule growth could be undertaken. A highly significant correlation coefficient ( $r_{yx} = 0.98$ ,  $p < 0.001$ ; d.f. 309) was obtained from the correlation analysis of head capsule width and larval instar. A comparison of estimation methods permits us to decide whether a linear or exponential function better describes larval growth in *Cx. quinquefasciatus*. The linear regression equation is  $Y = 0.285X - 0.027$ , where Y = head capsule width (mm) and X = larval instar, while the exponential equation is  $Y = 0.200 \cdot 1.563^x$ . With both equations it is possible to obtain a predicted head width for the 4 instars (Table 2). Total error reaches nearly 40% using the first equation, while it is only 4% with the second. Therefore, a better fit is given by the exponential function, as illustrated by the exponential curve (Fig. 2), which fits very closely the pattern outlined by the mean

Table 1. Determination of head capsule widths of *Cx. quinquefasciatus* larvae and comparison of observed widths by sex.

Larval instars	Number of measurements (n)	Observed width (mm)	Coefficient of variation (%) (1)	♂		♀		Significance of the difference between sexes
				n	Observed width	n	Observed width	
L <sub>1</sub>	80	0.311 ± 0.013	4.13	30	0.312	21	0.315	(2)
L <sub>2</sub>	88	0.493 ± 0.021	4.22	35	0.490	22	0.497	(2)
L <sub>3</sub>	83	0.775 ± 0.047	6.05	35	0.758	23	0.800	(3)
L <sub>4</sub>	60	1.182 ± 0.053	4.49	36	1.165	21	1.215	(3)

(1) Coefficient of variation  $(V_d) = \frac{100 s_x}{\bar{X}}$  (2)  $p > .05$  (3)  $p < .01$ .

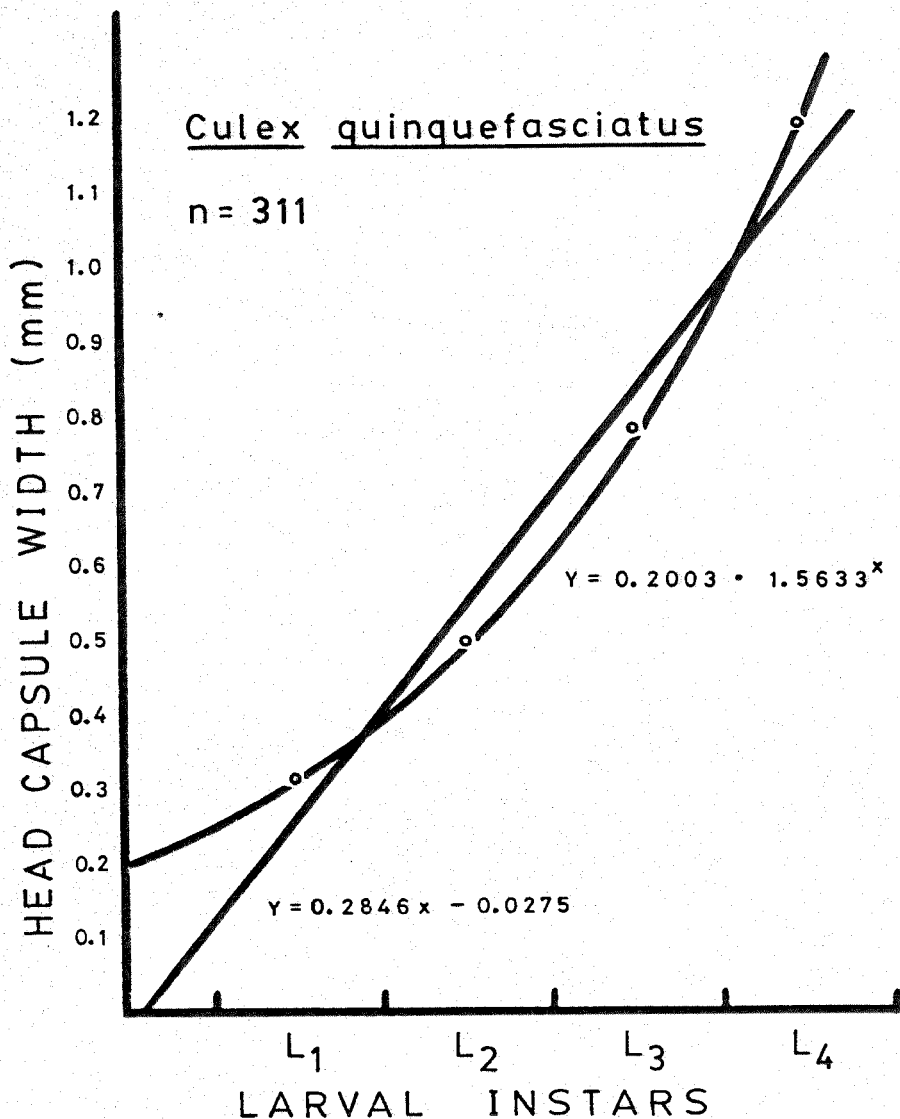


Fig. 1 Distribution of *Cx. quinquefasciatus* larvae according to head capsule width (mm).

Table 2. Comparison of observed head capsule widths of *Cx. quinquefasciatus* larvae with head capsule widths calculated using Dyar's rule and regression analysis.

Larval instars	Observed width (mm)	Exponential Function (Dyar)		Regression	
		Calculated width	% error	Calculated width	% error
L <sub>1</sub>	0.311	0.313	0.7	0.257	17.4
L <sub>2</sub>	0.493	0.490	0.7	0.542	9.9
L <sub>3</sub>	0.775	0.765	1.3	0.826	6.6
L <sub>4</sub>	1.182	1.196	1.2	1.111	6.0
			3.9		39.9
Mean growth ratio = 1.56 (Dyar)		Y = 0.200 • 1.563 <sup>x</sup>		Y = 0.285 X - 0.027	
				r <sub>yx</sub> = 0.98	

values observed for each instar. Similar results had previously been recorded for *Cx. territans* (de Oliveira and Durand 1978).

In spite of these results, it should be remembered that "Dyar's rule" is not an absolute law but rather a tool which is useful but not applicable in every case. For example, some Hymenoptera follow Dyar's rule while others do not (Ghent 1956, de Oliveira 1972). Furthermore, Deshmukh et al. (1977) have shown that variations of temperature and food type can influence the type of growth of the head capsule of a species of Lepidoptera.

### CONCLUSION

The separation of larval instars is necessary for many practical purposes in our work with the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.). For example, mosquito larvae of different instars are exposed to fungal spores. Because the duration of larval development is variable, a separation of larval instars using the larval age was impractical, so we are currently using head capsule width to separate the instars. Non-overlapping values of head capsule width make it possible to determine with certainty the larval instar which is being studied.

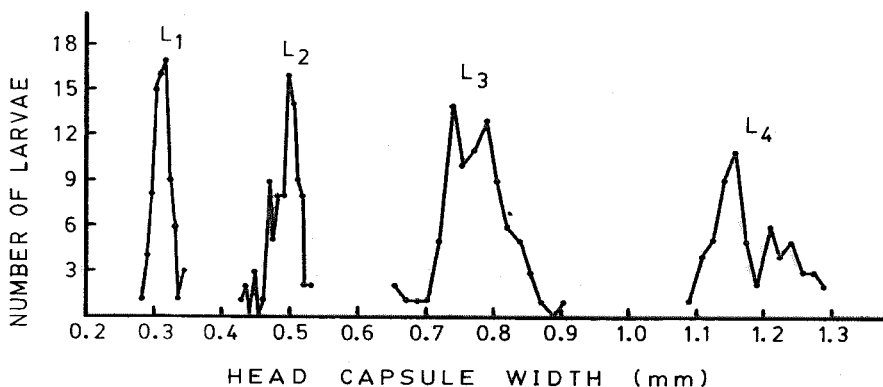


Fig. 2 Head capsule growth in relation to larval development.

In addition to providing us with a useful larval separation method, results on sexual dimorphism and head capsule growth add to our basic knowledge of the species.

#### ACKNOWLEDGMENTS

The authors express their appreciation to Dr. D. de Oliveira for comments, especially on the statistical treatment of data, and to Dr. K. Al-Aidroos for comments and help in translation of the article, as well as for help in the establishment of a full-scale mosquito culture. Thanks are also due to Dr. D. W. Roberts and Dr. A. R. Barr, from whom we received the initial eggs needed, and to all the students who helped take care of the culture. The FCAC programme of the Ministère de l'Éducation du Québec provided research support and also a graduate scholarship for P. Deslongchamps.

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