A STUDY OF THE RATE OF GROWTH OF TWO SCLEROTIZED REGIONS WITHIN LARVAE OF FOUR SPECIES OF MOSQUITOES

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The observations leading to the following study were made independently by the joint authors and arose from two unconnected studies of mosquito larvae in which measurements of the larvae were a part of the routine. Upon discussion and comparison of data it was decided that, in spite of a difference in approach and method, the information should be pooled in order that a more complete study might be presented.

It is the objective of this paper to determine whether the measurements of head capsule width and air siphon length in different instars are so distinctly separated as to allow one to ascertain from these measurements the stadia or number of larval instars present.

MATERIALS AND METHODS

The larvae of *Aedes aegypti* L., *Ae. trivittatus* (Coq.), *Culex apicalis* Adams and *Anopheles quadrimaculatus* Say were measured for head capsule width and, with the exception of *An. quadrimaculatus*, for air siphon length. The larvae of *Ae. aegypti* and *Ae. trivittatus* were killed in K. A. A. D. killing mixture (Peterson, 1943). They were measured under a binocular microscope in which an ocular micrometer was inserted in one of the 15× oculars. In all the first instar larvae examined the objective used was 2× under which the ocular micrometer was calibrated to 0.09 mm per small division. The remaining instars were examined under a 1× objective with the micrometer calibrated to 0.045 mm. The larvae of both species were obtained from reared material, those of *Ae. trivittatus* from the eggs of wild, gravid females and those of *Ae. aegypti* from a culture maintained in the Department of Zoology and Entomology at the Ohio State University.

The larvae of *Culex apicalis* and *Anopheles quadrimaculatus* were killed in a solution containing six parts water, three parts ninety-five percent alcohol and one part formalin. They, too, were measured under a binocular microscope; however, in this case a 10× micrometer ocular was used with the opposite ocular blacked out. An objective of 48 mm focal length was employed in all measurements. The micrometer ocular was calibrated to 0.0216 mm per small division on the scale. The specimens of *C. apicalis* were taken from a field collection of larvae found in a road side permanent pool. Since several previous and succeeding collections from this pool yielded other species of *Culex* only rarely it was considered that all the instars were a satisfactory representation of the single species. The larvae of *An. quadrimaculatus* were taken from a culture maintained in the same laboratory with that of *Ae. aegypti*.

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TABLE I

Aedes aegypti

INSTAR 1

Air Siphon	Head Capsule Width				Total
	202	225	247	270	
180	1	4	0	0	5
202	0	4	1	1	6
225	0	3	2	1	6
247	0	0	1	4	5
270	0	0	5	5	10
Total	1	11	9	11	32

Mean head capsule width: $h = 246$
Standard deviation of head capsule width: $s_h = 20.6$
Mean air siphon length: $a = 231$
Standard deviation of air siphon length: $s_a = 33.4$
Correlation coefficient between head capsule width and air siphon length: $r = .70$

INSTAR 2

Air Siphon	Head Capsule Width				Total
	360	405	450	495	
360	7	15	1	0	23
405	3	14	3	1	21
450	0	3	1	1	5
Total	10	32	5	2	49

$h = 402$
$s_h = 28.5$
$a = 388$
$s_a = 30.3$
$r = .36$

INSTAR 3

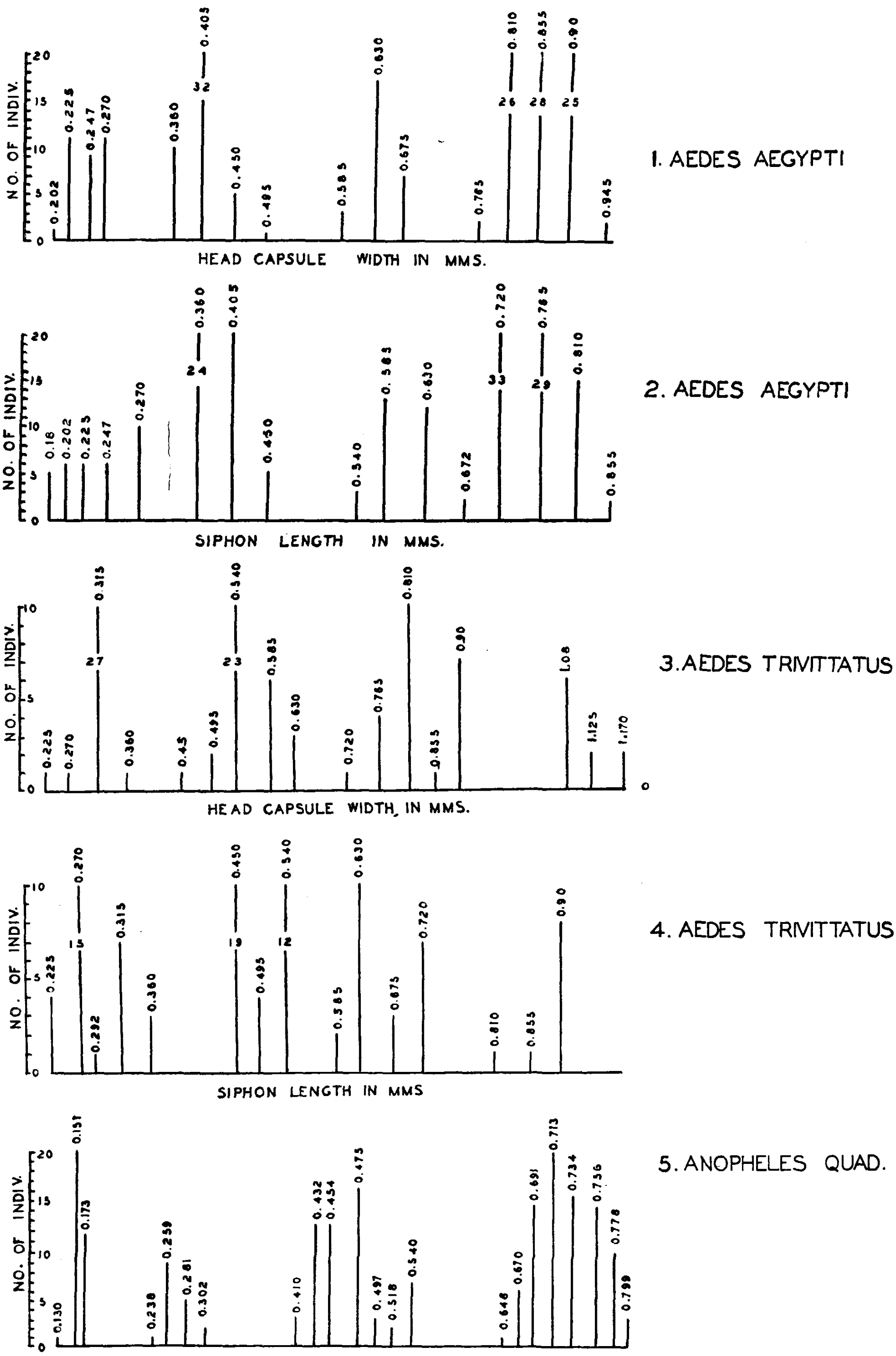
Air Siphon	Head Capsule Width			Total
	585	630	675	
540	0	3	0	3
585	3	9	1	13
630	1	6	6	13
Total	4	18	7	29

$h = 635$
$s_h = 27.8$
$a = 601$
$s_a = 30.1$
$r = .34$

INSTAR 4

Air Siphon	Head Capsule Width					Total
	765	810	855	900	945	
675	0	1	0	1	0	2
720	1	18	11	3	0	33
765	1	7	11	11	0	30
810	0	0	5	9	1	15
855	0	0	1	1	1	3
Total	2	26	28	25	2	83

$h = 854$
$s_h = 40.7$
$a = 756$
$s_a = 40.1$
$r = .51$



FIGS. 1–5. Histograms of the head capsule widths and air siphon lengths of *Aedes aegypti*, *Aedes trivittatus* and *Anopheles Quad.*

TABLE II

Aedes trivittatus

INSTAR 1

Air Siphon	Head Capsule Width				Total	
	225	270	315	360		
225	1	0	3	0	4	h =312
270	0	1	14	0	15	s_h = 20.2
292	0	0	1	0	1	a =284
315	0	0	7	0	7	s_a = 37.6
360	0	0	2	1	3	r = .42
Total	1	1	27	1	30	

INSTAR 2

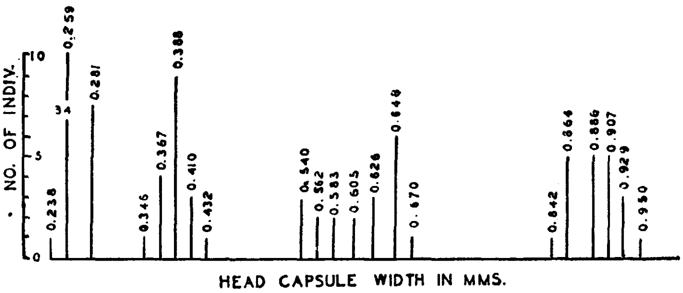
Air Siphon	Head Capsule Width					Total	
	450	495	540	585	630		h =550
450	1	2	14	4	0	21	s_h = 36.3
495	0	0	1	1	0	2	a =483
540	0	0	8	1	3	12	s_a = 42.7
Total	1	2	23	6	3	35	r = .57

INSTAR 3

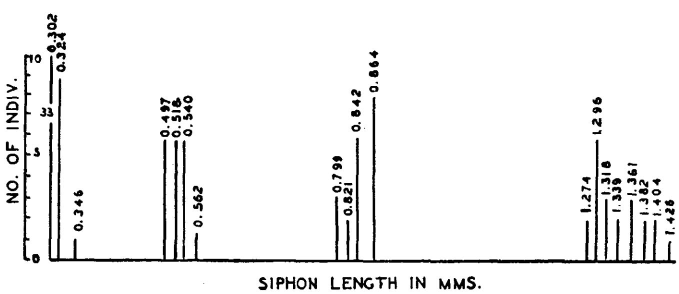
Air Siphon	Head Capsule Width					Total	
	720	765	810	855	900		
585	0	1	1	0	0	2	h =828
630	1	1	6	0	2	10	s_h = 55.5
675	0	1	0	0	2	3	a =667
720	0	1	3	0	3	7	s_a = 55.4
810	0	0	0	1	0	1	r = .32
Total	1	4	10	1	7	23	

INSTAR 4

Air Siphon	Head Capsule Width			Total	
	1080	1125	1170		h =1107
					s_h = 37.9
810	1	0	0	1	a = 887
855	1	0	0	1	s_a = 30.4
900	4	2	2	8	r = .35
Total	6	2	2	10	



6. CULEX APICALIS



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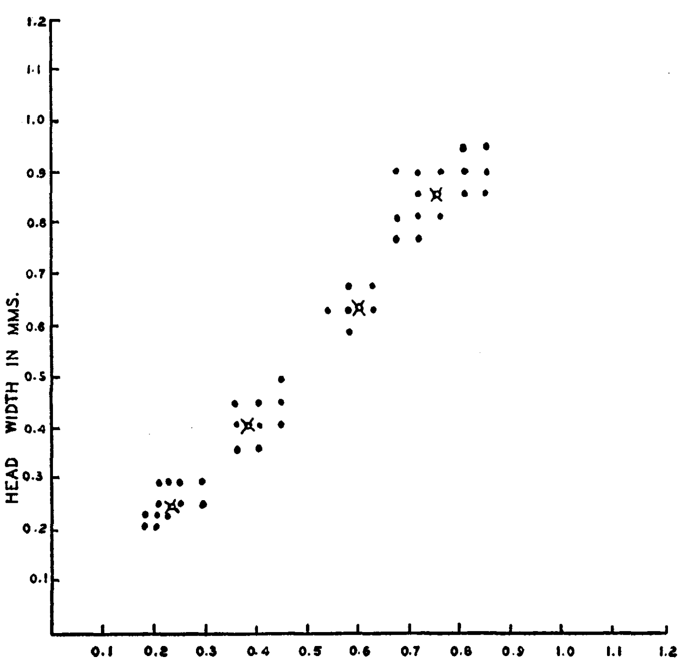


8. AEDES AEGYPTI

FIGS. 6–7. Histograms of the head capsule widths of air aiphon lengths of *Culex apicalia.*
FIG. 8. Scatter diagram of *Aedes aegypti.*

TABLE III

Culex apicalis

INSTAR 1

Air Siphon	Head Capsule Width			Total	
	238	259	281		
302	1	32	0	33	h =262.7
324	0	2	7	9	s_h = 9.33
346	0	0	1	1	a =307.9
					s_a = 10.6
					r = .81
Total	1	34	8	43	

INSTAR 2

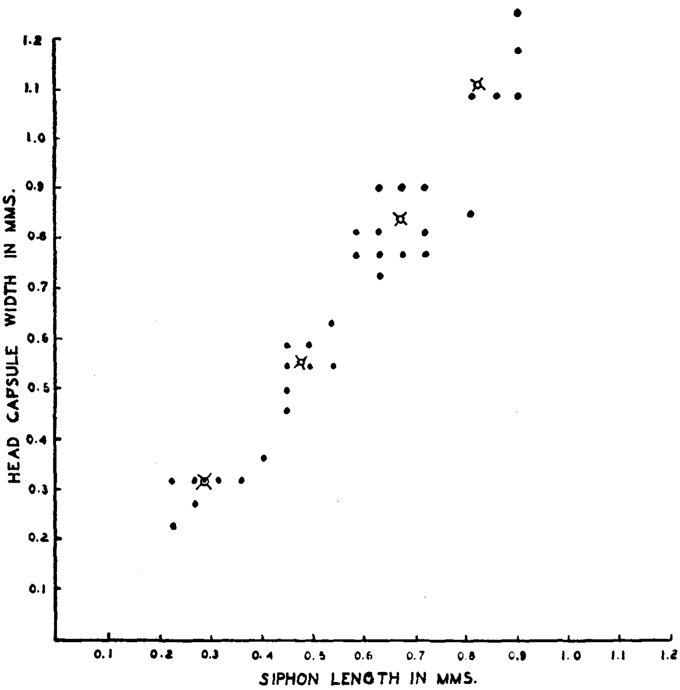
Air Siphon	Head Capsule Width					Total	
	346	367	388	410	432		
497	0	2	4	0	0	6	h =387.6
518	0	2	1	2	0	5	s_h = 20.2
540	1	0	4	1	0	6	a =520.8
562	0	0	0	0	1	1	s_a = 20.8
							r = .33
Total	1	4	9	3	1	18	

INSTAR 3

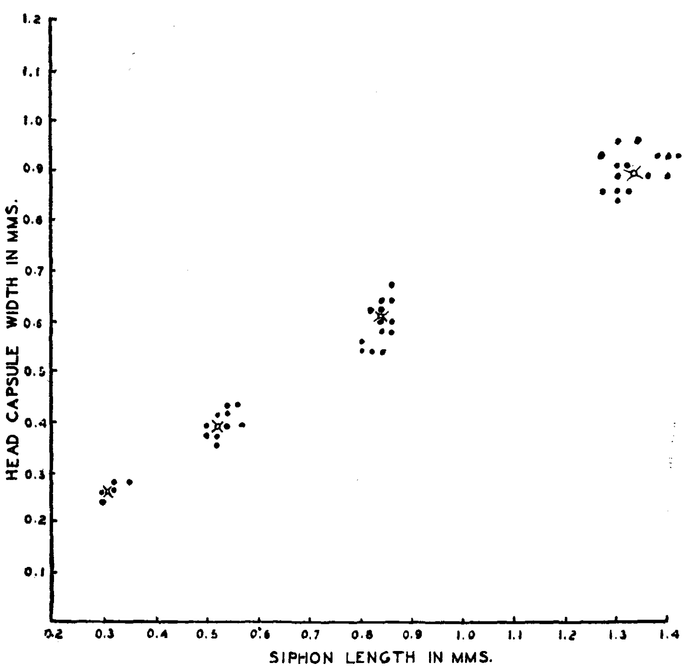
Air Siphon	Head Capsule Width							Total	
	540	562	583	605	626	648	670		
									h =610.2
799	1	2	0	0	0	0	0	3	s_h = 27.7
821	1	0	0	0	1	0	0	2	a =843.5
842	1	0	1	1	2	1	0	6	s_a =237
864	0	0	1	1	0	6	1	9	r =237
Total	3	2	2	2	3	7	1	20	

INSTAR 4

Air Siphon	Head Capsule Width						Total	
	842	864	886	907	929	950		
1274	0	2	0	0	0	0	2	h = 893.8
1296	1	2	1	2	0	0	6	s_h = 27.7
1318	0	1	0	2	0	0	3	a =1335.1
1339	0	0	0	1	0	1	2	s_a = 45.6
1361	0	0	3	0	0	0	3	r = .56
1382	0	0	0	1	1	0	2	
1404	0	0	1	0	1	0	2	
1426	0	0	0	0	1	0	1	
Total	1	5	5	6	3	1	21	



9.AEDES TRIVITTATUS



10.CULEX APICALIS

FIGS. 9–10. Scatter diagrams of *Aedes trivittatus* and *Culex apicalis*.

In the case of the larvae of *C. apicalis*, *An. quadrimaculatus* and *Ae. aegypti* the instars of each species were mixed together and withdrawn at random for measurement. The larvae of *Ae. trivittatus* were taken from predetermined instar groups. One hundred and two examinations were made in the case of *C. apicalis*, one hundred and ninety-six of *An. quadrimaculatus*, one hundred and ninety-two of *Ae. aegypti* and ninety-eight of *Ae. trivittatus*.

Measurements were made of the head capsules of the four groups represented in the region of greatest width, which occurred commonly between the ocular areas.

TABLE IV

Anopheles quadrimaculatus

INSTAR 1

Head Capsule Width			Total	
130	151	173		
1	21	12	34	$h = 158.2$ $s_h = 11.5$

INSTAR 2

Head Capsule Width				Total	
237	259	281	302		
1	9	5	2	17	$h = 269.4$ $s_h = 17.3$

INSTAR 3

Head Capsule Width							Total	
410	432	454	475	497	518	540		
3	13	13	17	3	2	7	58	$h = 467.8$ $s_h = 36.1$

INSTAR 4

Head Capsule Width								Total	
648	670	691	713	734	756	778	799		
1	6	15	21	16	15	10	3	87	$h = 727.2$ $s_h = 34.8$

The air siphons were measured from the point of juncture at the eighth abdominal segment to their tips.

Tables I-IV comprise a presentation of the head capsule and air siphon measurements in the form of frequency tables. The measurements are rounded off to three places and recorded in thousandths of a millimeter. Together with the frequency table will be found the means, standard deviation of the two variables and correlation coefficient.*

*The tables and statistical analysis included herein were prepared by the Statistical Laboratory, Ohio State University, Columbus, Ohio.

From the data in Tables I–IV, Table V has been compiled. The ratios refer to the ratio between the weighted mean of one instar and that of the preceding one. Difference refers to the differences between the weighted means.

TABLE V

	Head Capsule Width			Air Siphon Length		
Instar	Mean	Difference	Ratio	Mean	Difference	Ratio
Aedes aegypti						
1	246			231		
2	402	156	1.634	388	157	1.680
3	635	233	1.580	601	213	1.549
4	854	219	1.345	756	155	1.258
Aedes trivittatus						
1	312			284		
2	550	238	1.763	483	199	1.701
3	828	278	1.505	667	184	1.381
4	1107	279	1.337	887	220	1.330
Culex apicalis						
1	263			308		
2	388	125	1.475	521	213	1.692
3	610	222	1.572	844	323	1.620
4	894	284	1.466	1335	491	1.582
Anopheles quadrimaculatus						
1	158					
2	269	111	1.703			
3	468	199	1.740			
4	727	259	1.553			

DISCUSSION OF DATA

The distribution of the measurements of both the head capsule widths and air siphon lengths were plotted from the data in Tables I–IV. In addition, scatter diagrams were made by plotting the head capsule widths against the siphon lengths for *Aedes aegypti*, *Ae. trivittatus* and *Culex apicalis*.

Upon examination of the histograms (Plates I and II, Fig. 1–7) of both the head capsule widths and air siphon lengths it becomes apparent that these measurements are relatively constant within the four stadia. This observation corresponds with that of Dyar (1890) who, in his study of lepidopterous larvae, found in those examined that the sclerotized parts did not change in area during a stadium but rather the change occurred with ecdysis. In all cases, except the air siphons of the third and fourth instars of *Aedes aegypti*, there is a marked difference between the extremes.

It is considered that the accompanying scatter diagrams (Plates II and III, Fig. 8–10) serve to sum up the entire situation rather adequately. When length of siphon is plotted against head width for all individuals in each instar, it becomes readily apparent that both increase in a definite, clear-cut manner with ecdysis and that no overlapping of extremes occurs except in the case of the air siphons of the third and fourth instars of *Aedes trivittatus*.

Gaines and Campbell (1935) in their study of the number of instars in the corn earworm, *Heliothis obsoleta* (Fab.), used the ratio of the means of measurements of successive instars as a means of judging the progression of head capsule widths. They found, as did Ripley (1930) and Forbes (1934) with other lepidopterous larvae, that the greatest proportionate growth tended to fall between the first and second instars and the least between the pennultimate and the last. This also appears to hold true for the four groups of larvae considered here. An exception does occur in the head capsule ratios of *An. quadrimaculatus* and *C. apicalis* in which the ratios of the second and third instars are greater than those of the first and second instars; however, since the other five sets of ratios show a consistent decrease in ratio and since in no case is the last ratio greater than those preceding it, an experimental error rather than a deviation from the expected trend may be inferred.

The coefficients of correlation (r) indicate only a low degree of correlation between the size of the head capsules and air siphons. From this evidence it is not possible to predict size of an individual head capsule from the size of the air siphon or vice versa within a stadium.

Although there is an apparent and distinct increase in head capsule width and air siphon length between each instar and its succeeding one, it is not possible to state the nature of this increase in terms of regular progression, either geometric or arithmetic. This condition may be due to the fact that our time increment was determined arbitrarily and not with consideration of the time spent by each individual larva in each of the four stadia.

For practical purposes, the means of the head capsule widths of all the species studied and the air siphon lengths of the culicid species may be used as a criterion for determination of the instar. Although the character of the head capsule is known to be used by many field workers as an indication of instar, no stated corroboration of this practice was found in the literature in hand.

SUMMARY

The head capsule widths and air siphon lengths of the species of mosquito larvae herein examined are relatively constant within the four stadia. There was, with one exception, no overlapping of the extremes of measurements between instars.

The ratio between means of measurements of successive instars tends to decrease with the advancement of the larval instars.

Correlation is low between the size of head capsule and air siphon within an instar.

Determination of the nature of the rate of growth during successive molts cannot be completely assayed without an accurate determination of the time spent by each larva in each stadium.

For practical purposes the width of the head capsule and/or the length of the air siphon in each of the species considered may be used in determination of the stadia and number of instars.

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