CHANGES IN COMB SPINE NUMBER DURING LARVAL DEVELOPMENT IN *AEDES AEGYPTI* (L.)

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ABSTRACT. 1. In the first larval instar of *Aedes aegypti* (Bangkok Din Dang strain) the comb carried 2–5 spines (mean 4.1). Plotted as a frequency distribution, comb spine number showed a single mode at 4 spines but the distribution was not normal.

2. The mean spine number doubled to 8.2 in the second instar, the range being 5–10, and the frequency distribution bimodal, with a principal mode at 8 and a lesser one at 10. At the level of the individual comb, the number sometimes increased by more or less than a factor of 2, or in a few cases remained the same.

3. The mean spine number increased to 8.5 at instar 3 and 8.6 at instar 4. The bimodality was maintained, being most distinct at instar 3. The principal mode continued to be at 8 spines in the final instar, although there was some move towards 10 spines.

4. The bimodality at instars 2–4 seems to have arisen partly as a result of a doubling in spine numbers from instar 1 but also partly because of a tendency for spine number to move towards 8 spines, and to a lesser extent towards 10 spines, during development. Thus the bimodality arises as a property of the developmental system.

The combs are two rows or patches of spines found on the eighth abdominal segment of larvae of Culicine mosquitoes. The function of the combs is not known, but they may be employed in cleaning the head setae and particularly the mouth brushes (Hopkins 1952). In *Aedes aegypti* they consist of 2 single rows (left and right) of broad, slightly curved spines or scales directed posteriorly. They somewhat resemble the thorns of a rose except that there are lateral spines on the main "thorn" towards its base (Figs. 1 and 2).

The number of spines has been counted at the 4th instar and found to vary between 4 and 18 (La Casse and Yamaguti 1955, Wood 1975). However, the distribution of spine number is not normal but bimodal at 8 and 10 spines (Wood 1975). Combs with other than 8 or 10 spines are relatively unusual. There is a low but significant correlation in spine number between left and right sides.

The aim of this study has been to examine the basis of the bimodality by counting spines from the 1st instar onwards. As a working hypothesis it was proposed that the bimodal distribution at the 4th instar could have arisen from a unimodal one by a doubling of the spine number at an earlier stage. Thus, supposing most combs at the 1st instar carried 4 or 5 spines, this would lead to most combs at subsequent instars carrying 8 or 10 spines. In support of this hypothesis was the observation by Christophers (1960) that spine number in the 1st instar is approximately half that of subsequent instars: about 5 in the 1st instar, increasing to 8 or 10 in the 2nd, with some further increase in number later.

MATERIAL AND METHODS. The strain of *Aedes aegypti* investigated was Bangkok Din Dang, one of those studied by Wood (1975). The comb spines were counted on cast larval skins. First stage larvae were isolated in 4 x 1 inch tubes immediately after hatching, and there reared through the 4 instars to the pupal stage when they were sexed. Eggs were hatched in an infusion of hay; larvae were reared at $28 \pm 1°$ C and fed on powdered dog biscuit.

Only complete sets of spine numbers were used in the final analysis of results. For every instar and both sides of the body there were 8 counts. Sets of data which had counts missing for any reason were disregarded.

To prepare the comb for examination with the scanning electron microscope, larval skins were slowly air dried in dust-free conditions, attached to aluminum stubs and gold coated. The instrument used was a Cambridge "Stereoscan" Mark 2.

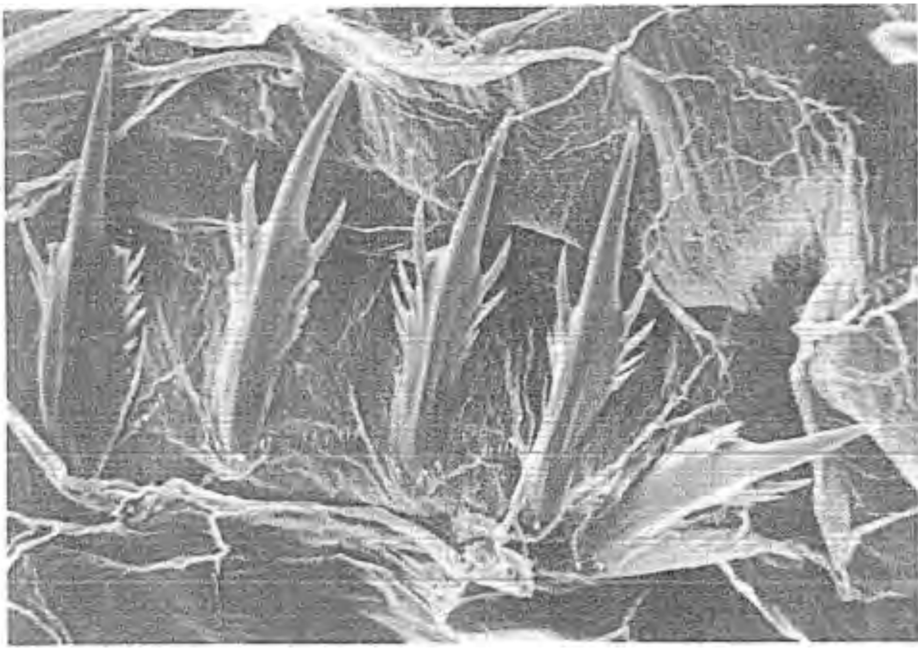

FIG. 1. The comb of *Aedes aegypti* fourth larval instar (X Ca. 160). Two "guide hairs" are included, numbered after Belkin (1962).

RESULTS. The distribution of spine number in the 4th instar was bimodal (Table 1). A total of 160 combs was examined; 104 (65%) carried 8 spines and only 13 (8.1%) carried other than 8 or 10. The distribution of spine number in instars 3 and 2 was also bimodal although the mean number was lower. In the 3rd instar 69.4% carried 8 spines and 6.9% carried other than 8 or 10. In the 2nd instar the corresponding figures were 61.3% and 17.5%. Thus the bimodality was most extreme in instar three.

The mean spine number doubled (from 4.1 to 8.2) between the 1st and 2nd instar, and increased slightly further in instars 3 (8.5) and 4 (8.6). The change in spine number between instars is shown in more detail in Table 2. It is evident that the doubling of the mean spine number between instars one and two is not always reflected by what happened at the level of the individual comb. Sometimes the number more than doubled (e.g., 2 to 6, 3 to 7 or 8 or 10, 4 to 9 or 10), sometimes it less than doubled (e.g., 4 to 6 or 7, 5 to 6 or 8 or 9), or even, in the case of some combs with 5 spines, remained unchanged. Overall we found substantially more eights in the 2nd instar than would occur by a simple doubling, and substantially fewer sixes and tens. It appears that development is directed towards 8 spines.

In a few combs the spine number doubled between the 2nd and 3rd instars but usually increased only slightly (e.g., 5 to 8 or 10, 6 to 8 or 9, 7 to 8 or 10, 8 to 9, 9 to 10). Occasionally spine number decreased between 2nd and 3rd instars (e.g., 10 to 8, 9 to 8). Note again the tendency to move towards 8 spines.

Further increases occurred in some combs between the 3rd and 4th instars (e.g. 6 to 8 or 10, 7 to 8, 8 to 9 or 10, 9 to 11, 10 to 12). In some cases the number was reduced (e.g., 9 to 8, 10 to 7, 8 to 7). Spine number was rather more variable in the 4th than in the 3rd instar and there

Table 1. The frequency of different comb spine numbers and the mean spine number in the four larval instars of *Aedes aegypti* (Bangkok Din Dang strain).

Spine number	Instar (left/right side) 1 L	1 Total	1 R	2 L	2 Total	2 R	3 L	3 Total	3 R	4 L	4 Total	4 R
2	2	(3)	1									
3	15	(28)	13									
4	41	(77)	36									
5	22	(5)	30	2	(3)	1						
6				5	(10)	5	0	(2)	2	0	(1)	1
7				2	(8)	6	0	(1)	1	1	(3)	2
8				51	(98)	47	54	(111)	57	52	(104)	52
9				3	(7)	4	5	(8)	3	4	(7)	3
10				17	(34)	17	21	(38)	17	22	(43)	21
11										0	(1)	0
12										1	(1)	0
Number of Combs	80	(160)	80	80	(160)	80	80	(160)	80	80	(160)	80
Mean spine number	4.0	(4.1)*	4.2	8.2	(8.2)*	8.2	8.6	(8.5)*	8.4	8.6	(8.6)*	8.6

* (L+R)/2.

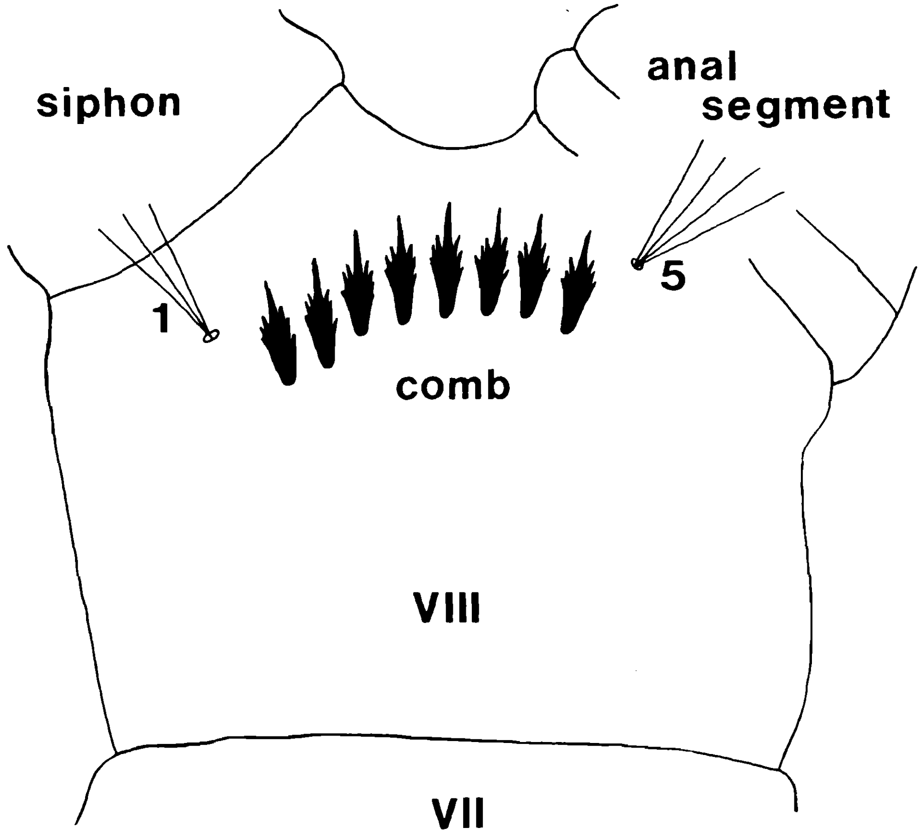

FIG. 2. Scanning electron micrograph of a fourth instar larva showing detail of spines (Ca. 720).

appeared to be some move away from 8 spines and towards 10 spines.

DISCUSSION. The distribution of spine number in the 1st instar showed a single mode at 4 spines, although the frequency histogram did not appear normal for the sample examined. At the 2nd, 3rd and 4th instar, spine number showed a bimodal distribution, with a principal mode at 8 spines and a lesser one at 10.

The mean spine number precisely doubled between the 1st and 2nd instars (from 4.1 to 8.2), but this doubling did not wholly explain the bimodality. This became evident from examining the progression in spine number through the instars within single combs. Thus, of the 98 combs with 8 spines at instar two, only 61 (62%) had 4 spines at instar one. Nevertheless, doubling must be a contributing factor. On the basis of chance alone, with no relation between spine number at 1st and 2nd instars, the expected proportion of 2nd instar combs with 8 spines coming from 1st instar combs with 4 spines would be lower, i.e., 48%, the frequency of 1st instar combs with 4 spines. The observed and expected values are significantly different ($\chi^2=8.01$, $P<0.01$), indicating that combs with 8 spines are more likely to derive from those with 4 than would happen by chance. The same is true in relation to combs with 10 spines in the 2nd instar. The proportion derived from 5-

Table 2. Changes in comb spine number between the first and subsequent instars. The frequencies of combs with different spine numbers are shown in the body of the table.

Number of spines on the comb of the first intar	Instar	Spine number										
		2	3	4	5	6	7	8	9	10	11	12
2	1	3										
	2					3						
	3							3				
	4							2		1		
3	1		28									
	2					4	3	20		1		
	3					2	0	25		1		
	4					1	0	25		2		
4	1			77								
	2					2	5	61	3	6		
	3						1	62	7	7		
	4						3	58	5	10	1	
5	1				52							
	2				3	1	0	17	4	27		
	3							21	1	30		
	4							19	2	30		1
2–5	1	3	28	77	52							
	2				3	10	8	98	7	34		
	3					2	1	111	8	38		
	4					1	3	104	7	43	1	1

spined 1st instar combs is 27/34 (79%) compared with 33% expected by chance. The difference between observed and expected is highly significant ($\chi^2=33.24$, $P<0.0005$).

By the 4th instar, only 58/104 (56%) of combs with 8 spines were derived from 1st instar larvae with 4 spines, which is not significantly different from what would have occurred by chance ($\chi^2=2.51$, $P>0.1$), whereas 30/43 (70%) of combs with 10 spines were derived from 1st instar larvae with 5, which is significantly different from what would be expected by chance ($\chi^2=26.25$, $P<0.0005$).

We may conclude that doubling of the spine number between the 1st and 2nd instars is an important contributory factor to bimodality in spine number distribution in instars 2 to 4. However, there also seems to have been an overriding tendency for spine number to move towards 8 (and to a lesser extent towards 10) at instars 2 to 4, whatever the number had been in the 1st instar.

In the final instar, 65% of combs carried 8 spines and only 8.1% other than 8 or 10. The present study was made in 1973; a comparable study on 4th instar larval skins, made in 1970 (Wood, 1975) in the same laboratory and under the same conditions gave corresponding values of 53.2% and 14.7%. Another strain, Trinidad-30, also examined in 1970, gave values of 56.3% and 14.9% (Wood, 1975). It may be concluded that bimodality is consistent between strains tested together but may be somewhat modified by environmental factors as yet undetermined.

Wood (1975) demonstrated that the bimodality was not due to sexual dimorphism and that it was unlikely that gene segregation was involved. Rather the evidence pointed to a developmental causation, i.e. that bimodality arose as a property of the developmental system. The present evidence seems to confirm this.

The range of comb spine number in the fourth instar larva in the present study is 6 to 12, precisely similar to that in the study of Wood (1975) when, as now, larvae were reared individually in tubes. However, Wood found an interesting difference in larvae reared in mass culture: the range was greater (Bangkok Din Dang: 4 to 18, Trinidad-30: 5 to 12) although the bimodality was still just as evident. Other authors have also found a wide range in larvae reared *en masse* e.g. 8 to 18 by La Casse and Yamaguti (1955), although most authorities (e.g., Christophers 1960) quote a range of 8–12. The factors responsible for the difference between tube- and mass-reared larvae in our study have yet to be determined.

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