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Larval and Pupal Chaetotaxy of *Anopheles nuneztovari* (Diptera: Culicidae)

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ABSTRACT

The chaetotaxy of three cytotypes of *Anopheles (Nyssorhynchus) nuneztovari* larvae and pupae was investigated. Cytotype A larvae have seta 8-M with fewer branches on average than do cytotype B and C larvae. Five setae were found to differ between male and female cytotype B larvae (5-C, 6-C, 6-Mx, 1-M, 0-VI). No easily discernable chaetotactic differences were found among pupae of cytotypes A, B, and C. A complete account of the chaetotaxy of the three cytotypes of *An. nuneztovari* larvae and pupae is given.

INTRODUCTION

The immature stages of anopheline mosquitoes often are more easily identified than are adult females (Faran 1980), and larval chaetotaxy is important for phylogenetic studies and species identification (Wolff & Nielsen 1977). The pupal stage of anopheline mosquitoes many times has been given cursory treatment in taxonomic studies, even though excellent characters may be found (Harrison & Peyton 1984). In recent years more attention has been paid to pupal taxonomy, e.g., the Gambiae Complex in Africa (Reid 1975a,b; Coetzee & Newberry 1980).

Anopheles nuneztovari Gabaldón has been implicated as a vector of human malaria parasites in a number of areas in South America, including Brazil, Peru, and Venezuela (Arruda et al. 1986, Gabaldón & Guerrero 1959, Hayes et al. 1987). Kitzmiller et al. (1973) described geographic variation of chromosomal banding patterns and determined that two forms were present. Conn (1990) called these two forms A (Amazonian) and B (western Venezuela southeast of the Andes); later she and coauthors described a third form, C, from Colombia and western Venezuela northwest of the Andes (Conn et al. 1993).

In his revision of the Albimanus Section of the subgenus *Nyssorhynchus*, Faran (1980) illustrated the larva and pupa of *An. nuneztovari* and described the taxonomically important setae, mentioning that larval specimens from Venezuela appeared to have fewer setal branches compared to larvae collected from other areas. This was in contrast to Root (1932) who stated that larval chaetotaxy did not appear to be very useful in the taxonomy of *Nyssorhynchus* mosquitoes. This study was initiated to determine whether differences in setal branching patterns exist among larval and pupal specimens of cytotypes A, B, and C, and to record the setal branching patterns of all setae.

MATERIALS AND METHODS

Host-seeking females of the three known cytotypes of An. nuneztovari were collected from the following localities: CYTOTYPE A - Puraquequara, Brazil (PR), and Victoria, Suriname (VC); CYTOTYPE B - Caño Amarillo, Venezuela (CA); CYTOTYPE C - Sitronela, Colombia (SI), and Río Socuavó, Venezuela (RS). Complete collection data for all sites has been published elsewhere (Hribar 1994). Females were transported to the laboratory in Vero Beach, provided with a blood meal, and permitted to oviposit. Eggs were hatched and progeny broods were reared in enamel pans at 26° C. Larvae were fed a mixture of liver powder and brewer's yeast. Fourth instars were removed from the pans and reared individually in 9 dram vials in order to associate larvae with known adult specimens. After pupation, larval exuviae were removed from vials and stored in ethanol. Pupae were permitted to develop until adult emergence, after which pupal exuviae were removed from vials and mounted on microscope slides with associated larval exuviae. Numbers of larval exuviae examined per collection site were, CA, 56; PR, 31; RS, 12; SI, 7; VC, 29. Total numbers of larval exuviae examined per cytotype were, A, 60; B, 56; C, 19. Numbers of pupal exuviae examined per collection site were, CA, 30; PR, 30; RS, 12; SI, 18. Thirty pupal exuviae were examined per cytotype.

Larvae.—Methodology generally followed that of Reid (1973), i.e., three larval exuviae from each collection site were examined and all branches of all setae were counted. Those setae that appeared to be different among collection sites were counted on a larger number of exuviae. The larval exuviae of CA specimens were matched with corresponding adults to determine sex. Setal branching differences between females and males were examined by using t-tests (SAS TTEST procedure) (SAS Institute 1985). In some cases there were unequal sample variances, in which case Satterthwaite's (1946) formula was used, resulting in noninteger degrees of freedom (SAS Institute 1985). Chaetotaxy of larvae of all cytotypes is presented in tabular form and is based on no fewer than three specimens per seta. Nomenclature for setae is that used by Harbach and Knight (1980). The seta that Faran (1980) called "bmh" has a long list of synonyms but actually is seta 6-Mx.

The utility of seta 8-M for distinguishing differences among the three cytotypes was determined by analysis of variance (SAS GLM procedure) and mean separations were performed by the Ryan-Einot-Gabriel-Welsch multiple range test (SAS Institute 1985). This seta was chosen for attention because it is conspicuous and branches are easily counted.

Pupae.—Methodology generally followed that of Reid (1975a,b), i.e., five exuviae from each collection site were examined and all branches of all setae were counted. A preliminary statistical analysis was conducted on all setae, and four setae differed among collection sites, viz., 2-I, 0-II, 2-IV, and 4-VII. These then were counted on a larger number of exuviae.

Differences were examined by analysis of variance and mean separations were performed by the Ryan-Einot-Gabriel-Welsch multiple range test. Chaetotaxy of pupae of all cytotypes is presented in tabular form and is based on no fewer than five specimens per seta.

RESULTS

Five setae were found to have differing numbers of branches for female and male larvae of CA specimens (Table 1). Three of these are cephalic setae (5-C, 6-C, and 6-Mx), one is thoracic (1-M), and one abdominal (0-VI). There is a large amount of overlap in number of branches between male and female specimens for all setae, i.e., 5-C (♀, 12-19; ♂, 11-17), 6-C (♀, 13-20; ♂, 11-17), 6-Mx (♀, 3-10; ♂, 3-6), 1-M (♀, 16-33; ♂, 24-31), and 0-VI (♀, 3-8; ♂, 3-5).

The branching pattern of 191 pairs of larval setae is presented in Table 2. Many setae present a wide range of variation in setal branching number, whereas others exhibit less variation in number of branches, and still others are constant in number of branches. Cytotype A larvae had seta 8-M with fewer branches than did cytotype B or C larvae ($F = 8.18$; df $= 2,106$; $P < 0.0005$) (Table 3).

The branching pattern of 96 pairs of pupal setae is presented in Table 4. Once again, many setae present a wide range of variation in setal branching number. When larger numbers of setae were counted, the differences seen for setae 2-I and 0-II were no longer significant. Apparent differences were seen for setae 2-IV and 4-VII, such that seta 4-VII appeared to have a greater number of branches on cytotype A pupae than did cytotype B and C pupae. However, when the data were reexamined, it was discovered that one specimen had an abnormally large number of branches for this seta relative to all other specimens. When this specimen was removed from the dataset and the analysis was rerun, there were no differences in number of branches for seta 4-VII among the three cytotypes. This seta is not included in the range given in Table 4.

Seta 2-IV is 1-3 branched for all cytotypes (Table 4). Cytotype C has a greater frequency of single branched setae than do cytotypes A and B; however, it is not possible to distinguish among the three cytotypes by using this seta.

DISCUSSION

The branching pattern of setae examined in this study appears to agree closely with those reported by Faran (1980) and Delgado and Rubio-Palis (1992), despite some statistical differences between sexes and among cytotypes. Environmental factors are known to affect some mosquito larval structures (Mattingly 1975). Minute particulate matter in the larval habitat can result in changes in size and numbers of branches of some setae of *Aedes aegypti* L. & *Ae. albopictus* Skuse (Colless 1956). The source of the differences in branching of setae is not known, but perhaps rearing larvae in the more confined space of vials rather than in more spacious pans could have such an effect. The wide range of overlap of setal branch numbers among the three cytotypes of *An. nuneztovari* indicates that setal branch numbers may be of limited utility for distinguishing among cytotypes.

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Table 1. Setal branching differences between female and male fourth instar *An. nuneztovari* larvae from Caño Amarillo, Venezuela.

Seta	Sex	N	Mean No. Branches	*t* value	df	P
5-C	♀	21	16	3.824	38.5	0.0005
	♂	20	13.9			
6-C	♀	21	16.7	3.803	39.1	0.0005
	♂	21	14.2			
6-Mx	♀	11	6.6	2.234	16.8	0.0322
	♂	11	4.8			
1-M	♀	22	29.3	2.113	40	0.0409
	♂	20	27.4			
0-VI	♀	21	4.8	2.339	35.1	0.0252
	♂	18	4.2			

Table 2. Chaetotaxy of *An. nuneztovari* fourth instar larvae.

	Cytotype		
Seta	A	B	C
Head			
0-C	1	1	1
1-C	1	1	1
2-C	1	1	1
3-C	1	1	1
4-C	1-5	1-5	2-4
5-C	12-19	11-19	13-18
6-C	13-19	11-20	14-21
7-C	14-23	15-24	19-26
8-C	2-3	2-7	3-6
9-C	3-8	2-9	4-7
10-C	2-3	2-5	3-4
11-C	24-45	24-48	29-43
12-C	2-5	1-8	3-7
13-C	3-6	2-6	4-5
14-C	2-4	2-6	2-3
15-C	1-4	1-4	1-5
6-Mx	3-8	3-10	5-9
Antenna			
1-A	4-7	3-9	4-8
2-A	1	1	1
3-A	1	1	1
4-A	2-3	2-4	2-3
6-A	1	1	1
Prothorax			
0-P	1-2	1-2	1
1-P	10-14	10-13	11-16
2-P	13-19	12-19	15-18
3-P	1	1	1
4-P	14-22	14-23	13-19
5-P	18-33	17-33	25-27
6-P	1	1	1
7-P	26-37	20-37	33-37
8-P	22-36	21-36	26-38
9-P	1	1	1
10-P	1	1	1
11-P	2	1-2	2
12-P	1	1	1
13-P	3-4	3-7	3-4
14-P	6-9	4-9	8-10
Mesothorax			
1-M	16-33	16-33	25-33
2-M	1-3	1-4	1-3
3-M	1	1	1
4-M	2-3	2-4	3-4
5-M	1	1	1
6-M	2-5	2-3	2-4
7-M	2-4	2-7	2-5
8-M	20-29	19-32	19-31
9-M	1	1	1
10-M	1	1	1
12-M	1	1	1
13-M	3-9	3-8	6-12
14-M	6-11	5-12	7-11
Metathorax			
1-T	1	1	1
2-T	1	1	1
3-T	9-18	10-17	13-17
4-T	3-4	2-4	4
5-T	26-39	21-41	37-39
6-T	2-6	2-4	2-3
7-T	30-37	29-42	31-35
8-T	28-41	31-43	26-38
9-T	1	1	1
10-T	1	1	1
11-T	2	2-3	2-4
13-T	2-3	2-5	2-4
Abdominal Segment I			
1-I	12-18	10-18	14-21
2-I	3-5	2-5	3-5
3-I	1	1	1
4-I	3-6	3-6	3-5
5-I	2-5	2-4	3-4
6-I	26-37	25-37	28-34
7-I	27-38	21-38	26-31
9-I	3-10	3-10	4-6
10-I	1	1	1
11-I	2-6	2-6	3-4
12-I	1-3	1-3	2
13-I	5-9	4-9	6-8

Abdominal Segment II			
0-II	4-7	5-6	4-7
1-II	19-25	16-25	17-24
2-II	2-7	3-7	3-6
3-II	1	1	1
4-II	3-6	3-6	4-5
5-II	5-9	3-9	5-8
6-II	29-36	28-36	30-36
7-II	31-38	27-38	29-36
8-II	1-3	1-3	2-3
9-II	5-10	5-11	5-8
10-II	2-3	1-4	3
11-II	1	1	1
12-II	1	1	1
13-II	7-14	5-14	7-12
14-II	1	1	1

Abdominal Segment III			
0-III	4-8	3-8	4-6
1-III	21-27	18-27	17-27
2-III	2-5	2-6	3-6
3-III	1	1	1
4-III	2-4	2-4	2-3
5-III	5-10	5-11	6-12
6-III	24-36	22-36	21-28
7-III	2-4	2-4	3
8-III	2-3	1-5	2-3
9-III	6-12	3-10	4-10
10-III	1	1	1
11-III	2-3	2-5	3
12-III	2-4	2-5	2-5
13-III	8-12	7-14	8-11
14-III	1	1	1

Abdominal Segment IV			
0-IV	4-7	4-7	3-4
1-IV	19-27	16-27	21-26
2-IV	1	1	1
3-IV	3	2-7	2-3
4-IV	2-4	1-4	2-5
5-IV	3-6	3-7	4-5
6-IV	1	1	1
7-IV	2-4	2-4	2-4
8-IV	2-3	2-3	2-3
9-IV	5-12	4-12	6-8
10-IV	1	1	1
11-IV	1-3	2-4	2
12-IV	2-5	2-6	3-4
13-IV	4-6	4-10	5-6
14-IV	1	1	1

Abdominal Segment V			
0-V	4-10	3-7	6-7
1-V	17-27	17-27	17-25
2-V	1-2	1-2	1
3-V	1	1-2	1
4-V	2-3	2-5	3-4
5-V	3-8	3-8	3-7
6-V	1	1	1
7-V	2-3	2-4	2-3
8-V	2-4	2-3	3
9-V	5-10	4-10	6-8
10-V	1	1	1
11-V	2-4	2-4	2-3
12-V	2-3	2-6	2-3
13-V	4-8	4-12	6-8
14-V	1	1	1

Abdominal Segment VI			
0-VI	4-8	3-8	5-7
1-VI	19-27	19-27	22-27
2-VI	3-6	1-10	4-5
3-VI	1	1	1
4-VI	1-2	1	1
5-VI	4-9	5-10	6-8
6-VI	1	1	1
7-VI	2-3	2-3	3
8-VI	2-3	2-3	2-3
9-VI	3-11	5-11	7-11
10-VI	2-5	1-5	2-6
11-VI	2-3	1-4	2-3
12-VI	1	1	1
13-VI	5-13	3-12	12-16
14-VI	1	1	1

Abdominal Segment VII			
0-VII	3-7	3-7	3-7
1-VII	21-26	17-26	21-27
2-VII	3-8	5-10	6-8
3-VII	2-4	1-4	3-4
4-VII	1	1	1
5-VII	5-9	3-9	8-10
6-VII	3-7	3-7	1-6
7-VII	3-11	3-8	5-7
8-VII	3-6	3-6	5-7
9-VII	4-8	2-9	7-9
10-VII	1-7	2-8	2-6
11-VII	1-2	1-4	2-4

12-VII	1	1	1
13-VII	5-9	4-9	5-7
Abdominal Segment VIII			
0-VIII	2-7	2-7	3-5
1-VIII	1	1	1
2-VIII	5-13	5-11	5-13
3-VIII	4-15	7-15	7-12
4-VIII	1	1	1
5-VIII	6-11	5-9	4-7
14-VIII	1	1	1
Spiracular Apparatus			
1-S	5-6	3-9	3-7
2-S	4-7	3-7	5-6
4-S	1	1	1
5-S	1	1	1
6-S	1	1-2	1-2
7-S	1-2	1-2	2
8-S	2-5	2-5	3-4
9-S	3-9	3-9	3-5
10-S	1	1	1
11-S	1	1	1
12-S	1	1	1
13-S	1	1	1
Abdominal Segment X			
1-X	1	1	1
2-X	17-22	15-20	14-18
3-X	6-10	7-9	8-9
4-X	8 pairs	8 pairs	8 pairs

Table 3. Differences in number of branches of seta 8-M among *An. nuneztovari* larvae.

Cytotype	N	Mean no. branches
B	47	25.8a
C	13	25.4a
A	49	23.2b

Means followed by the same letter are not different (Ryan-Einot-Gabriel-Welsch multiple range test, $P < 0.0005$).

Table 4. Chaetotaxy of *An. nuneztovari* pupae.

	Cytotype		
Seta	A	B	C
Cephalothorax			
1-C	1-3	2-3	1-2
2-C	1-4	2-3	2
3-C	2-3	2-3	2-3
4-C	2-3	2-3	2-4
5-C	2-4	2-3	2-4
6-C	1-2	1-2	1-2
7-C	2-3	2	2
8-C	1	1	1
9-C	2-3	2	2-3
10-C	1	1-2	1
11-C	2-8	3-5	4-6
12-C	1-3	1-2	1-3
Abdominal Segment I			
1-I	99-125	87-166	84-116
2-I	3-5	2-5	3-6
3-I	1	1	1
4-I	2-6	2-4	2-5
5-I	1-3	1-2	2-4
6-I	1	1	1
7-I	1-5	2-4	2-4
9-I	1	1	1
Abdominal Segment II			
0-II	3-5	3-5	3-4
1-II	4-9	5-9	6-8
2-II	4-6	4-6	6-7

3-II	1	1	1
4-II	2-5	2-6	2-5
5-II	2-4	2-4	2-3
6-II	1-2	1	1-2
7-II	2-3	2-3	1-3
9-II	1	1	1
11-II	1-5	2	2
Abdominal Segment III			
0-III	3-5	4-5	3-4
1-III	5-7	3-8	5-7
2-III	3-4	4-5	3-5
3-III	1	1	1
4-III	3-6	2-3	4-6
5-III	3-7	4-10	4-7
6-III	1-4	1-2	1-2
7-III	3-5	3-4	2-5
8-III	2-4	1-3	2-3
9-III	1	1	1
10-III	2-5	2-3	2-3
11-III	1-2	1	1
Abdominal Segment IV			
0-IV	3-5	3-5	4-5
1-IV	1	1	1
2-IV	1-3	1-3	1-3
3-IV	3-6	1-5	3-6
4-IV	1-4	3-4	3-5
5-IV	2-4	3-5	1-5
6-IV	1	1	1
7-IV	3-6	1-4	3-4
8-IV	1-3	1-3	2-3
9-IV	1	1	1
10-IV	1	1	1
11-IV	1	1	1
Abdominal Segment V			
0-V	3-5	3-5	3-4
1-V	1	1	1
2-V	1-2	1-2	1-2
3-V	2-3	1-3	2-3
4-V	2-5	3-4	3-4
5-V	1	1	1
6-V	1	1	1
7-V	1-4	1-3	2-3
8-V	2	1-2	2-3
9-V	1	1	1
10-V	1	1	1
11-V	1	1	1
Abdominal Segment VI			
0-VI	2-4	2-3	2-3
1-VI	1	1	1
2-VI	1-2	1-3	1-2
3-VI	1-3	1-3	2-4
4-VI	1-2	2	1-2
5-VI	1	1	1
6-VI	1	1	1-2
7-VI	1-2	1-3	1-2
8-VI	1-2	2	2-3
9-VI	1	1	1
11-VI	1	1	1
Abdominal Segment VII			
0-VII	3-5	2-5	3-4
1-VII	1	1	1
2-VII	1-3	1-4	1-3
3-VII	2-4	1-4	3-4
4-VII	1-3	1-3	1-3
5-VII	1	1	1
6-VII	1-2	2	1-2
7-VII	1	1	1
8-VII	3-5	3-4	2-4
9-VII	1	1	1
10-VII	1-2	1-2	1-2
11-VII	1	1	1
Abdominal Segment VIII			
0-VIII	1-2	1-3	1-2
4-VIII	2-4	1-4	1-4
9-VIII	1	1	1
14-VIII	1	1	1
Abdominal Segment IX			
1-X	2-3	2	2-3
Paddle			
1-P	1	1	1
2-P	1-2	1-2	1