VARIATION IN THE LENGTH OF THE MEDIAN PALE BAND ON THE PROBOSCIS OF *AEDES TAENIORHYNCHUS*[1]

THOMAS P. GARGAN, II AND KENNETH J. LINTHICUM

U.S. Army Medical Research Unit (WRAIR), Kenya, Box 401, APO New York 09675 and U.S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD 21701-5011

Aedes (Ochlerotatus) taeniorhynchus (Wiedemann) is a common pestiferous mosquito-species found along the coasts and inland saline areas from New Hampshire to Brazil, and from California to Peru, as well as the Antilles and the Galapagos Islands (Knight and Stone 1977, Burger 1981). The presence of a well-developed, median, pale-scaled band on the proboscis (BP) of adult females is an important diagnostic character used to separate *Ae. taeniorhynchus, Ae. (Och.) mitchellae* (Dyar), *Ae. (Och.) sollicitans* (Walker) and *Ae. (Och.) nigromaculis* (Ludlow) from other Nearctic species of the genus *Aedes* (Carpenter and LaCasse 1955, Darsie and Ward 1981, Matheson 1944). During an arbovirus research project involving identification of field-collected mosquitoes, several *Aedes* specimens were encountered which could not be identified by using the keys of Carpenter and LaCasse (1955) and Darsie and Ward (1981). These specimens lacked pale scales on the proboscis, but, based upon detailed descriptions by Carpenter and LaCasse (1955) and Belkin et al. (1970), they were eventually determined to be *Ae. taeniorhynchus*. The purpose of this paper is to describe the variation in the size of the BP in *Ae. taeniorhynchus*.

Mosquitoes were collected by carbon dioxide-baited (0.5 kg dry ice) CDC light traps located near salt marshes on the Chincoteague National Wildlife Refuge on Assateague Island, Virginia (37° 55′ N, 75° 21′ W) from July through September, 1978. The 533 specimens examined in this study were collected by routinely removing random samples from pools to be assayed for virus. Specimens were mounted on paper points and examined under a stereoscopic dissecting microscope. Measurements of the length of the proboscis and the distances from the tip of the proboscis to the distal and proximal ends of the band were made using a calibrated ocular micrometer. Both the band length and the position of the band on the proboscis could be calculated from the measurements. These specimens have been deposited in the U.S. National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Data were analyzed on an Amdahl 470-V7 computer with various statistical procedures from SAS (Helwig and Council 1979) and BMD-P (Dixon 1983) programs.

There was considerable variation in the size of the median pale-scaled BP of *Ae. taeniorhynchus* (Fig. 1). Ten of the 533 (1.9%) specimens examined did not have a median pale-scaled BP; the proboscis was completely covered with dark scales (Fig. 1C). There was considerable variation in the size of the BP in the remaining 523 specimens. Most had a well-developed BP (Fig. 1A); however, some individuals had a very small BP (Fig. 1B). There were 6 specimens in which the BP did not completely encircle the proboscis. There was reduced pale-scaling on the dorsal surface and increased pale-scaling on the lateral and ventral surfaces of the proboscis in these individuals.

Statistical analysis on the relationship between the lengths of the BP and the proboscis were based on a sample size of 523 individuals. The 10 individuals which did not have a BP were excluded from the analysis. The initial Kolmogorov-Smirnov tests for goodness of fit (Daniel 1978) indicated that the distribution of the proboscis and BP lengths varied significantly from a normal (Gaussian) distribution. Attempts to normalize the data by various transformations such as logarithmic, square-root and power functions were unsuccessful. Therefore, nonparametric statistical procedures were used to analyze the data. A Spearman rank correlation test indicated that the lengths of the BP and proboscis were slightly correlated ($r = 0.41$, $p < 0.01$). It was not possible to linearly predict the length of the BP based upon the length of the proboscis because of this low correlation. Because there was no Gaussian distributions of the proboscis and BP lengths, the medians, not the means, of these lengths were chosen to measure central tendency. Descriptive statistics for the length of the proboscis and the BP are shown in Table 1.

The distribution of the ratio of BP length to proboscis length (BP/P ratio) was also tested for normalcy by the Kolmogorov-Smirnov test. While this distribution was not significantly different ($n = 523$, D max $= 0.037$, $p = 0.068$) from a Gaussian distribution, the D max value was very close to being rejected. A plot of the distribution of the BP/P ratio is shown in Fig. 2. The distribution of this ratio is slightly skewed to the left. Correlation analysis showed there was very low correlation ($r = 0.11$, $p = 0.009$) between the BP/P ratio and the length of the

[1] The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

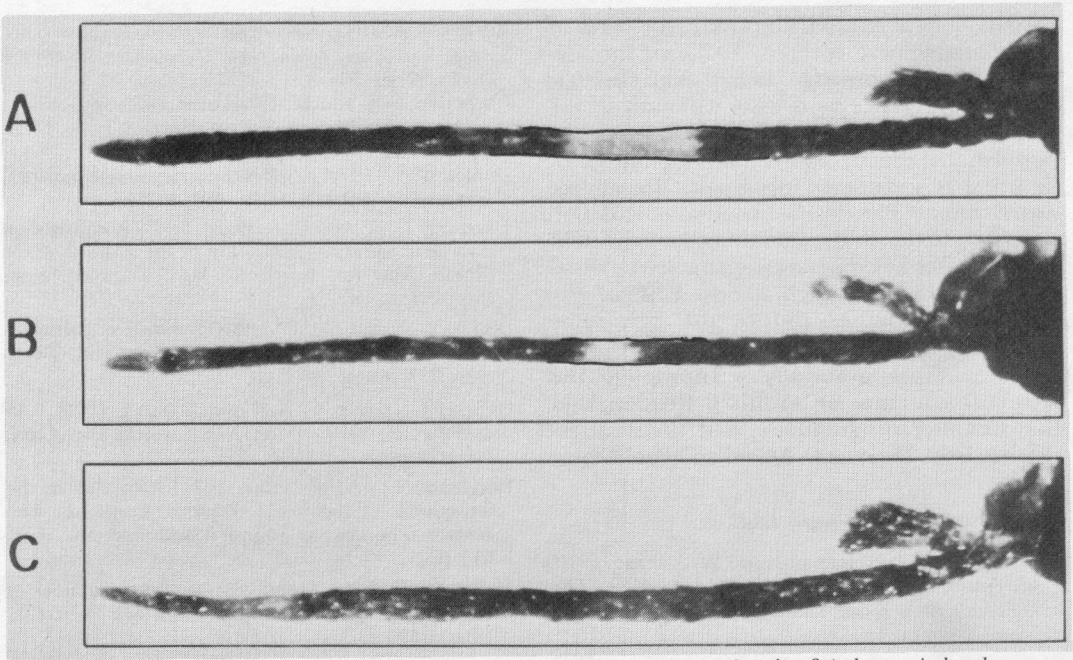

Fig. 1. Variation in the size of the median pale-scale band on the proboscis of *Aedes taeniorhynchus*.

proboscis. Descriptive statistics for the BP/P ratio are shown in Table 1.

For the 10 mosquitoes without a BP, the median proboscis length was 2.24 mm; the minimum and maximum values were 2.08 and 2.38 mm, respectively. The measurements for the lengths of the proboscis were very similar from mosquitoes with (Table 1) and without a proboscidial band.

The low correlation between the BP/P ratio and the length of the proboscis indicated that, for any particular proboscis length, the variation and distribution of the length of the BP was similar. The 25% and 75% quantiles for the median length of the proboscis, the BP, and the BP/P ratio showed that the range in variation was very small (Table 1).

Specimens of *Ae. taeniorhynchus* that do not have a median pale-scaled BP can create considerable problems in routine identification of field-collection specimens. These specimens were keyed to *Ae. riparius* Dyar and Knab by using Carpenter and La Casse (1955). According to the key of Darsie and Ward (1981), specimens with either a few pale scales or no BP at all keyed to the *Ae. vexans-cantator* couplet if the basal pale band of the hindtarsomere covered ≤0.2% of the segment, and to either *Ae. alponotum* Dyar (without lower mesanepimeral setae) or *Ae. increpitus* Dyar (with lower

Table 1. Descriptive statistics for the lengths (mm) of the proboscis, the pale-scaled band on the proboscis (BP), and the ratio of the length of BP to the proboscis (PB/P ratio) in female *Aedes taeniorhychus*.

Diagnostic character	Quantiles				
	Minimum	25%	Median (50%)	75%	Maximum
Proboscis	1.52	2.05	2.18	1.28	2.64
BP	0.06	0.20	0.26	0.30	0.53
BP/P ratio	0.03	0.10	0.12	0.14	0.23

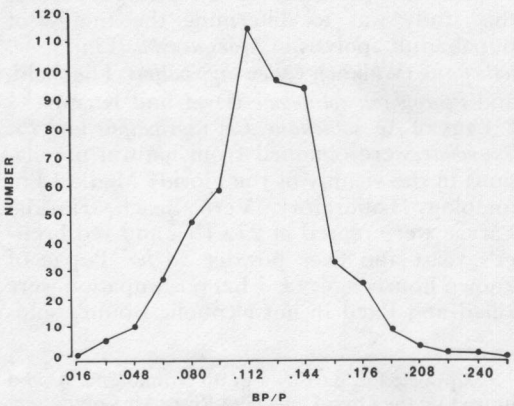

Fig. 2. Plot of the distribution of the ratio of the length of the median pale-scale band on the proboscis to the length of the proboscis (BP/P ratio) in *Aedes taeniorhynchus*.

mesanepimeral setae) if the basal pale band of the hindtarsomere covered ≥0.3% of the segment. To our knowledge, Belkin et al. (1970) is the only reference to discuss variation in the size of the BP. These authors state that in some populations, the BP shows considerable variation and is sometimes incomplete dorsally or nearly absent. The detailed analysis of variation described in this study should facilitate the utilization of this key diagnostic character by demonstrating that although almost 98% of the specimens examined did fit the key, some specimens could not be identified.

The authors gratefully acknowledge the technical assistance of Ardith J. Regdon, and the computer programming and statistical assistance from Dwayne D. Oland and Paul Gibbs.

References Cited

Belkin, J. N., S. J. Heinemann and W. A. Page. 1970. Mosquito studies (Diptera, Culicidae). XXI. The Culicidae of Jamaica. Contrib. Am. Entomol. Inst. (Ann Arbor) 6:1–458.

Burger, J. F. 1981. New records of mosquitoes (Diptera: Culicidae) from New Hampshire. Entomol. News 92:49–50.

Carpenter, S. J. and W. J. LaCasse. 1955. Mosquitoes of North America (North of Mexico). Univ. Calif. Press, Berkeley, 360 pp., 127 pl.

Daniel, W. W. 1978. Applied nonparametric statistics. Houghton Mifflin Co., Boston, 510 pp.

Darsie, R. F., Jr. and R. A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Mosq. Syst. Suppl. 1:1–313.

Dixon, W. J. (ed.). 1983. BMDP statistical software, 1983 printing with additions. Univ. Calif., Berkeley, 733 pp.

Helwig, J. T. and K. A. Council (eds.). 1979. SAS user's guide 1979 edition. SAS Institute Inc., Cary, NC 921 pp.

Knight, K. L. and A. Stone. 1977. A catalog of the mosquitoes of the world (Diptera: Culicidae). 2nd edition. Thomas Say Found. 6, Entomol. Soc. Am., 611 pp.

Matheson, R. 1944. Handbook of the mosquitoes of North America, 2nd edition. Comstock Publ. Co., Ithaca, 314 pp.

TIMING OF PUPAL-ADULT APOLYSIS IN MOSQUITOES[1]

E. D. WALKER[2] AND W. S. ROMOSER[3]

Apolysis, the separation of cuticle and epidermis prior to new cuticle formation, is a key, morphologically evident event which signals the initiation of molting (Hinton 1973). Despite the developmental significance of this event, it has been little studied in mosquitoes (Walker and Romoser 1982). The purpose of this study was to determine the timing of pupal-adult apolysis in *Aedes aegypti* (Linn.), *Ae. sollicitans* (Walker), *Culex nigripalpus* Theobald, and *Psorophora columbiae* (Dyar and Knab).

Eggs of *Ae. sollicitans, Cx. nigripalpus* and *Ps. columbiae* were obtained from natural populations in the vicinity of the Florida Medical Entomology Laboratory, Vero Beach, Florida. Larvae were reared at 27±1°C. and fed brewer's yeast and liver powder *ad lib.* Pupae of known hourly ages (± 1 hr) postpupation were killed and fixed in hot alcoholic Bouin's solution, dehydrated and infiltrated with paraplast, cut into 7.0 μm serial sagittal sections, mounted on slides with Mayer's albumin, and stained according to the modified azan trichrome technique (Hubschman 1962). Specimens of *Ae. aegypti* Rockefeller strain were prepared similarly, except that a dioxane-paraffin dehydration and infiltration method was used, and the slides were stained with Masson's triple stain[4]. Specimens of ages spanning the entire pupal stadium of each species were prepared.

Four integument sites were examined, at 1000X with a light microscope, on the midsagittal section of each specimen: (1) scutum, (2) third abdominal tergum, (3) seventh abdominal sternum, and (4) anterior wall of ventral air space. These different sites were examined to take into account variation in timing of apolysis about the pupal body (Walker and Romoser 1982).

[1] Supported in part by a grant from Sigma Xi and in part by the Ohio University Research Committee.

[2] Vector Biology Laboratories, Dept. of Biology, University of Notre Dame, Notre Dame, IN 46556.

[3] Dept. of Zoological and Biomedical Sciences, Ohio University, Athens, OH 45701.

[4] Lane, J. E. 1963. The development of the oesophageal diverticula in the yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae). Ph.D. Dissertation, The Ohio State University, Columbus, OH, 99 pp.