ARTICLES

PRELIMINARY OBSERVATIONS ON THE USE OF ELECTROPHORESIS FOR SEPARATING AND IDENTIFYING SEVERAL GENERA AND SPECIES OF TAIWANESE MOSQUITOES [1]

JOHN L. MCDONALD, EVE TANG AND JOHN H. CROSS
Medical Ecology Department, U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan, Republic of China

Many groups of complexes of medically important arthropods are sometimes morphologically indistinguishable, or if distinguishable the conventional characteristics used for identification are sometimes not entirely reliable. Often identification is simply an element of judgment on the part of the taxonomist. Although the chemical composition of some arthropods has been studied, only recently have investigators directed their efforts toward the application of biochemical methods to taxonomy. In studies by Micks *et al.* (1966a) it was shown that utilizing chromatographic techniques, biochemical methods could be used to differentiate *Anopheles gambiae* subspecies indistinguishable by conventional taxonomy. In addition, Ross (1968) also working with the *Anopheles gambiae* complex used cellulose acetate membrane electrophoresis and was able to show differences in egg-protein between species A and B of the complex. Micks *et al.* (1966b) demonstrated biochemical differences between strains of *Aedes aegypti,* and Chen (1967) working with autogenous *Culex pipiens molestus* and anautogenous *C. p. fatigans* found differences in hemolymph protein by disc electrophoresis.

By the use of immunodiffusion and disc electrophoresis Zaman and Chellappah (1963) and Desowitz (1969) showed alterations in protein constitution in the developmental stages of *C. p. fatigans* and *Armigeres subalbatus.* Kimura *et al.* (1971) on the other hand, working with egg and pupa extracts of mosquitoes from the *Culex pipiens* complex were unable to note electrophoretic differences between the eggs and pupae tested.

In dealing with arthropod-borne diseases it is necessary to identify the particular species to be controlled to determine the best approach to the problem (Davidson, 1964). It is apparent, therefore, that more accurate methods are needed for differentiating vector populations at the subspecies levels (Micks *et al.,* 1966a).

The purpose of this paper is to present preliminary observations on the electrophoretic patterns observed in larvae, pupae, and adults of some mosquitoes of Taiwan.

Mosquitoes used were from colonies of species native to Taiwan established in the insectory at the U. S. Naval Medical Research Unit No. 2 in Taipei. Mosquito extracts were prepared as follows: larval extracts were prepared from fourth instar larvae only; pupae were 24–36 hours old to give them time to digest food taken before pupation; adults were also 24–36 hours old and were given no food except a 10 percent sucrose solution. The larvae and pupae were placed in distilled water for 1–2 minutes to remove, by washing, any exterior protein contamination. Adults were not washed but were placed in a cold chamber for 10 minutes to immobilize

[1] This study was supported in part through funds provided by the Bureau of Medicine and Surgery, Navy Department, for Work Unit MR-005.09.01-0122B.

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large.

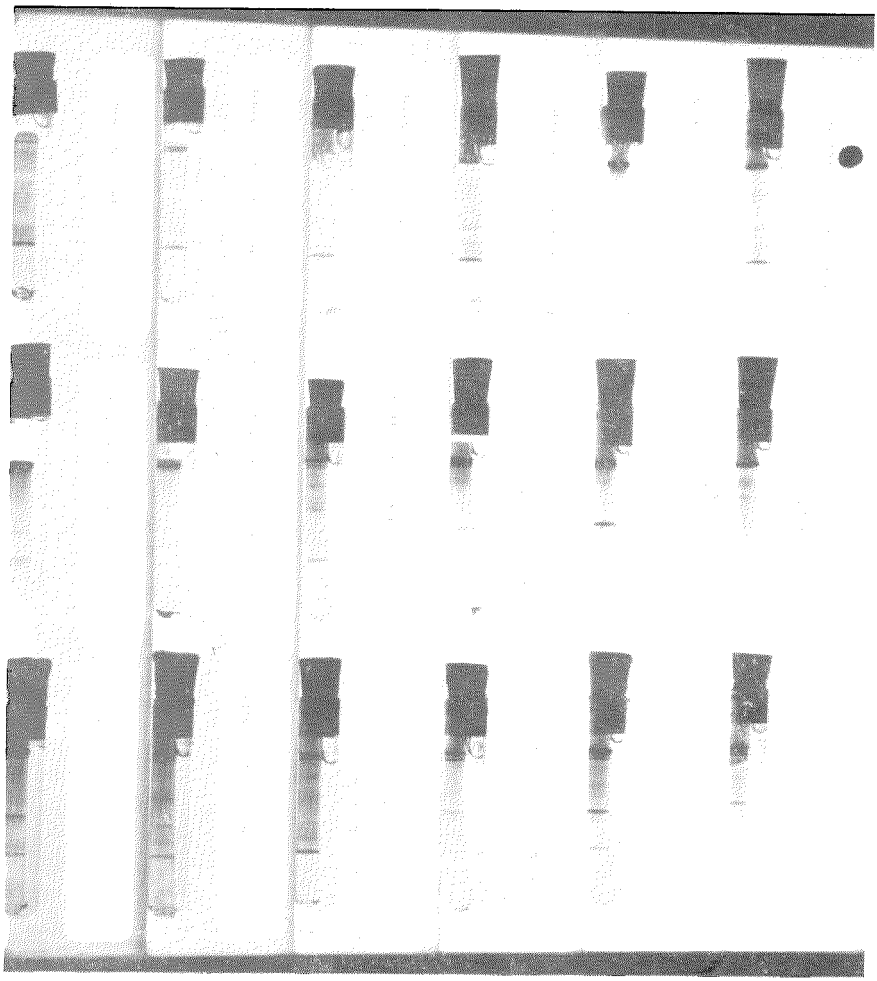
Reprint requests to Publications Office, NAMRU-2, Box 14, APO San Francisco 96263.

them. Specimens of the respective instars were placed in 2 ml. saline (0.9 percent NaCl) and ground in a glass tissue grinder at 1725 rpm while in an ice bath until homogenous. This mixture was sonified for 30 seconds and the homogenates centrifuged under refrigeration at 3500 rpm at 2° to 4° C for 10 minutes. Immediately after centrifugation the homogenate was filtered through a "rough" millipore filter.

The resultant supernatant was subjected to the Folin reagent test to determine the protein content expressed in mg/ml. In all instances the resultant protein extracts used were not less than 3.75 mg/ml nor more than 6.0 mg/ml. Because of the variation in the physical size as well as

in the constituent proteins different numbers of larvae, pupae, or adults were used to maintain these criteria. The supernatants were then collected for disc electrophoresis.

Polyacrylamide electrophoresis using 7.5 percent acrylamide gel according to the method of Davis and Takada (1969) was employed. N, N, N, N′ Tetramethylethylenediamine 0.03 ml/100 ml was used in the lower gel to inhibit polymerization. Tris-glycine buffer with a pH range of 8.2 to 8.4 was used in the well of a Canalco® electrophoresis outfit Model



FIGS. 1 and 2.—Gel columns of six of the genera and eleven species of mosquitoes examined.

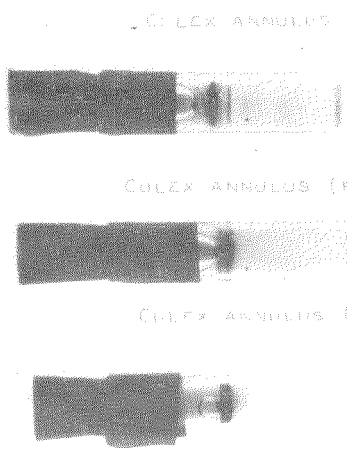


FIG. 3.—A typical variation in the disc pattern demonstrated by *Culex annulus* larva, pupa, and adult stages.

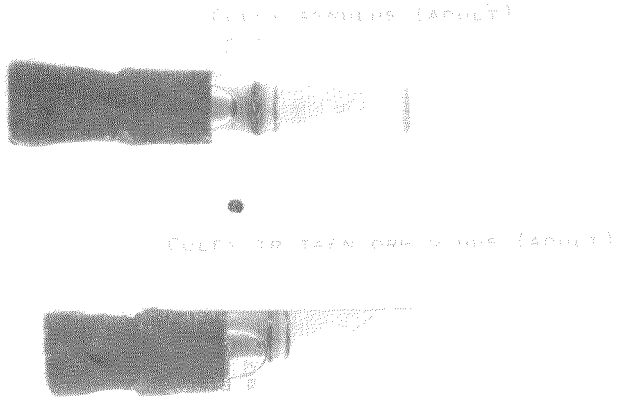


FIG. 4.—The basic pattern difference between specimens of adult *Culex annulus* and *Culex nigripalpus* is seen.

1200. Protein extracts were added to the upper gel and a 5 mA electric current per column was applied until the tracking dye (bromphenol blue) had traveled 35 mm down the lower gel. Gel columns were removed from their respective glass columns and stained for one hour in Amido-Schwartz stain then electrically destained in 8 percent acetic acid solution.

Figures 1 and 2 show the gel columns of six of the genera and eleven species studied and clearly demonstrate different disc patterns found in different genera, different species, and different instars within a given species. Figure 3 depicts a typical variation in the disc pattern demonstrated by *Culex annulus* larva, pupa, and adult stages. The individual basic pattern differences between specimens of adult *Culex annulus* and *Culex tritaeniorhynchus* are shown in Figure 4. Their characteristic disc patterns and those of the other instars were consistent as long as there were no variations in the age of the specimens or in their care prior to preparation of the respective extracts.

SUMMARY

The results of these preliminary studies show distinct protein patterns demonstrated by disc electrophoresis between species of Taiwan mosquitoes. These results confirm similar observations by other investigators using different species of mosquitoes, and offer further evidence that biochemical methods are of value to the taxonomist in the identification of arthropods.

Literature Cited

Chen, P. S. 1967. Electrophoretic patterns of larval haemolymph-protein in autogenous and anautogenous forms of *Culex pipiens* L. Nature 215:316–317.

Davidson, C. 1964. *Anopheles gambiae,* a complex of species. Bull. Wld. Hlth. Org. 31: 625–634.

Davis, G. M. and Takada, T. 1969. *Oncomelania hupensis nosophora* electrophoretic separation of foot proteins of laboratory-reared and field-collected specimens. Exp. Parasit. 25: 193–201.

Desowitz, R. A. 1969. Developmental changes in the protein constitution of a mosquito (*Armigeres subalbatus*) as revealed by disc electrophoresis. J. Parasit. 55:476.

Kimura, T., Yoshii, A. and Tipton, V. J. 1971. Disc-electrophoretic analysis of some numbers of the *Culex pipiens* complex. J. Med. Ent. 8:1–6.

Micks, D. W., Rehmet, A., Jennings, J., Mason, G. and Davidson, G. 1966a. A chromatographic study of the systematic relationship within the *Anopheles gambiae* complex. Bull. Wld. Hlth. Org. 35:181–187.

Micks, D. W., Rehmet, A. and Jennings, J. 1966b. Biochemical differentiation of morphologically indistinguishable strains of *Aedes aegypti* (Diptera: Culicidae). Ann. Ent. Soc. Amer. 59:239–242.

Ross, G. C. 1968. Egg protein of species A and B of the *Anopheles gambiae* complex: a pilot experiment to show fractionation by electrophoresis on cellulose acetate membrane. Bull. Wld. Hlth. Org. 38:324.

Zaman, V. and Chellappah, W. T. 1963. Gel-diffusion studies with mosquito (Diptera: Culicidae) antigens. I. Antigenic analysis during metamorphosis. Exp. Parasit. 13:108–112.