Protein Band Studies of Fourth Instar Larvae in the Aedes varipalpus Group[1]

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ABSTRACT. Morphologically the larvae of Ae. monticola and Ae. varipalpus are very similar to each other but distinct from Ae. sierrensis. Very little similarity was found among these three species as a result of a study of their protein bands.

The most comprehensive taxonomic study of the varipalpus group to date was published by Arnell and Nielsen (1972). The methods used by them were primarily those of classical comparative morphology. Five species were considered to be members of this group, viz., Aedes laguna Arnell and Nielsen, Aedes monticola Belkin and McDonald, Aedes varipalpus (Coquillett), Aedes deserticola Zavortink, and Aedes sierrensis (Ludlow).

The present investigation was undertaken to provide biochemical data, in the form of protein bands produced by disc gel electrophoresis, to determine if there was a correlation between biochemical and morphological characteristics among the species in the varipalpus group.

Some researchers have found consistencies between biochemical and morphological data from mosquitoes and others have found inconsistencies. For example, Warren and Breland (1969) studied protein patterns using starch gel electrophoresis. The high degree of similarity in biochemical data for adult Ae. triseriatus and Ae. hendersoni was consistent with the morphological similarity that exists between these two species. In contrast, Trebatoski and Haynes (1969) used a comparative enzyme study by means of starch gel electrophoresis and found that "Ae. pseudoscutellaris, which is morphologically more similar to Ae. scutellaris and supposedly more closely related to it phylogenetically, showed a higher degree of biochemical affinity to Ae. albopictus."

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MATERIALS AND METHODS

Specimens used in this investigation were larvae of Ae. sierrensis, Ae. monticola, and Ae. varipalpus which had been collected, identified, and made available to the author by Dr. Lewis T. Nielsen, University of Utah. Unfortunately Ae. laguna and Ae. deserticola were not available for study.

Fourth instar larvae were selected and rinsed three times in distilled water and allowed to stand for one hour in the third rinse prior to the maceration of the specimens. Fifteen larvae of each of the three species were placed in separate small Potter-Elvehjem tissue grinders with one drop of saturated phenylthiorurea and 0.1cc of gel buffer. Homogenation was carried out in an ice bath for 30 minutes. The homogenized samples were placed in capillary tubes and then centrifuged for 5 minutes at 12,000 g (about 9,500 rpm).

The electrophoretic apparatus consisted of a linear chamber manufactured by the C. L. Davis Co., Lincoln, Nebraska. An electric current was provided by a Thomas Electrophoresis Power Supply (Model 21). Gel tubes (5mm X 75mm) and destaining tubes (7mm X 80mm) were made from glass tubing.

The electrophoretic method used was basically that of Ornstein and Davis (1962), but it also included modifications of the methods described by Smithies (1955, 1959), Wang and Patton (1968), and Dajmal and Brooks (1968). Gel tubes consisted of a column of 7% acrylamide gel (pH 8.8-8.9). Homogenized mosquito tissue samples (0.10cc) were placed at the top of the gel columns. The samples were carefully overlayed with 10% sucrose solution to prevent the convection of the sample material into the electrode compartment. The tubes were then placed through the openings in the linear chamber and a drop of electrode buffer (pH 8.1-8.3) was added to the top and the bottom of each tube to avoid the entrapment of air.

Electrophoresis was carried out using constant current at 1 ma per tube for 15 minutes to concentrate the sample and then for an additional 75 minutes at 13 ma per tube until the tracking dye (5% bromophenol blue, pH 8.1-8.3) had migrated to a line 1 cm from the anodic end of the tube. After electrophoresis the tubes were placed in cold water and the gels removed and placed in the staining solution (1% Aniline Blue-Black) for one hour. Destaining was accomplished by running an electric current at 10 ma per gel through the destaining tubes placed in the chamber with 7.5% acetic acid in the buffer reservoirs. After destaining the gels were placed in glass vials with 15% acetic acid for storage.

The distance traveled by the tracking dye was used to determine the relative distance of migration (relative mobility) of the protein bands. The tracking dye always produced a single dense band. Relative mobilities (Rmb) were calculated as decimal fractions of the mobility of the tracking dye. Distances were measured in millimeters from the leading edge of the tracking dye to the center of the protein band. In all of the runs normal human serum diluted 1:20 was used as a control.

RESULTS AND DISCUSSION

Four gels were obtained for each of the three species. The number of bands found were 5 in Ae. sierrensis, 4 in Ae. monticola, and 11 in Ae. varipalpus. In addition to the differences in the number of bands, the three species also differed from each other to some extent in the staining intensity and relative mobility of the bands (Fig. 1).

According to Arnell and Nielsen (op. cit.) larvae of Ae. varipalpus and Ae. monticola are very similar in morphological features and cannot always be reliably separated, but Ae. sierrensis is distinctively different from these two species.

Protein band studies suggest that all 3 species are distinct. Indeed, it is perplexing that Ae. varipalpus and Ae. monticola which are so similar in morphology are so strikingly different in the characteristics of their protein bands. To speculate about the genetic relationships among these 3 species based solely on protein band characteristics would be premature since this study was limited in scope. Additional work should be done which would include all 5 species and an identification of specific proteins (e.g., enzymes) that could be compared between species. Perhaps more precise biochemical data resulting from such a study would correlate better with the morphological data and allow conclusions to be made about the genetic relationships among the species in the group.

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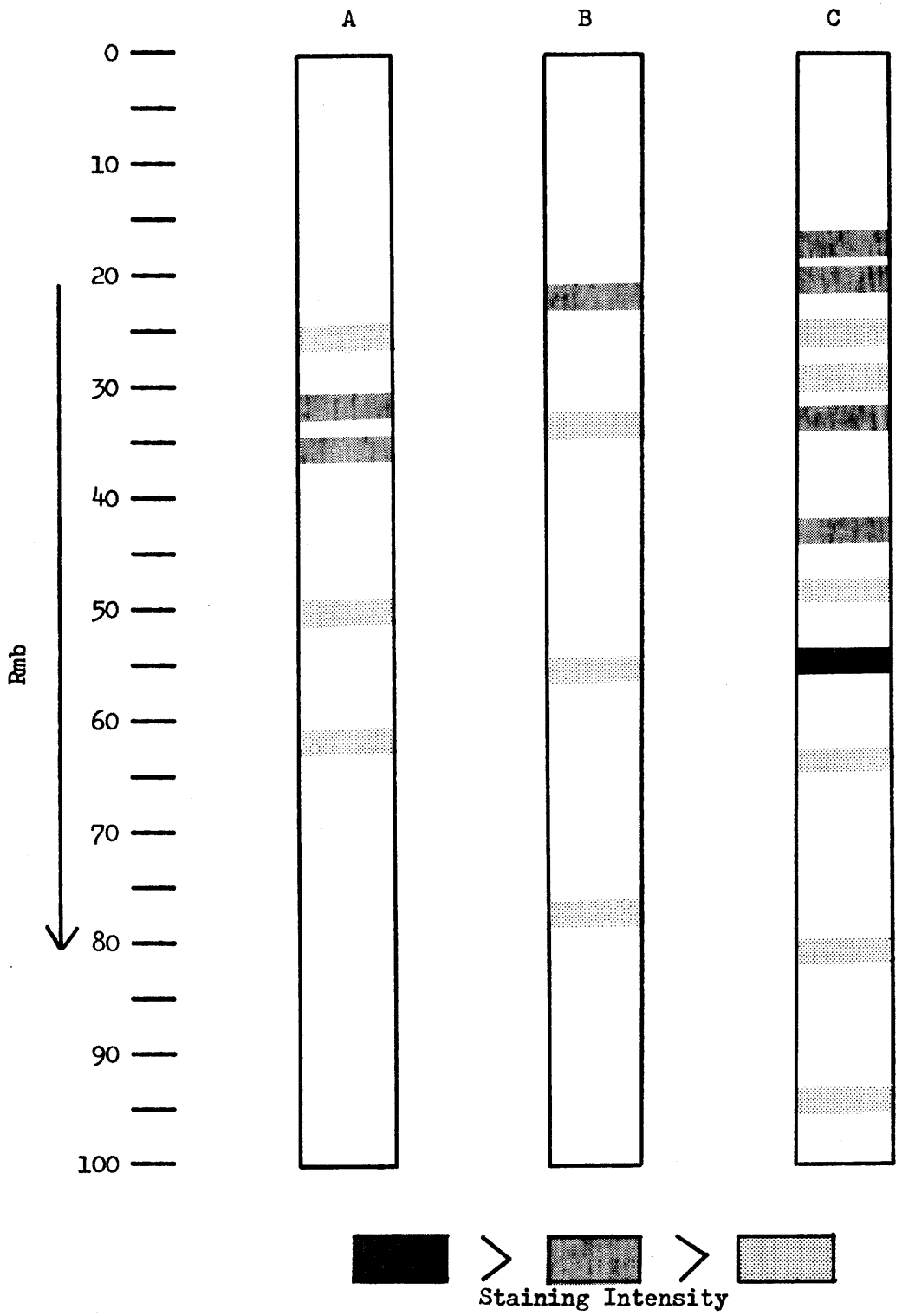

Fig. 1. Diagram of disc gel electropherograms showing relative mobilities (Rmb) and staining intensities of protein bands for fourth instar larvae of Ae. sierrensis (A), Ae. monticola (B), and Ae. varipalpus (C).