tested under the same conditions used with his current isolate SSII-1. The range in response demonstrated with spores of SSII-1 in our current study can encompass the difference between the results of Singer (1973) and of Kellen et al. (1965).

Although spores of *B. sphaericus* can initiate growth in the presence of selected microbial floras, the fact that there are some natural floras; i.e., F4 (sewage pond flora), that inhibit replication, dictates that before *B. sphaericus* (SSII-1) can be reliably applied as a mosquito larval field control measure, the problem of "flora" inhibition should be solved or that a sufficiently high control dosage be utilized; i.e., based on $ED_{50} = \pm 7 \times 10^5$ cells/ml or ED_{95} of 3×10^6 cells/ml.

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PROTEIN BAND STUDIES OF THE SUBSPECIES IN THE *AEDES ATROPALPUS* GROUP[1]

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ABSTRACT. A study of the 4 subspecies in the *Aedes atropalpus* group was conducted using acrylamide gel electrophoresis to separate soluble proteins. Four anodically migrating protein bands were observed in whole-body homogenates of each subspecies. Differences in rates of migration and staining intensities of the protein bands were sufficient to differentiate the subspecies. Therefore, the biochemical data tend to support the 4 subspecies rather than the 2 species concept of the group.

Despite the recent attention given to the taxonomy of the *Aedes atropalpus* group its status still is not clear. O'Meara and Craig (1970) proposed 4 subspecies based on distinctive features in morphology, physiology, and behavior. All 4 subspecies were interfertile when crossed in the laboratory.

Zavortink (1972) made an extensive study of geographical distribution and morphological features and concluded that 2 distinct species were involved rather than 4 subspecies. The type-form *Ae. a. atropalpus* (Coquillett) was designated as *Ae. atropalpus* (Coquillett). Because no reliable distinguishing morphological characteristics could be found, the 3 remaining subspecies were synonomized under a reinstated species, *Ae. epactius* Dyar & Knab.

Recently, Brust (1974) presented data supporting the 2 species concept of the group. From matings in the laboratory it was shown that *Ae. a. epactius, Ae. a. perichares,* and *Ae. a. nielseni* crosses had a high genetic affinity, while *Ae. a. atropalpus* had a low genetic affinity when crossed with the other 3. Scanning electron micrographs of the chorionic sculpturing of the eggs revealed that *atropalpus* was distinct,

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but the eggs of *epactius, perichares,* and *nielseni* were essentially indistinguishable.

The purpose of the present investigation was to use a biochemical approach to the study of classification of the *Ae. atropalpus* group, viz., a comparison of protein bands of the 4 subspecies.

MATERIALS AND METHODS. Eggs of *Ae. a. atropalpus, Ae. a. epactius, Ae. a. nielseni* and *Ae. a. perichares* were obtained from mosquitoes colonized by Dr. G. B. Craig, Jr., University of Notre Dame. The collection site for each subspecies was *atropalpus,* Essex Co., Massachusetts; *epactius,* Travis Co., Texas; *nielseni,* Grand Co., Utah; *perichares,* Metapan, El Salvador.

Eggs were hatched by submerging them in deoxygenated water for 24 hr. Larvae were reared in enamel pans with ca. 2 liters of water, fed liver powder, and maintained at 27±1°C with a photoperiod of 16 hr light and 8 hr dark.

Fourth instar larvae were selected and rinsed 3 times in distilled water and allowed to stand for 1 hr in the third rinse prior to the maceration of the specimens. Only larvae were used in this study for 2 reasons. First, the immature stages of mosquitoes tend to have more protein bands than adults (Warren and Breland 1969); therefore, more bands might be present to compare in the larval stage. Second, the morphological features used by O'Meara and Craig (op. cit.) to distinguish 4 subspecies in the *Ae. atropalpus* group were based on adult females; they found almost no differences in the 4th instar larvae. It seemed worthwhile to compare the biochemical and morphological similarity of the larval stage. Twenty larvae of each of the 4 subspecies were placed in separate small Potter-Elvehjem tissue grinders with 1 drop of saturated phenylthiorurea and 0.1cc of gel buffer. Homogenation was carried out in an ice bath for 30 min. The homogenized samples were placed in capillary tubes and then centrifuged for 5 min at 9500 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 75 mm) and destaining tubes (7mm x 80mm) were made from glass tubing. The electrophoretic method used was that of Lunt (1976). 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 min to concentrate the sample and then for an additional 75 min 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 1 hr. 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 7% 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. Ten electrophoretic runs were made. Each run consisted of 3 gel tubes per subspecies. The replicates were pooled and average values determined for each protein band. In all of the runs normal human serum diluted 1:20 was used as a control.

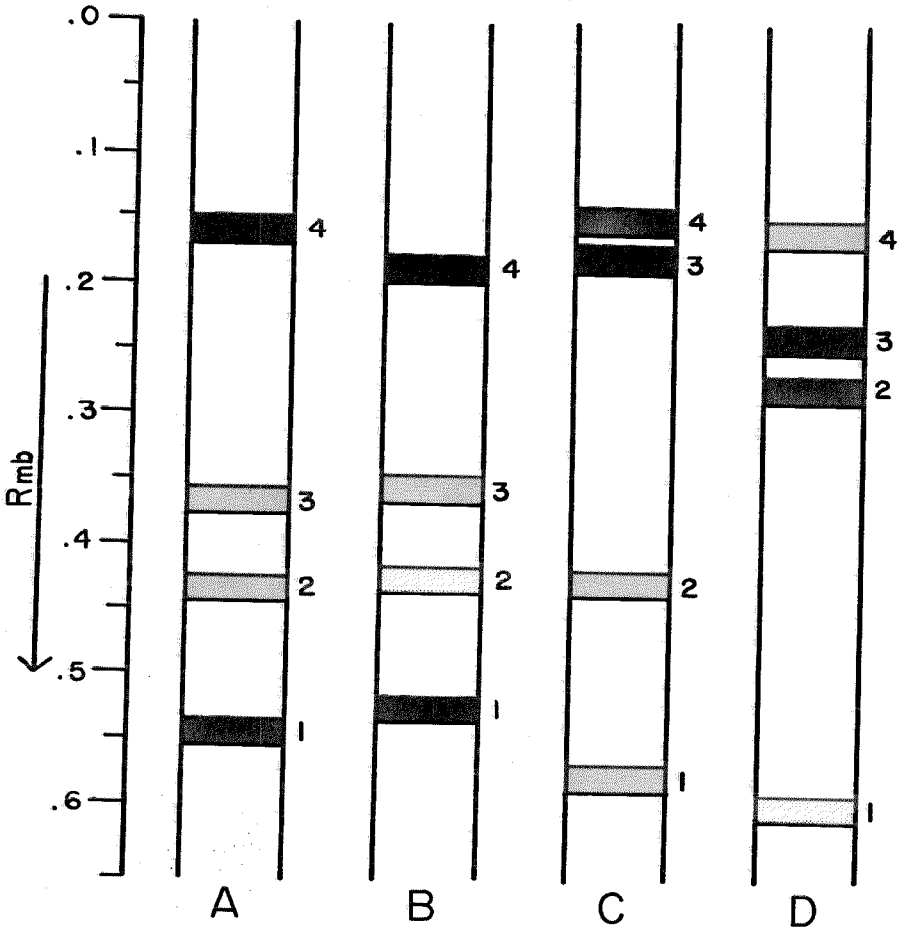

Fig. 1. Diagrammatic protein electropherograms of 4th instar larvae of the subspecies *atropalpus* (A), *epactius* (B), *nielseni* (C) and *perichares* (D).

RESULTS AND DISCUSSION. Four protein bands were found for each subspecies; however, the staining intensity and relative mobility of the bands appeared to be unique to each subspecies (Fig. 1).

As a means of making an overall comparison, the subspecies were examined for similarities in relative mobility (Rmb) and staining intensity of the protein bands. Rmb's differing by less than 0.020 mm were considered to be similar (+) but not necessarily identical. Furthermore, 4 different staining intensities were discernible and comparable bands with the same

Table 1. Comparison of 4th instar larvae of the subspecies *atropalpus* (A), *epactius* (B), *nielseni* (C) and *perichares* (D) based on relative mobility and intensity of the protein bands (left and right column respectively, under each pair of subspecies being compared).

	Comparison between subspecies					
Band number	A & B	A & C	A & D	B & C	B & D	C & D
1	− +	− −	− −	− −	− −	+ −
2	+ −	+ +	− −	+ −	− −	− −
3	+ +	− −	− −	− −	− −	− +
4	− +	+ −	+ −	− −	− −	+ −
Total similarity	5	3	1	1	0	3

intensity were considered to be similar (+) but not necessarily identical (Table 1). Some interesting biochemical relationships appeared to exist between the subspecies: (1) high similarity between *atropalpus* and *epactius;* (2) lower but about equal similarity between *nielseni* and *atropalpus,* and between *nielseni* and *perichares;* (3) still lower but about equal similarity between *atropalpus* and *perichares,* and between *epactius* and *nielseni;* and (4) virtually no similarity between *epactius* and *perichares.*

The method of comparing protein bands was biased towards emphasizing similarities. However, the differences between the 4 subspecies were as great as the differences between full but similar species reported by Warren and Breland (op. cit.). Therefore, the biochemical data tend to support the 4 subspecies rather than the 2 species concept of the *A. atropalpus* group.

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