ARTICLES

ELECTROPHORETIC PATTERNS IN MOSQUITOES

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The family Culicidae is one of the best known groups of organisms. Knowledge of this family has been gained from almost all of the classical approaches, such as adult and larval anatomy, ecology, etc. It was believed by the writers that the taxonomy of the family was worked out well enough on these classical bases to be a prime target for biochemical analysis, both to test the established relationships, and as a test of the validity of these biochemical methods themselves. In central Texas several groups of mosquitoes are available, some of which occupy strategic positions in taxonomic problems.

Several investigators have studied mosquito proteins by various biochemical methods. Clark and Ball (1956) utilized free and paper electrophoresis to study *Culex pipiens* and *Culex stigmatosoma* (now *C. peus*). Chen (1959) compared the autogenous *Culex pipiens molestus* and the anautogenous *Culex fatigans (Culex pipiens quinquefasciatus)* by paper electrophoresis. Other workers have approached the same problems with serological techniques. Zaman and Chellappah (1962, 1963, 1964, 1965) studied cross reactions between larval stages and eggs in species of *Culex* and *Armigeres.* Downe (1963) compared adult females of four species of *Aedes* in the subgenus *Ochlerotatus* by the precipitin technique. Fox, Knight, and Bayona (1963) utilized agar-gel diffusion technique in their study of the relationships between *Aedes aegypti, Culex quinquefasciatus, Aedes taeniorhyncus,* and *Culicoides furens.* Smith and Silverman (1966) studied metamorphic antigens in species of *Anopheles* and *Aedes* by serological techniques.

In the present study an attempt has been made to establish electrophoretic protein patterns for selected examples of several genera of mosquitoes by means of the starch-gel technique, to discover at which stages of development sexual differences are discernable, and at what developmental stage different species are most easily distinguished.

MATERIALS AND METHODS. All mosquitoes were collected from nature with the exception of *Culex pipiens molestus,* which was maintained as a laboratory colony. This colony of *Culex pipiens molestus* has since been maintained by the senior author.

Other mosquitoes were collected from nature in the following manner. *Culex* and *Culiseta* were collected predominantly as egg rafts; these hatched and were reared in the laboratory. Species of *Aedes, Anopheles, Orthopodomyia* and some *Psorophora* were collected as larvae. Each of the above was then reared in the laboratory to the desired stage of development by standard methods. *Toxorhynchites,* the larvae of which are carnivorous, were collected as larvae, isolated and fed *Orthopodomyia* larvae or curly wing mutants of *Drosophila melanogaster. Psorophora ciliata* larvae are also carnivorous and were isolated and fed *Psorophora* and *Aedes* larvae from their native habitat.

Larvae were identified when they attained the fourth (last) instar. Pupae were placed in stender dishes and covered with a bell jar. Twenty-four-hour old adults were then collected in an aspirator and killed with chloroform. Fresh materials were always used in preference to frozen material, as a certain loss of electrophoretic band sharpness was experienced with frozen or cold stored samples. In cases where storage of samples was neces-

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ary, short-term cold storage at 1–2° Centigrade was used.

Larvae and pupae were rinsed with three changes of distilled water before preparation. Preliminary experimentation with various food deprivation periods before preparation showed gut contents of larvae to have no evident effect on electrophoretic pattern. Larvae were, however, isolated from their food supply one hour before preparation. Adults were unfed from time of eclosion until preparation.

Ten to 20 mosquitoes (0.15–0.25 gram) were required for a single electrophoretic examination, depending on the size of the species. *Aedes aegypti*, being a small species, required more individuals than *Culiseta inornata*, a large species. Required amounts of mosquito tissue of a given developmental stage and sex were placed in Kontes Potter-Elvehjem tissue grinders (5 cc. to 8 cc. size) with a standard appropriate amount (0.20 cc.) of gel buffer saturated with phenylthiourea crystals and homogenized with the grinders placed in an ice bath. Clark and Ball (1956), Laufer (1962) and Hubby (1963) have given precedents for utilizing homogenated whole organisms for soluble protein studies. This practice has been limited to small insects. Loughton (1965) states that results obtained from homogenates compare favorably to hemolymph preparation. Each homogenized sample was placed in a small wide mouth glass bottle and extracted in the presence of cyanide vapors for 30 minutes at 5° Centigrade. This treatment prevents melanin formation in the samples.

After the above treatment, the samples were placed in capillary tubes and centrifuged at 7000 r.p.m. for 10 minutes. The clear samples were ready for use in a previously prepared starch-gel.

The electrophoretic method used was that of Smithies (1955, 1959). The technique utilized was the vertical electrophoresis which gives improved resolution over the previously used horizontal method. The equipment was very similar to that described by Smithies (1959). The bridge buffer was tris (hydroxymethyl) aminomethane and boric acid; tris was of ionic strength 0.114 and the borate molarity 0.05. The pH of the bridge buffer was 8.55. The gel buffer was also tris-borate, but ionic strength of the tris was 0.0114 and borate molarity was 0.005. The starch (Starch-Hydrolyzed, Connaught Medical Research Laboratories, Toronto, Canada) was made at 12 percent in gel buffer.

The runs were done in the refrigerator at 5° C., buffers and gel equipment being previously equilibrated at this temperature. Runs were either 5½ hours at 5 MA constant current, 240 V. initially (7.4 V./cm), or 3½ hours at 8 MA constant current, 380 V. initially (12V./cm). The source of electric current was a Beckman Model RD-2 Duostat regulated power supply.

Each gel was stained for 30 minutes with Amido Black (napthol blue black) for proteins. Photographs using Kodak High Contrast Copy Film were used for permanent records.

Human serum diluted 1:10 or 1:20 with gel buffer was used in the gels as an index of comparison of migration rates and as a control for judging performance of each run against the well-known human serum electrophoretic pattern.

Representative species of the following genera were used in this study: *Toxorhynchites, Psorophora, Orthopodomyia, Culiseta, Aedes, Culex* and *Anopheles*. Major protein zones resolved on stained starch-gels have been assigned arabic numerals following common practice, the number one (1) in each case being the fraction nearest the origin and of the lowest electrophoretic mobility. Relative amounts of protein in any given zone or band are expressed by three progressively darker mechanical zones. Those mosquitoes examined for soluble proteins are given in Table 1. The electrophoretic mobilities given are calculated as decimal fractions of the mobility of human albumin (1.00), measured to the leading edge of the stained fraction. Mosquito fractions are measured to the center of each fraction. The mobility of human albumin is indi-

TABLE 1.—Median measurements of adult electrophoresis zones.

	Aedes zoosophus		*A. hendersoni*		*A. triseriatus*		*A. atropalpus*		*A. vexans*		*A. aegypti*	
Fraction	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.30	0.30	0.30	0.30	0.30	0.30	0.38	0.38	0.25	0.25	0.39	0.39
2	0.38	0.38	0.38	0.38	0.38	0.38	0.48	0.48	0.34	0.34	0.81	0.81
3	0.48	0.48	0.48	0.48	0.48	0.48	0.82	0.82	0.40	0.40	1.08	1.08
4	0.82	0.82	0.82	0.82	0.82	0.82	1.07	1.07	0.67	0.67	....	....
5	1.06	1.06	1.08	1.08	1.04	1.04	....	....	0.89	0.89	....	....
6	....	....	....	....	....	....	....	....	1.04	1.04	....	....

	Anopheles pseudopunctipennis		*Anopheles quadrimaculatus*		*Psorophora ciliata*	
Fraction	Male	Female	Male	Female	Male	Female
1	0.08	0.08	0.09	0.09	0.10	0.10
2	0.18	0.18	0.18	0.18	0.18	0.18
3	0.28	0.28	0.28	0.28	0.29	0.29
4	0.38	0.38	0.34	0.34	0.43	0.43
5	0.61	0.61	0.47	0.47	0.56	0.56
6	0.72	0.72	0.77	0.77	0.78	0.79
7	0.85	0.85	0.85	0.85	0.89	0.89
8	1.05	1.05	1.05	1.05	1.10	1.10

	Toxorhynchites r. sept.		*Orthopodomyia signifera*		*Culiseta inornata*		*Culex pipiens*		*Culex tarsalis*	
Fraction	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.31	0.33	0.34	0.34	0.37	0.37	0.31	0.31	0.39	0.34
2	0.34	0.37	0.45	0.45	0.78	0.78	0.84	0.84	0.85	0.85
3	0.74	0.74	0.77	0.77	1.09	1.11	1.03	1.03	1.03	1.03
4	0.96	0.96	0.97	0.97	1.15	1.19	....	....	....	....
—1	—0.04	—0.04	....	....	....	....	....	....	....	....
—2	—0.15	—0.15	....	....	....	....	....	....	....	....
—3	—0.32	—0.31	....	....	....	....	....	....	....	....
—4	—0.45	—0.46	....	....	....	....	....	....	....	....
—5	—0.57	—0.57	....	....	....	....	....	....	....	....

cated in the figures by a thin horizontal line. The point of origin is the black line at the lower edge.

RESULTS. The most distinctive protein patterns obtained in this study were from *Tox. rut. sept.* (Fig. 1 A, B). This was the only species of *Tox.* to be tested. It is readily apparent that *Toxorhynchites* has, in addition to fractions that migrate toward the anode (as does human albumin), five fractions that migrate toward the cathode (as does human gamma globulin). Those fractions bearing a negative number (Table 1) are those that migrate toward the cathode. Fraction 4 was closely associated with another band in the fourth instar larvae and pupae; this band has a relative mobility of 1.02. Fraction 3 has appeared as a double band in some but not all immature stages. Band 2 shows a distinct sexual dimorphism in adults, relative mobilities being 0.34 for males and 0.37 for females. Fraction 1 also exhibits a similar difference in mobility. This fraction shows a concentration difference between males and females most strikingly in fourth instar larvae sexed by dissection and observation of gonads. At this time, fraction 1 of the female is in higher concentration than in the male. This mobility difference extends to the adult stage. Fraction 1 occurs in *Tox. rutilus* in a higher concentration than in any other species of mosquito that was tested.

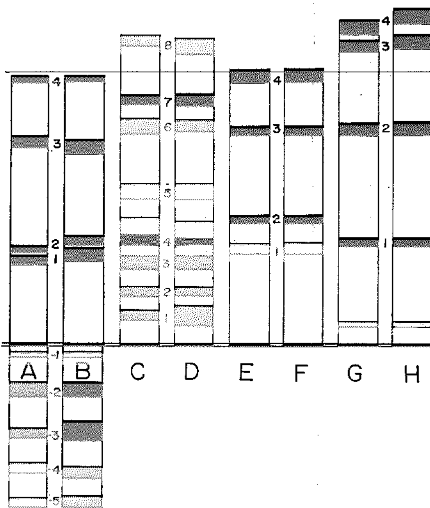

FIG. 1.—Diagram of starch-gel electrophoresis of adult mosquitoes
A. *Toxorhynchites rutilus septentrionalis*, male, B. *Toxorhynchites rutilus septentrionalis*, female, C. *Psorophora ciliata*, male, D. *Psorophora ciliata*, female, E. *Orthopodomyia signifera*, male, F. *Orthopodomyia signifera*, female, G. *Culiseta inornata*, male, H. *Culiseta inorata*, female

Minor fractions, not shown in the figures, migrating toward the anode include one in the female pupa and adult. Mobility of this fraction is 1.38. Two other minor fractions are seen in the larval stage, one between the origin and fraction 1 and the other above fraction 4.

As mentioned previously a group of bands in this species migrates toward the cathode (Fig. 1 A, B). Fraction —1 may represent debris but has been included because it appears consistently and stains darkly. Fractions —2, —3 show concentration differences between the sexes, the female having stronger concentration in all stages. Fractions —3, —4 are in smaller concentration than those above but also exhibit a smaller concentration in the male. Photographs show these differences more clearly than the diagram. In many male larvae examined, the cathode bands were barely visible.

Soluble proteins were also measured in *Tox. rutilus.* Female adults have 12.5 miligrams percent soluble protein or about two and one half times that of *Culex* adults which were calculted at 5.0–5.5 milligrams percent by the biuret method.

Only one species of *Culiseta, Culiseta inornata,* was studied. *Culiseta inornata* adults (Fig. 1 G, H) show sexual dimorphism in the mobility of the fastest fractions, the male fractions 3 and 4 being somewhat lower in mobility than the female. The same fractions (3 and 4) are also notable for their mobility as compared to other species; *C. inornata* had the high-

est recorded major band for any mosquito examined. Mobility was 1.19 in the female, the male was 1.15. Band 4 in the male seems to be reduced in amount compared with that of the female. This band also appears strongly only at the adult stage; however, trace mounts are evident in pupae. Late fourth instar larvae have this fraction in small amounts but it is absent in third instar larvae. Band 3 appears in heavy concentration at least as early as the third instar. The concentration of this band in the adult male is only slightly less than that of the female (1.09 to 1.11). Band 2 appears consistently as a major band as early as the third instar larval stage; there is no sexual dimorphism in mobility. However, this band is broader and less distinct at the larval and pupal stages of females.

Band 1 consistently shows higher concentration in the female adult than in the male. Larvae of both sexes show two faint bands occurring between bands 1 and 2. The upper and more diffuse of these bands all but disappears in the adult while the lower of these remains as a very faint thin band.

Two species of *Culex, C. pipiens quinquefasciatus* and *C. pipiens molestus,* were available in sufficient numbers for detailed study. The discussion of these two subspecies of the *Culex pipiens* complex has been combined because they possess identical starch-gel patterns (Fig. 2 C, D). These two fractions exhibit no sexual dimorphism and do not appear to change appreciably during development and metamorphosis. Band 1 is double in larvae and pupae and is in heaviest concentration in the 24-hour-old adult female. In the gravid female band 1 becomes indistinct and is greatly reduced in concentration. Eggs in this species have very small amounts of bands 1, 2 and 3, and a predominant amount of two new bands appears above and below band 1; this situation is currently being studied in more detail. The two species of *Culex* have two very slight narrow bands appearing just above band 1 at mobilities of 0.37 and 0.43. Perhaps the light band of 0.43 mobility is comparable to one of the specific egg proteins as it has about the same mobility.

Another species, *Culex tarsalis,* was briefly examined. *Culex tarsalis* exhibits a sexual dimorphism in the slowest fraction (Fig. 2 A, B). This fraction is also the heaviest of the major bands. According to these tests, the species of the genus *Culex* that were investigated have relatively few major protein bands.

Orthopodomyia signifera shows no sexual dimorphism in adult protein patterns (Fig. 1 E, F). The fastest migrating fraction (4) has a mobility almost equal to that of human serum albumin. This band becomes dense only in the adult stage; in the larval stage it is much lighter. Band 3 is dense in the larval stage and continues dark through the adult stage. Band 1 is slightly more dense than band 3 and remains unchanged in development and metamorphosis. Between bands 3 and 2 of the larva, there is another dense band,

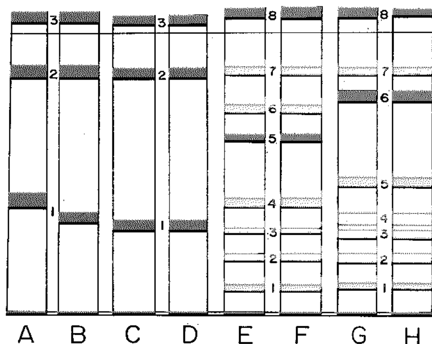

FIG. 2.—Diagram of starch-gel electrophoresis of adult mosquitoes
A. *Culex tarsalis,* male, B. *Culex tarsalis,* female, C. *Culex pipiens,* male, D. *Culex pipiens,* female, E. *Anopheles pseudopunctipennis,* male, F. *Anopheles pseudopunctipennis,* female, G. *Anopheles quadrimaculatus,* male, H. *Anopheles quadrimaculatus,* female

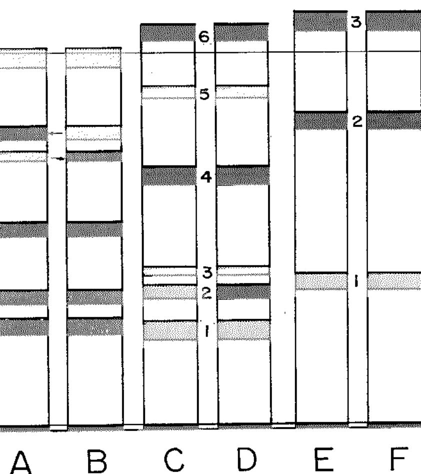

FIG. 3.—Diagram of starch-gel electrophoresis of mosquitoes
A. *Orthopodomyia signifera,* fourth instar larva, B. *Orthopodomyia alba,* fourth instar larva, C. *Aedes vexans,* male adult, D. *Aedes vexans,* female adult, E. *Aedes aegypti,* male adult, F. *Aedes aegypti,* female adult

which is seen in the larval diagrams (Fig. 3 A) but it disappears at the pupal stage and does not occur in the adult. Band 1 is a very dense band in the fourth instar larval and pupal stage but nearly disappears in the adult stage.

It is of interest to compare the larvae of *Orthopodomyia signifera* and *Orthopodomyia alba,* another species that was studied. Band 3 of *Orthopodomyia signifera* (Fig. 3 A, arrow) is present as a very minor band in *Orthopodomyia alba* whereas another dense band of slightly lower mobility is seen in *Orthopodomyia alba* (Fig. 3 B, arrow). This heavy band is present in *Orthopodomyia signifera* as a minor band. All other bands have a good correlation in both species of *Orthopodomyia.* Insufficient numbers of adult *O. alba* were obtained for testing.

Psorophora ciliata was the only species of this genus tested, and only a few adults were available; no larvae were tested. This species has a high number of bands; two are very heavy and five are somewhat lighter (Fig. 1 C, D). Band 8 is of intermediate concentration and is slightly different in concentration between the sexes. Band 7 is the most concentrated fraction in this species. Band 6 is present in about the same concentration as band 8; band 5 is in lower concentration than those above. Band 4 is as heavy as band 7 and seems to grade at both the leading and trailing edges into a diffuse area; the appearance of the gels suggests that a diffuse amount of protein is present in the whole lower half of the electrophoretic pattern in this species. Bands 3, 2, and 1 are also intermediate in concentration and vary slightly in band thickness. The pattern in this species sets it apart somewhat from other mosquitoes tested because of the large number of bands in the intermediate range of concentration.

The specimens of *Anopheles pseudopunctipennis* that were tested were collected locally; *Anopheles quadrimaculatus* was available only as frozen specimens. *Anopheles pseudopunctipennis* and *Anopheles quadrimaculatus* adults have a total of eight protein bands, none of which exhibit a sexual dimorphism (Fig. 2 E–H). Band 8 is a dense band in both species. Band 7 is of intermediate concentration and has identical mobility in both species. Band 6 is more dense in *A. quadrimaculatus and* has a greater mobility (0.77) in this species than in *A. pseudopunctipennis* (0.72). Band 5 exhibits a reciprocal situation with this band being more dense and having greater mobility (0.61) in *A. pseudopunctipennis* than in *A. quadrimaculatus* (0.47). Band 4 varies slightly, *A. pseudopunctipennis* being of intermediate density. Bands 3 and 2 are of light density and have identical relative mobilities. Band 1 is of intermediate concentration and has a mobility of 0.08 in *A. pseudopunctipennis* and 0.09 in *A. quadrimaculatus.*

Four of the five species of the subgenus *Finlaya* occurring in the United States

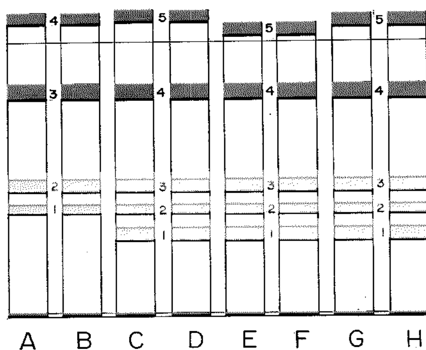

FIG. 4.—Diagram of starch-gel electrophoresis of adult mosquitoes
A. *Aedes atropalpus,* male, B. *Aedes atropalpus,* female, C. *Aedes hendersoni,* male, D. *Aedes hendersoni,* female, E. *Aedes triseriatus,* male, F. *Aedes triseriatus,* female, G. *Aedes zoosophus,* male, H. *Aedes zoosophus,* female

were examined. These were *Aedes atropalpus, Aedes hendersoni, Aedes triseriatus,* and *Aedes zoosophus.* In addition, *Aedes aegypti* (subgenus *Stegomyia*) and *Aedes vexans* (subgenus *Aedimorphus*) were also studied.

Electrophoretic patterns of the subgenus *Finlaya* are presented in figure 4 and *A. aegypti* and *A. vexans* are in fig. 3. As can be seen, the patterns are very similar. No sexual dimorphism was noted in electrophoretic mobility. Two major bands were found in adults. The fastest migrating band varied only slightly within this subgenus: *Aedes hendersoni* (1.08) had the highest mobility; *Aedes atropalpus* (1.07) and *Aedes zoosophus* (1.06) were intermediate; *Aedes triseriatus* was most divergent in this band (1.04). The other major band had a mobility of 0.82 in all members of this subgenus. The three tree-hole breeding members examined, *Aedes hendersoni, Aedes triseriatus,* and *Aedes zoosophus,* showed bands 3, 2, and 1, which had mobilities of 0.48, 0.38, and 0.30, respectively. The rock-hole breeding member of this group, *Aedes atropalpus* (Fig. 3) differed slightly in that it showed two of these three bands but lacked one. Some very indefinite bands were found between band 1 of *Aedes atropalpus* and the origin slot.

Developmental changes have been followed in *Aedes hendersoni, Aedes triseriatus,* and *Aedes zoosophus.* Bands 2 and 3 are extremely dark in fourth instar larvae and pupae and resemble both in appearance, density, and mobility those of larval stages of *Orthopodomyia.*

Band 4 is a major band throughout metamorphosis, being dense in larvae, becoming less dense in pupae and dense once more in adults. Band 5 is relatively weak in larvae, weaker in pupae, and dense in adults.

A number of faint bands appear in larvae, one with a mobility of 0.95 which becomes diffuse at the pupal stage and disappears at the adult stage. Some bands of high mobility are noted. One band (mobility of 1.35) appears in larval and pupal stages, another of mobility 1.20 appears with less density in larvae but disappears in pupae. These faint, rapidly migrating bands may represent transient developmental proteins.

Adults of *Aedes aegypti* (Fig. 3 E, F) have three bands, two major and one intermediate. Band 3 corresponds to band 5 of *Aedes hendersoni* with a mobility of 1.08, band 2 with a mobility of 0.81 corresponds to band 4 of the *Finlaya* group. Band 1 has the same mobility as band 2 of *Finlaya* but is of intermediate density while adults of *Finlaya* have three weak bands.

Aedes vexans (Fig. 3 C, D) has one dense band (band 6). Its mobility is the same as that of band 5 of *Aedes triseriatus.* Band 5 is of low density, and band 4 is dense. Band 3 is faint. Band 2 exhibits a sexual dimorphism; the mobility is the same (0.34) in both sexes but the band is denser in the female. In the male the density is intermediate; band 1 is intermediate in density in both sexes.

DISCUSSION. A major consideration in a study of this type is the level of discrimi-

nation of the technique utilized, that is, at what taxonomic level does this technique distinguish groups of organisms? The results of previous studies indicate that the level of discrimination depends on both the group under study and what technique is applied. Starch-gel electrophoresis of mosquito proteins seems to distinguish adults at the species and species-group level. Patterns remain the same under given identical conditions. The "type" pattern for a given taxonomic group holds for major bands within a subgenus such as *Finlaya* of the genus *Aedes* in most cases examined. Within the subgenus *Finlaya* there was variability within a small range in the fastest migrating major band, although the other major band showed no variability. Within all the species tested in *Aedes* there was also a trend for certain bands to be repeated in different species. At the higher levels generic trends were apparent. For example, species of *Anopheles,* considered to be a primitive genus, had eight bands while at the other end of the already proposed (Ross, 1951; Thurman, 1959) evolutionary spectrum, species of the more advanced *Culex* and *Aedes* showed only two to three bands. Strictly speaking, patterns are species specific and within the same subgenus tend to be more similar to each other than to species outside that subgenus.

A single zone resolved on an electrophoretic pattern does not necessarily mean that only one type or species of protein appears in that zone, but that several proteins of similar migration rates may be represented. The absence of a band may mean that the fraction is either absent or present in such trace amounts that it is not identifiable by the methods employed.

In the present study, bromophenol blue, another dye commonly used to bind human albumin as a marker, was often added to insect samples. This material had no affinity for any bands, and it migrated unbound far in advance of the protein bands. Thus none of the mosquitoes had any fraction bindable to bromophenol blue which indicates the absence of any fraction similar to albumin.

In all species of mosquitoes examined, one major band lies near the mobility of albumin. Table 1 summarizes these data. The highest mobility of 1.19 was in *Culiseta inornata,* while *Toxorhynchites rutilus septentrionalis* and *Orthopodomyia signifera* had lower values of 0.96 to 0.97. The majority of the mosquitoes examined had mobility values between 1.03 to 1.10. There is no significance attached to the position of this fraction, except that possession of a fraction with a mobility near that of albumin seems to be a trend within the family Culicidae.

The electrophoretic pattern of *Toxorhynchites r. sept.* is the most distinctive of any mosquito examined in this study. The anodiacally migrating fractions (Fig. 1, bands 1, 2, 3, 4) are not especially unusual, with the exception of two bands (bands 1, 2) which were exceptionally dense, particularly in the female. The unusual feature was that there were proteins that migrated in the same direction (toward the cathode) as human gamma globulin, a feature not found in any other mosquito at this pH (8.55). This feature alone would place *Toxorhynchites* in a position apart from all other mosquitoes examined. A total of five bands migrated in this direction (Fig. 1, band —1, —2, —3, —4, —). While these cathodically migrating bands invite comparison with gamma globulin, the direction of migration at this pH is their only known similarity. The important distinction is the fact that gamma globulin migrates as an indistinct swath and is made up of a heterogeneous group of molecules; the cathodically migrating bands in *Toxorhynchites,* concentrated heaviest in females, on the other hand, are very distinct, clear-cut, and repeatable.

Species of the genus *Toxorhynchites* occupy a distinct ecological niche; they are unusual in many ways other than the distinctive protein pattern. The larvae are completely carnivorous, utilizing mosquito larvae, adult flies, or each other as food. Their diet is, therefore, protein rich. The adults, unlike most other mosquitoes, never feed on blood. The lack of

an adult blood meal and high protein concentration in this mosquito indicate that a mechanism operates here similar to that proposed by Laufer (1963) and Telfer (1954) for the saturnid moths, that is, in *Toxorhynchites* a storage of protein reserve may be built up by feeding larvae which is of use to the adults for reproduction and as a supply of amino acids. Experiments with varying food type made no appreciable change in electrophoretic pattern.

Psorophora ciliata is also carnivorous in the larval stage. When examined by starch-gel electrophoresis this species lacked the cathodically migrating bands. However, it had a rather large number of less densely staining bands, indicating relatively greater amount of soluble proteins than culicine mosquitoes. However, *Psorophora ciliata* must have a blood meal before yolk deposition can occur, as must most mosquitoes. The suggestion is made, therefore, that the cathodically migrating bands of *Toxorhynchites* represent storage proteins that are utilized in later adult life for reproductive purposes, a situation that does not occur in *Psorophora ciliata.*

The status of *Orthopodomyia signifera* and *Orthopodomyia alba* has been debated since *O. alba* was originally described. The two are thought to be closely related and it has been suggested that *Orthopodomyia alba* is a genetic variant of *Orthopodomyia signifera* (Jenkins and Carpenter, 1946). The adults are morphologically indistinguishable; by contrast the fourth instar larval stages of the two differ in coloration and other features, and they are easily distinguishable with the unaided eye. It has also been found that the larvae are distinguishable in all stages beginning with the first instar (Wilkins and Breland, 1951).

The electrophoretic differences between *Orthopodomyia alba* and *Orthopodomyia signifera* (Fig. 3) are greater than differences among the species of the *Finlaya* group of *Aedes;* most of these *Aedes* species can be distinguished from each other by adult as well as larval features. It is believed that the differences in protein patterns between *Orthopodomyia alba* and *Orthopodomyia signifera* should be considered as added evidence that the two should be regarded as distinct species.

Three species of the subgenus *Finlaya* have presented taxonomic problems in the past and some of these problems have not yet been solved. These species are *Aedes zoosophus, Aedes triseriatus* and *Aedes hendersoni.* All of these species breed in tree holes, and larvae of all three often occur in the same cavity. The adults of *Aedes zoosophus* are easily distinguishable from the other two, but until some years ago, features used to distinguish the larvae of this species and *Aedes triseriatus* were unreliable. The larvae, while similar, are now distinguishable (Breland, 1949). These two species are considered closely related to each other, but since the adults are easily distinguishable there has never been any suggestion that the two should be regarded as the same species.

The situation with respect to *Aedes triseriatus* and *Aedes hendersoni* is somewhat more complex. Until recently *Aedes hendersoni* was considered variously as a synonym or a variety of *Aedes triseriatus.* Recently, however, *Aedes hendersoni* has been raised to full specific rank (Breland, 1960), on the basis of larval differences, as well as adult differences in certain parts of the ranges of the two species. In central Texas, there is a broad overlapping of the ranges of the two, larvae are distinct, but the adults are very difficult to distinguish from each other. True *hendersoni* is considered to occur in Colorado and adjacent areas, whereas, unsolved taxonomic problems still exist in the group in Central Texas and other areas where its range overlaps that of *Aedes triseriatus.*

The starch-gel patterns of the adults of *Aedes triseriatus, Aedes zoosophus,* and *Aedes hendersoni* showed variation in one band, this being the most rapidly migrating one (Fig. 4, band 5). *Aedes hendersoni* and *Aedes zoosophus* are more similar in this band, whereas *Aedes triseriatus* is different from both. Larval protein differences are not as obvious as are adult differences, but even here, the larvae of *Aedes hendersoni* and *Aedes zoosophus* are more

similar to each other than either is to *Aedes triseriatus*.

It is not considered that these results solve all the problems relative to the *hendersoni* complex, but it is suggested that they give added evidence that *Aedes hendersoni* and *Aedes triseriatus* are distinct species, even in the central Texas area. Perhaps the more sensitive technique of enzymatic anaysis on gels would help further to clarify the situation.

Two major publications have appeared on evolution within the family Culicidae. Additional evidence has been obtained from the present work. Data from protein patterns and serological evidence, to be reported elsewhere, lend support to parts of the generic evolutionary scheme of Thurman (1959) as opposed to the scheme of Ross (1951). Thurman groups *Orthopodomyia* and *Culiseta* within the tribe Culisetini; *Culex* is placed in the tribe Culicini. Ross proposed a phylogenetic tree in which *Culex* and *Culiseta* are located side by side as being derived from similar ancestors. *Orthopodomyia* is shown as arising early from the Culicinae. *Culiseta,* according to the present data, is more closely related to species within the genus *Orthopodomyia* than to species of *Culex*. Both from protein band correspondence (none shared with *Culex,* two shared with *Orthopodomyia*) and from unpublished serological cross-reaction data a greater degree of serological cross reactions with *Orthopodomyia;* less cross-reaction with *Culex*. These results agree with the ideas of Laufer (1964) who proposed in a very similar case that electrophoretic mobilities had become modified through mutation and evolution, and as the species became more divergent from a common ancestor, the antigenic sites have undergone less alteration. The above-mentioned serological data also show that *Orthopodomyia* bears a certain measure of serological relationship to *Toxorhynchites,* which seems to agree with the phylogeny of Ross.

Sexual dimorphisms in protein patterns have been noted for several species of mosquitoes in the present study, most apparent in *Culiseta inornata* and *Toxorhynchites r. sept.;* these differences were both qualitative and quantitative. These results differ markedly from the predictions of Stephen and Steinhauer (1957) and Steinhauer and Stephen (1959) that within a species protein pattern differences would be quantitative only. The development of these sexual differences has been worked out in some detail for *Culiseta inornata,* where sex can be determined as early as the third instar by observing the testis through the integument. Sexual differences become apparent only in the adult, and then only in fractions three and four. Some pupae show one of these dimorphic fractions but this is variable. The evidence from the present work and the results of others (e.g. Telfer, 1954; Laufer, 1960a) indicate that differences between males and females of a given species may be both quantitative and qualitative. The results presented herein will perhaps help to clarify the problem of little cross reactivity of male and female antisera described by Smith and Silverman (1966).

In the species studied in various stages of the life cycle, patterns of mosquitoes do change during development, individual fractions appearing and disappearing at various stages in the life cycle independently of each other. This situation suggests that each protein fraction is under independent genetic and hormonal control. Results of the present study indicate that the immature stages of mosquitoes contain a greater number of protein fractions than the adult stage. These results are somewhat at variance with the reports of Whittaker and West (1962) and Laufer (1964), working with moths, that band number increased at the adult stage. This disagreement may be due to differences in the type of life history of moths and mosquitoes.

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