ELECTROPHORETIC TECHNIQUES FOR THE GENETIC STUDY OF MOSQUITOES

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ABSTRACT. The techniques of starch gel electrophoresis have become widely used in studying the genetic biology of a wide number of species. In the following paper, we describe the techniques and materials in use in our laboratory to conduct electrophoresis on mosquitoes of the genera *Culex*, *Anopheles* and *Aedes*. A brief survey is included on electrophoretic studies in mosquitoes. Our survey indicates that most of these studies have concentrated on polymorphisms in only 2 proteins and provides a guide for those seeking alternative techniques.

Genetic studies of mosquito species have been retarded by the lack of efficient, accurate and dependable methods by which to identify individual gene loci and their allelic arrays. The application of electrophoretic techniques to identify variable (polymorphic) gene loci in mosquitoes seems currently the best way to resolve this problem. These techniques have been available since 1961 and are now widely used in the study of the genetics and evolutionary biology of a wide range of organisms. It is only in the past few years, however, that they have become widely applied to the study of mosquitoes; the techniques (including histochemical stains) hold great potential for the study of phylogenetic relationships, for investigating species homologies, for studying ecological-genetic relationships, and for many other purposes as well. Our laboratory has been interested in different aspects of these problems in both *Drosophila* and various mosquito species. Because few studies to date have aimed at surveying a great number of electrophoretic loci in mosquitoes, we became interested in establishing a set of techniques which would enable the surveying of a wide range of protein loci from a single mosquito at a reduced cost. We report here our success to date, summarizing our techniqies for starch gel electrophoresis and providing a description of our standard equipment (Appendix 1) including how to make those items not generally available and where to buy items which are. The items we list are not meant as an endorsement; other pieces of equipment and/or biochemicals may be substituted with a few exceptions; we list these only so that other workers who wish to establish an electrophoresis laboratory will have some feeling for the necessary equipment and chemicals. In addition, we include a table of published studies reported by other authors using similar techniques and a brief review. These not only will give the reader an idea of the wide applicability and importance which the electrophoretic approach is gaining in mosquito biology, but also enable the reader to have at hand a ready bibliography of alternative techniques to ours.

EQUIPMENT PREPARATION

Before sample preparation, every effort should be made to have all the required solutions and equipment prepared, as many proteins are unstable at room temperature.

The electrophoresis apparatus consists of 2 electrode chambers (see Figure 1) containing a buffered electrolyte solution from Table 1, an electrical power source (i.e., a Heathkit model IP-17 power supply) and the U-molded gel. The gel serves as a bridge between the electrode chambers (Figure 2). The electrical current serves to set up a charged field in the gel

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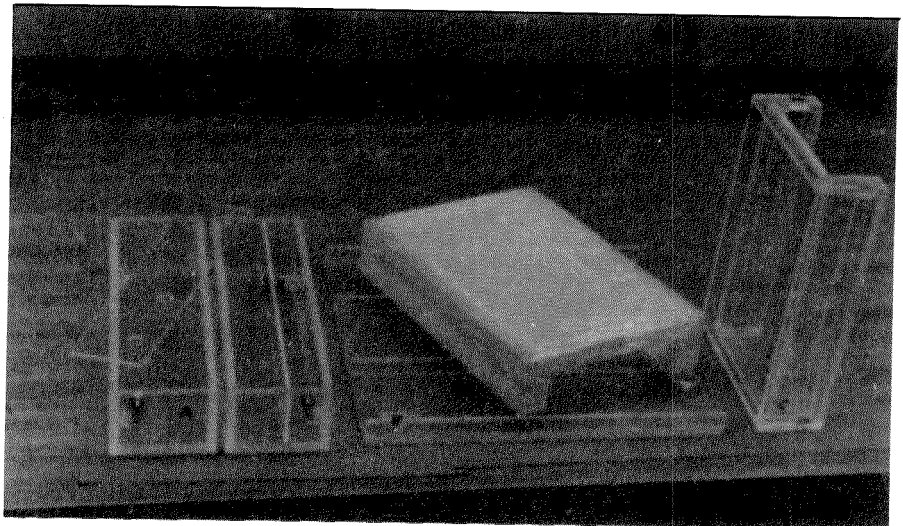

Figure 1. Photograph of a complete electrophoresis unit with an extra U-mold present. A, the two electrode (bridge) buffer chambers with one of the removable dividers lying across the top. B, the support plate which holds the electrode chambers in the outside channels formed by the support rails. C, two U-molds are shown, one containing a poured, solidified starch gel covered with saran wrap. Note that the bottom slot along each leg of the gel-containing mold has been taped shut. The tape will be removed just prior to the electrophoretic run as this is the portion of the gel which will extend into the electrode chamber buffer to complete the electrical circuit. The U-molds shown are for 1 cm thick gels.

Table 1. Gel and bridge (electrode chamber) buffers. All water used should be distilled and/or deionized.

System	Buffer Type	Adjust to pH	Chemical Ingredients
LIOH	gel	8.45	.074 M Trizma Base (9.00 g/L) .009 M Citric Acid H_2O (1.9 g/L)
	Electrode	8.25	.036 M Lithium hydroxide (1.50 g/L) .194 M Boric Acid (12.00 g/L)
CA-8	gel	8.45	.074 M Trizma Base (9.00 g/L) .009 M Citric Acid H_2O (1.9 g/L)
	electrode	8.10	1.37 M Trizma Base (166.40 g/L) .314 M Citric Acid·H_2O (66.00 g/L)
	Note: Cathode tray; dilute stock 1:3 (v/v) with dd H_2O. Anode tray; dilute stock 1:4 (v/v) with dd H_2O.		
CA-7	gel	7.10	.009 M Trizma Base (1.10 g/L) .003 M Citric Acid H_2O (0.63 g/L)
	electrode	6.90	.135 M Trizma Base (16.35 g/L) .040 M Citric Acid H_2O (8.40 g/L)

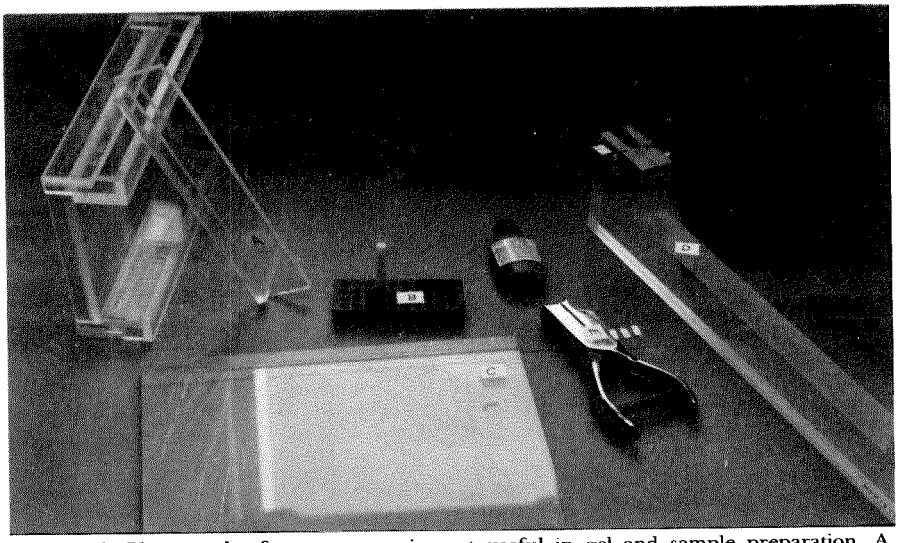

Figure 2. Photograph of accessory equipment useful in gel and sample preparation. A, L-shaped slot guide leaning against a U-mold; B, a nylon grinding block containing 4 rows of 1/4 inch wide holes each countersunk (beveled) at the top (note the nylon grinding peg inserted in one of the holes); C, a 1.5mm cutting guide with only one of the 1.5mm raised edges in view. Sunlight from a nearby window is being reflected off the cutting guide. D, a special plexiglass gelslicer; the banjo wire it holds is drawn through a gel to obtain a gel slice after the gel is placed on the cutting gude. In the background can be seen a small bottle of RBY reference dye (Gelman Instruments, Ann Arbor, MI) and a drill-like apparatus to hold the grinding peg seen in B. Item E is a 4 × 10mm paper punch, available from M. C. Mieth Manufacturing (Port Orange, FL), which is used to make the paper inserts that the homogenized sample is drawn up into.

in which the protein molecules migrate and separate depending on:

(a) the number of positive versus negatively charged amino acids present in the protein;

(b) the size and configuration of the molecule with respect to the pore size of the gel matrix;

(c) the ionic and steric forces created by the buffer system and its specific pH;

(d) other, unknown factors.

Each electrode chamber contains a platinum electrode (20 gauge platinum wire) attached to a banana plug set in 1 end of the chamber. A set of 2 chambers will hold one gel. About 250 ml of electrolyte is placed in each chamber for an electrophoretic run and is good for about 3 runs. The gel mold is in the shape of an inverted U, hence our name U-mold for this component. The parts to make a complete electrophoretic unit, as shown in Figure 1, are listed in Table 2. Note that the design of the U-mold facilitates completion of the electrical circuit when the gel is placed so as to bridge the chambers.

The apparatus must be cooled, to prevent heat degradation of the proteins, by placing it in a controlled environment, (such as a refrigerator), and by placing ice trays on top of the gel.

GEL AND ELECTRODE BUFFER SYSTEMS

The 3 buffer systems we find most useful in analysis of mosquito protein sys-

tems are listed in Table 1. The first one is modified after Ashton and Braden (1961) and is discontinuous in nature; that is, the bridge (electrode) buffer is different from the gel buffer. The second system (CA-8) is a continuous system with the bridge buffer having the same composition as the gel buffer, but being much more concentrated. This system is modified from Shaw and Prasad (1970) and has the unique feature of having a higher ionic concentration in the cathodal tray than in the anodal buffer chamber. Finally, the third system (CA-7) is a continuous system with a much lower ionic concentration and pH than CA-8.

Table 3 lists the enzyme or protein systems which can be analyzed on each buffer system. Note that some systems can be analyzed on the same gel (i.e., *Pgm* and *Pgi* on system LIOH). Since a maximum of 6 gel slices can be obtained without much difficulty from each, 1 cm thick gel, this "double-staining" technique enables one to stain for 5–8 protein systems on each gel. Some of these systems in turn code for more than 1 locus. The technique thus maximizes the amount of information to be gained from a single small organism such as a mosquito.

GEL AND SAMPLE PREPARATION

Gels are prepared using the appropriate gel buffer for the protein system under analysis (see Tables 1 and 3). An 11.5% (w/v) solution of electro-starch (Otto Hiller, Madison, Wisconsin) is

Table 2. Component specifications to build one complete U-mold and gel chamber assembly. See Figure 1 for a complete unit. All components are made from ¼ inch plexiglass with one exception (see below).

U-mold component	Number per U-mold	For a 6 mm thick gel[1]
Base	1	11.5 x 20.3 cm
Inside U-wall	2	3.1 x 20.3
U-floor	2	1.3 x 20.3
Endplate	2	.6(1) x 11.5
Outside U-wall	2	2.5(2.8) x 20.3
Wall support (L-Shaped)	4	3.8(4.1) x .6 x 2.5(2.8) x .65 x 1.3 x 1.3

Gel chamber component	Number per set of 2 chambers	Component Dimensions
Base	2	5.1 x 22.5 cm
Sidewalls	4	5.1 x 22.5
Endplate[2]	4	5.1 x 4.5
Removable divider[3]	2	4 x 21.8
Electrode holder	4	1 x 1
Platinum wire, 20ga.	50 cm long	25 cm per chamber
Banana plugs, male	2	one per chamber, to screw through endplate
Support plate	1	23 x 23
Support rails	4	1 x 23

[1] For a 1 cm thick gel, substitute the dimension in the parenthesis for the one immediately preceding it. All component dimensions are in cm.

[2] These must have a 3 mm groove cut 2 cm from and parallel to the 4.5 cm edge. For each chamber, one endplate must be tapped near the top between the divider groove and the edge in the narrow (2 cm wide) portion to receive a banana plug.

[3] Made of ⅛ inch plexiglass with four ⅜ inch holes tapped at even intervals along the length and 1 cm from the edge.

Note: In some instances, we have used standard U.S. measurements rather than metric units because the plexiglass is sold or cut in these units rather than in metric.

heated over a gas flame with continuous swirling in a 1000 ml Erlenmeyer flask until a vigorously boiling, clear, viscous solution is obtained (4–5 min). The starch solution is then degassed via an aspirator attached to a water faucet until large bubbles form at the surface of the cooked solution (about 60 sec). The solution is then poured into a U-mold which has the slots along the bottom of the sidewalls taped shut with a double layer of three-inch masking tape. To conserve starch, it is wise to cook only that amount which matches the investigators' analytical desires. In our laboratory, we have gel molds which form gels 1 cm thick (approximate volume of cooked and degassed starch 425 ml; yield = six, 1.5 mm thick gel slices obtained by slicing the gel in a horizontal plane as described in the next section), gels 6 mm thick (approximate volume of cooked and degassed starch 325 ml; yield = three, 1.5 mm thick gel slices) and gels 3 mm thick (approximate volume of treated starch 210 ml; yield = two, 1.5 mm thick gel slices). Table 2 includes the components and their dimensions to make the 1 cm and 6 mm thick U-molds for the gels.

After pouring, the gel is cooled at room temperature for 1 hr, covered with saran wrap to prevent excessive dehydration,

Table 3. Three buffer systems and the protein systems which they separate. See Table 1 for a description of each buffer system.

Buffer System	Enzyme Classification Number	Protein	Number of Loci encoded[1]	
LIOH	3.1.3.2	Acid Phosphatase[3]	(Acph)	1
	1.1.1.1	Alcohol dehydrogenase[3]	(Adh)	1
	1.2.3.1	Aldehyde oxidase	(Aldox)	1
	3.1.3.1	Alkaline phosphatase[3]	(Aph)	1–2
	3.2.1.1	Amylase	(Amy)	1
	3.1.1.1	Esterase	(Est)	4–6
	———	General protein	(cbb)	1–2
	2.6.1.1	Glutamate oxaloacetate transaminase	(Got)	2
	3.4.11	Leucine aminopeptidase	(Lap)	2–4
	1.1.1.40	Malic Enzyme	(Me)	1
	1.1.1.73	Octanol dehydrogenase[3]	(Odh)	1
	3.4.11	Peptidase	(Pep)	2
	2.7.5.1	Phosphoglucomutase	(Pgm)	1
	5.3.1.9	Phosphoglucose isomerase	(Pgi)	1
	5.3.1.1	Triose phosphate isomerase[3]	(Tpi)	1
	1.2.1.37	Xanthine dehydrogenase	(Xdh)	1–2
CA-7[2]	4.2.1.2	Fumerase	(Fum)	1
	1.1.1.49	Glucose-6-phosphate dehydrogenase[3]	(G-6-pdh)	1
	1.1.1.8	α-Glycerophosphate dehydrogenase	(α-G-pdh)	1
	1.1.1.42	Isocitrate dehydrogenase	(*T*dh)	2
	2.7.1.1	Hexokinase	(Hk)	2–4
	1.1.1.44	6-phosphogluconate dehydrogenase	(6-pgdh)	2
CA-8	2.7.4.3	Adenylate kinase	(Adk)	1–2
	4.1.2.13	Aldolase[3]	(Ald)	2
	1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase[3]	(gly-3-pdh)	1
	1.1.1.30	Hydroxybutyrate dehydrogenase[3]	(Hbdh)	1
	1.1.1.37	Malic dehydrogenase	(Mdh)	2

[1] May vary with the species or the developmental stage analyzed.

[2] The 6 enzymes may also be analyzed on CA-8; although separation of bands is better on CA-7, CA-8 may have sharper bands.

[3] These enzymes have been noted to have generally weaker activity on these buffer systems although banding is often very good.

and cooled for another hour (the latter period may be hastened by placing the gel in a refrigerator for 30 min after covering). A vertical slot is then made 2 in from 1 end of the cooled gel with a safety razor blade using a straight-edged ruler as a guide (our laboratory uses an L-shaped slot guide of plexiglass; see Figure 2). The samples will be placed in this slot prior to electrophoresis. About 30 samples can be placed on a single gel.

The samples are prepared as follows: Whole organisms or tissue samples are placed in a nylon block containing 4 rows of nine, 1/4 inch holes about 1/3 of an inch deep and slightly beveled at the top (Figure 2). A nylon peg (2 inches long, 1/4 inch in diameter) is then inserted into the holes and used to homogenize the samples. The peg may be attached to a multi-speed, drill-like apparatus to facilitate homogenization. (Samples may also be ground by hand on a 9-hole spot plate using a hand-held glass rod with a rounded end.) In either case about 25 microliters of distilled water or buffered gel solution (Table 1) is added to the sample prior to homogenization.

After homogenization, the homogenate is soaked in 10 × 4 mm paper wicks for placing in a 1 cm deep gel. The wicks may be Whatman #1 for enzymes with strong to moderate activity, or Whatman #3 for enzymes with weak activity. These wicks are then placed in the sample slot on the prepared gel for electrophoresis. In most cases, 2 wicks may be obtained per sample and each inserted into a different gel. For gels of lesser thickness, wicks correspondingly shorter than 10 mm must be used. The gel in the U-mold is covered with Saran wrap and may now be placed so as to form the bridge between the electrode chambers (Figure 1). The power supplies are then turned on. Running times will vary somewhat depending on how well standardized the power supplies are. For the systems listed, the buffer front for system LIOH will run about 10 cm at 75 mA in 5 hr, CA-8 will run about 10 cm at 90 mA in 5 hr, and CA-7 will run about 10 cm in 4 hr at 75 mA. For overnight runs, these times may be extended by cutting the mA (i.e., at 30 mA, the LIOH buffer front will migrate 10 cm in 15 hours). For all systems, we find it advisable to include a wick (insert) containing a tracking dye (i.e., RBY dye from Beckman Company). Included as sample number 31, the dye marks how fast the buffer front is moving on systems CA-7 and CA-8. The actual buffer front is not visible on the last 2 systems as it is on LIOH where the borate ions tend to mark this moving zone as a thin brown line.

GEL STAINING

After electrophoresis, the power is turned off and the gel removed from the electrophoresis apparatus. The ends of the gel beginning at where the gel bends downward as it follows the inverted U-mold, are then cut off and discarded. Next, the paper wicks are removed and the gel and back slice (that narrow piece of gel between the cut at the cathodal end and the slot for the sample wicks) are placed upon a 1.5 mm cutting guide (Figure 2). A length of 4 pound test nylon fishing cord or banjo string (.009 width) is used to slice the gel horizontally into five-six, 1.5 mm thick gel slices. These slices are then placed in individual staining boxes (A401 or A206, Vlcheck plastics, Middlefield, Ohio). Each slice can then be stained for a particular protein according to the specifications in Tables 3 and 4.

Care should be taken to include the back slice for staining when a new enzyme or a new organism is being examined as some proteins will migrate cathodally instead of anodally in an electric field with a basic pH. Generally speaking, aldolase, glutamate oxaloacetate transaminase, malate dehydrogenase and glyceraldehyde-3-dehydrogenase have loci which migrate very near to the point of origin on these systems for mosquitoes.

When staining is finished, the stain can be aspirated off and discarded. A few stains may be saved and used over after filtering (i.e., those containing TPN or

Table 4. Stain solutions adapted from Brewer (1970) or Shaw and Prasad (1970). All water used should be distilled and/or deionized as some metals will inhibit enzyme activity. See Table 5 for other solutions used below. Most of the biochemical reagents in this list may be obtained from Sigma Company, St. Louis, Missouri. * = photosensitive stain

Protein	Rating	Technique	Stains best in
Acph	2	Presoak gel 20 minutes in solution 10. Discard solution. To a 50 ml mixture of solution 8 and 9 add: 50 mg sodium-α-naphthyl acid phosphate, 100 mg Polyvinylpyrrolidone, 30 mg Fast Blue BB or RR. With filtering, pour over gel and incubate for 3 hours at 37°C. Remove stain and add solution 18.	adults or larvae
Adk*	2	To 35 ml of solution 13 add: 5 ml $MgCl_2$ solution 16, 200 mg α-D+glucose, 40 mg ADP, 1 ml solution 20, 1 ml solution 21, 40 units Glucokinase, 3 ml solution 23. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml solution 19 and incubate for 2 hours more. Remove stain and add solution 18.	adults
Adh*	2	To 35 ml of solution 13 add: .5 ml isopropyl alcohol (2-propanol), 1 ml solution 20, 2 ml solution 22. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml solution 19 and incubate for 1 hour more. Remove stain and add solution 18. NOTE: This enzyme may be stained with *Xdh, Odh,* or *α-gpdh* as long as the band zones don't overlap.	larvae
Aldox	1	To 35 ml of solution 14 add: 0.20 ml of Benzaldehyde, 1 ml solution 20. Pour over gel and incubate at 37°C for 15 minutes. Add 0.5 ml solution 19 and incubate 30 minutes more. Remove stain and add solution 18.	adults
Ald*	2	To 35 ml of solution 13 add: 200 mg Na_4 Frutose-1, 6-diphosphate; 1 ml solution 20, 2 ml solution 22; 50 units glyceraldehyde-3-phosphate dehydrogenase. Pour over gel and incubate at 37°C for 25 minutes. Add 0.5 ml solution 19 and incubate for 1 hour more. Remove stain and add solution 18.	adults
Amy	1	This protein must be run on Acrylamide gels. To 50 ml solution 13 add: 10 ml of a 10% starch solution which has been boiled in a sodium phosphate, pH 6.5 solution and cooled. Pour over gel and incubate at room temperature for 45 minutes. Pour off and add a (1% KI plus 1% I_2) solution of Potassium Iodide-Iodine for 30 minutes. Bands are clear on a dark background and will fade quickly.	larvae
Ap	2	Presoak gel 30 minutes in solution 15. Discard solution. To 50 ml of gel buffer (System A, TABLE 1) add: 10 drops solution 16, 10 drops solution 17, 100 mg Polyvinylpyrrolidone (optional), 50 mg Na-α-naphthyl acid phosphate, 50 mg Fast Blue RR. Pour over gel and incubate for 3 hours at 37°C. Remove stain and add solution 18.	larvae
Cbb	1	This stain works best in 4th instar larvae. Pour 50 ml of solution 27 over gel. Incubate at room temperature for 3 hours, then pour off and save for reuse. Rinse gel with solution 18 at 30 minute intervals until gel is cleared of background stain.	larvae

Table 4. Continued

Protein	Rating	Technique	Stains best in
Est	1	To 50 ml of $NaPO_4A$ (solution 6) and 10 ml of $NaPO_4B$ (solution 7) add: 40 ml H_2O, 2 ml solution 4, 2 ml solution 5. Pour 20 ml on gel and incubate at room temperature for 15 minutes. Add 50 mg Fast Blue RR to remaining 30 ml, shake and filter onto gel. Incubate at 37°C for 45 minutes. Remove stain and add solution 18.	larvae or adults
Fum	2	To 35 ml of solution 13 add: 50 mg of fumaric acid, 1 ml solution 20, 1 ml solution 22, 75 units malate dehydrogenase. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml solution 19 and incubate for 2 hours. Remove stain and add solution 18. NOTE: This protein has weak activity and care must be taken in order to prevent it from being confused with *Mdh* which sometimes appears on the same gel.	adults
G-6-pdh*	2	To 35 ml of solution 13 add: 50 mg of Glucose-6-phosphate, 1 ml solution 20, 1 ml solution 21. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml solution 19 and incubate for 2 hours more. Remove stain and add solution 18. NOTE: This system can sometimes be stained with *6-pgdh* on same gel by adding 6-phosphogluconic acid to above.	adults
Got	1	To 50 ml of solution 13 add: 1 ml solution 11, 1 ml solution 12, 10 mg Pyridoxal-5'-Phosphate. Pour over gel and incubate for 30 minutes at 37°C. Add 50 mg Fast Blue RR Salt and incubate for 2 hours more. Remove stain and add solution 18.	adults
G-3-pdh*	1	To 35 ml of solution 14 add: 80 mg Na_4 Fructose-1, 6-diphosphate $6H_2O$, 35 units Aldolase, 1 ml solution 20, 2 ml solution 22, 100 mg Sodium Arsenate. Pour over gel and incubate for 20 minutes at 37°C. Add 0.5 ml solution 19 and incubate 2 hours more. Remove stain and add solution 18.	
α-gpdh*	1	To 35 ml solution 14 add: 50 mg α-glycerophosphate, 1 ml solution 20, 2 ml solution 22. Pour over gel and incubate for 30 minutes at 37°C. Add 0.5 ml solution 19 and incubate for 1 hour more. Remove stain and add solution 18. NOTE: This system may sometimes be stained simultaneously with *Mdh* by adding 1 ml DL-malic acid to above.	adults
Hk*	1	To 35 ml of solution 14 add: 50 mg α-D+-Glucose, 1 ml solution 16, 1 ml solution 20,1 ml solution 21, 2 ml solution 23, 40 mg ATP. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml of solution 19 and incubate for 1 hour more. Pour off stain, filter, and save for reuse. Add solution 18.	adults or larvae
Hbdh	2	To 35 ml of solution 13 add: 100 mg NaCl, 100 mg β-hydroxybutyric Acid, 0.2 ml solution 16, 1 ml solution 20, 2 ml solution 22. Pour over gel, incubate for 30 minutes at 37°C. Add 0.5 ml solution 19 and incubate for 2 hours more. Remove stain and add solution 18.	adults

Table 4. Continued

Protein	Rating	Technique	Stains best in
Idh*	1	To 35 ml of solution 14 add: 50 mg DL-Na_3-Isocitric Acid, ½ ml solution 17, 1 ml solution 20, 1 ml solution 21. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml of solution 19 and incubate for 1 hour more. Remove stain and add solution 18.	adults
Lap	1	Add 50 ml solution 10 for 15 minutes. Pour off and to 50 ml of solution 3 add: 1 ml solution 1. Pour over gel and incubate at room temperature for 15 minutes. Add ¾ ml solution 2 *or* add 50 mg of Fast garnet GBC. Incubate at 37°C for 2 hours. Remove stain and add solution 18.	larvae
Mdh*	1	To 35 ml of solution 14 add: 1 ml solution 20, 1 ml solution 22, 1 ml DL-malate solution 24. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml solution 19. Incubate 30 minutes more, drain off stain, filter and save for reuse. Add wash (solution 18).	adults
Me	1	To 35 ml solution 14 add: 1 ml solution 20, 1 ml solution 21, ½ ml DL-malate solution 24. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml solution 19. Incubate 30 minutes more, drain off stain, filter and save for reuse. Add solution 18.	
Odh*	2	Dissolve 2 ml Octanol in 2 ml Ethanol: Methanol (1:1) mixture. Add this to: 35 ml solution 13, 1 ml solution 20, 2 ml solution 22. Incubate at 37°C for 45 minutes. Add 0.5 ml solution 19 and incubate for 2 hours more. Pour off stain, filter and save for reuse. Add solution 18. NOTE: *Adh* may appear on gel also due to presence of Ethanol. *Xdh* may be stained for by dissolving hypoxanthine in Ethanol also.	adults
Pep	2	To 35 ml Trizma solution 14 add: 20 mg dipeptide (i.e., leucyl-alanine, phenylalanine-proline, etc.), 40 mg peroxidase, 40 mg amino acid oxidase (snake venom). After 30 minutes add: 20 mg 0-dianosidine (dissolved in 10 ml of stain buffer). Incubate at room temperature for 1 hour. Remove stain and add a wash made of 1:1 methanol and H_2O.	adults or larvae
Pgi*	1	To 35 ml solution 14 add: 4 ml solution 16, 1 ml solution 20, ½ ml solution 21, 1 ml solution 23, 30 mg Fructose-6-phosphate. Incubate for 30 minutes at 37°C. Add 0.5 ml solution 19 and incubate for 10 minutes. Pour off stain and add solution 18. NOTE: This can be stained on *Pgm* gel slice by adding Fructose-6-phosphate after *Pgm* bands appear.	adults
Pgm*	1	To 35 ml solution 14 add: 4 ml solution 16, 1 ml solution 20, 1 ml solution 21, 2 ml solution 23, 2 ml solution 25, 2 ml solution 26. Pour over gel and incubate for 30 minutes at 37°C. Add 0.5 ml solution 19 for 1 hour more. Remove stain, filter and save for reuse. Add solution 18. NOTE: This stain *must have good quality G-6-pdh* to work.	adults

Table 4. Continued

Protein	Rating	Technique	Stains best in
6-pgdh*	1	To 35 ml solution 14 Add: 50 mg 6-phosphogluconic Acid, 1 ml solution 20, 1 ml solution 21. Pour over gel and incubate at 37°C for 30 minutes. Add 0.5 ml solution 19 and incubate for 1 hour more. Remove stain and add solution 18. NOTE: This stain can sometimes be stained with G-6-pdh on the same gel by adding Glucose-6-phosphate to above.	adults
Tpi	2	To 35 ml solution 14 add: .5 gr α-Na glycerophosphate .2 gr Na *pyruvate,* 2 ml solution 22, 100 units *α-gpdh,* 100 units *Ldh.* Incubate this solution for 75 minutes at 37°C. Stop reaction by addition of concentrated HCl until pH = 2.0. Then quickly readjust to pH 8.0 with NaOH. Add: 1 ml solution 20, 2 ml solution 22, 50 mg Sodium Arsenate, 100 units glyceraldehyde-3-phosphate dehydrogenase. Pour over gel. After 30 minutes add .5 ml solution 19. After 1.5 hours, pour off and add solution 18.	adults
Xdh*	2	To 35 ml solution 13 add: 100 mg Hypoxanthine, 1 ml solution 20, 2 ml solution 22. Pour over gel and incubate at 37°C for 40 minutes. Add .05 ml of solution 19 and incubate for 2 hours more. Remove stain, filter, and save for reuse. Add wash to gel. NOTE: *Adh* will appear on gel also due to presence of Ethanol. *Xdh* may also be stained with *Odh.*	adults (developmental form differs with mobility)

Ratings: 1 In regular use in our lab, displays good banding patterns, found in all mosquitoes investigated to date.
2 Usually displays good banding patterns, not found in all species of mosquitoes investigated to date, possibly because of sub-optimal buffer conditions.

DPN) at the discretion of the experimenter. Although this can cut chemical costs by as much as one-quarter, care should be exercised as some stains have ingredients which are sensitive to temperature. Note that all gel slices should be stained in the dark whenever possible as some stains are photosensitive. We stain most of our enzymes in a 37°C incubator which not only maintains a dark state with the door closed but also provides a good temperature for the biochemical reactions.

After staining, about 50–75 ml of wash solution (Table 5, solution 18) should be poured on the gel slice. This will serve the purpose of clearing background stain from the gel slice and act to preserve it. However, it is a good idea to record (score) the data before adding the wash solution, as some stains will fade in wash within several hours. All TPN and DPN dependent enzymes will fade at room temperature over several months. It is wise to photograph or make a drawing of the gel slice as soon as possible. For photographs, Pan-X panatomic black and white film serves well. If it is desirable to save the gels, it is best to wrap them in Saran wrap and store them at a very cool temperature after soaking in wash solution overnight.

THE USE OF ACRYLAMIDE GELS

Acrylamide has become extremely useful in electrophoresis. It offers a better method to control pore size of the gel, leading to clearer and sharper banding patterns in some instances. Being transparent, it facilitates quantification of proteins by densitometric methods. In addi-

Table 5. Stock solution. All water used should be deionized and distilled.

Solution Number	Name	To Make	To Use
1	L-leucyl-β-naphthlamide (Lap substrate)	1 gr./40 ml H_2O	1 ml/50 solution 3
2	Black K Salt (Lap stain)	1 gr./25 ml H_2O	¾ ml/50 ml solution 3
3	Maleate buffer (Lap stain buffer)	24.2 gr. Trizma Base (Sigma) 23.2 gr. Maleic Acid, 1000 ml H_2O; pH = 5.2	50 ml, use with solutions 1 and 2.
4	β-naphthyl Acetate (*Est.* substrate = red bands on gel)	1 gr./50 ml Acetone	1 ml/50 ml (see solution 7)
5	α-naphthyl Acetate (*Est* substrate = black bands)	1 gr/25 ml Acetone + 25 ml H_2O	1 ml/50 ml (see solution 7)
6	$NaPO_4$ A (*Est* stain buffer, acidic pH)	0.2 M Monobasic Sodium Phosphate (13.2 gr/500 ml H_2O) Store in dark bottle	Mix 50 ml $NaPO_4$ A with 10 ml $NaPO_4$ B (solution 7), then add 40 ml H_2O (pH = 6.4)
7	$NaPO_4$ B (*Est* stain buffer, basic pH)	0.2 Dibasic Sodium Phosphate (26.81 gr/500 ml H_2O) Store in dark bottle	Mix 10 ml to 50 ml solution 6, add 40 ml H_2O (pH = 6.4), add substrates to this solution
8	Acetate A (*Acph* stain buffer, acidic pH)	23.1 ml Glacial Acetic Acid/2 L H_2O	Mix 15 ml Acetate A with 35 ml solution 9, add 50 ml H_2O
9	Acetate B (*Acph* stain buffer, basic pH)	27.2 gr Sodium Acetate/1 L H_2O	(See solution 8)
10	Borate 5.0 (*Acph* and *Est* buffer)	30.9 gr. Boric Acid/1 L H_2O (0.5M) Bring to pH = 5.0 with 1 M Trizma Base	See stains for Acph, Est and Lap
11	L-aspartic Acid (*Got* substrate)	100 mg/ml H_2O; Adjust to pH 7.0 with 1 M Trizma Base	Add 1 ml to 50 ml solution 12
12	α-ketoglutaric Acid (*Got* substrate)	75 mg/ml H_2O, bring to pH 7.0 with 1 M Trizma Base	add 1 ml to 50 ml solution 13
13	Trizma buffer 8.0 (*Got* and *Odh* stain buffer)	24.2 gr. Trizma Base (0.2 M), 11.8 ml concentrated HC1, 1000 ml H_2O	Mix 50 ml with solutions 11 and 12, pH=8.0
14	Trizma buffer 7.5 (general stain buffer)	12.1 gr. Trizma Base (0.1 M), 7.5 ml concentrated HC1 to pH 7.5, 1000 ml H_2O	Mix 35 ml with TPN and DPN dependent stains (see Table 3)
15	Trizma buffer 10.4 (*Ap* presoak buffer)	24.2 gr. Trizma Base (2 M)/1 L H_2O pH = 10.4	pour 50 ml on gel for ½ hour, pour off, then add stain for *Ap*
16	$MgCl_2$	2.03 gr. (0.1 M)/100 ml H_2O	See stains, Table 3
17	$MnCl_2$	4.9 gr. (0.25 M)/100 ml H_2O	See stains, Table 3

Table 5. Continued

Solution Number	Name	To Make	To Use
18	Wash solution	6 parts Anhydrous (Absolute) Methanol 5 parts H_2O 1 part Glacial Acetic Acid (for acrylamide gels, 10% glycerol in 8% Acetic Acid)	pour 50 ml on gel after staining; leave on overnight
19	Phenazine Methosulfate (PMS)	1 gr./100 ml H_2O	0.5 ml/35 ml stain (see Table 3)
20	MTT Tetrazolium (stain for TPN and DPN dependent stains)	1 gr./100 ml H_2O	1 ml/35 ml stain (see Table 3)
21	Triphosphopyridine Nucleotide (TPN)	1 gr./100 ml H_2O	1 ml/35 ml stain (see Table 3)
22	Diphosphopyridine Nucleotide (DPN)	1 gr./100 ml H_2O	2 ml/35 ml stain (see Table 3)
23	Glucose-6-phosphate dehydrogenase (*G-6-pdh*)	1000 units/100 ml H_2O	Use 3 ml/35 ml stain (see Table 3)
24	DL-Malate (substrate for *Me* and *Mdh*)	268.2 gr. (2.0 M/1 L H_2O) Adjust to pH = 7.0 with 150 gr. NaOH	Use 1 ml/35 ml stain
25	α-D-Glucose-1,6-diphosphate (*Pgm* substrate)	22 mg (0.0004 M)/100 ml H_2O	Add 3 ml/35 ml solution 14
26	α-D-Glucose-1-phosphate (*Pgm* substrate)	1.7 gr. (0.046 M)/100 ml H_2O	Add 3 ml/35 ml solution 14
27	Coomassie Brilliant Blue *cbb* (General Protein stain)	1 gr./1 L solution 18	Pour 50 ml over gel (save for reuse)
28	Ammonium Persulfate APS	10 gr./100 ml H_2O (10% solution) This solution should be used within 1 week	Use 0.4 ml/100 ml gel buffer to make Acrylamide gels (see section V, text)

tion, starch sometimes has ions present which will interfere with some protein assays. Acrylamide is also easier to handle (i.e., the gel can be prepared and ready for use in 20 min as it requires no cooking), and it runs electrophoretically faster than starch.

Acrylamide does have several inherent problems. It is quite toxic, and care should be taken to avoid contact with bare skin and to prevent inhalation. Because of its high tensile strength, acrylamide is extremely hard to cut in a horizontal plane. For this reason, it is difficult to use in population surveys where more than 3 protein systems are under analysis, although it will work fine where time, expense, and sample sizes are not limiting factors.

In general, those protein systems assayable on starch also work on acrylamide. However, care should be taken to keep the amount of ammonium persulfate (APS) to a minimum to prevent APS inhibition; it is sometimes wise to first run a current of 50–60 mA through the gel to clear it for 3 hrs before samples are placed in the gel. We have found that a shallow gel mold (i.e., a thinner gel slab) yields better defined protein systems. For this reason, we routinely use slab molds about 19.5 cm × 17.5 cm × 2 mm (volume = 100 ml) in size.

The amount of acrylamide (Cyanogum

41, Fisher Scientific, Fair Lawn, N.J.) will vary in concentration depending on the gel pore size required for proper separation of allozymic proteins. Generally, the larger the protein molecule, the larger the pore size should be to prevent molecular fractionation.

The same gel and electrode buffers used in starch gels can be utilized for acrylamide gels. The following method has been adapted for use in this lab and has been modified from Raymond and Weintraub (1959):

To 100 ml gel buffer add, *in order:*

6–11 grams Cyanogum 41, dissolve: (determine concentration which yields the best separation) 0.1 ml of TEMED (N,N,N',N'-tetramethylethylenediamine, Eastman Kodak, Rochester, N.Y.), 0.4 ml 10% APS.

Quickly mix and pour into gel mold. Cover mold with glass plate (195 cm × 18 cm), taking care that no air bubbles occur at the interface of the glass and the gelling solution. Let set for 20–30 minutes, then gently remove the glass plate. Use the gel as directed in the above sections.

ELECTROPHORETIC STUDIES OF MOSQUITOES

An increasing number of isozyme studies have been done in mosquitoes of the genera *Anopheles, Culex,* and *Aedes* over the past 12 years. These investigations have focused on species relationships, formal genetics, surveys of natural populations for genetic variability and studies on reproductive biology and behavior. We have summarized the allelic, structural and some linkage estimates for most of these studies in Table 6.

It is interesting to note that of the 27 loci which we are publishing techniques for less than half of them have been studied to any extent and for some of these, the linkage information is only inferred. In fact, studies of the esterases and of *Pgm* account for half of the studies listed in Table 6.

This table does not list loci for which no strong genetic data exist but which have nevertheless proved valuable with respect to systematic comparisons. One of these is *Ap,* which Bianchi (1965, 1966, 1968a) and Bianchi and Pirodda (1968) used to differentiate species of the European *maculipennis* complex of the anophelines. It is likely that electrophoresis will continue to be a valuable tool in this area as it has proved valuable in differentiating not only anopheline species (the studies by Bianchi and by Coluzzi et al. 1971a, 1971b, by Bullini and Coluzzi 1973, by Mahon et al. 1976, by Narang 1970, and by Joslyn, 1977), but also species of the genera *Aedes* (Saul et al. 1978) and of the genera *Culex* (Miles 1974).

With two exceptions, studies on differences in the proteins between closely related species have been aimed primarily at recognizing diagnostic loci (i.e., after Ayala and Powell 1972). However, these cannot prove of great aid in studying evolutionary relationships between species since the comparison of only 1–3 loci does not enable us to have any confidence in our estimates. Reliability requires not only that we sample a large number of loci which would be representative of a species gene pool, but also that we have some numerically valid way of interpreting the genetic data and drawing a picture of the evolutionary relationships. The numerical techniques which have proven of value in this regard are those of Nei (1972, 1973) and Rogers (1972). Only two attempts have been made to study species relationships in mosquitoes using these techniques and incorporating many loci. The first of these was by Miles (1972) on the Australian *Cx. pipiens* complex. The second was just finished in our laboratory by Joslyn (1977) who studied 3 species of nearctic anophelines. Because of the questions which currently exist with respect to the evolutionary relationships in the different genera of mosquitoes, we anticipate more of this type of in-depth and exhaustive surveys in the future. Eventually, the problem of genetic variants hidden within the different electrophoretic forms observed to date will also have to be addressed and their

Table 6. Structure and allelic components of enzymatic loci in different mosquito species.

Enzyme	Species	Linkage	No. of codominant alleles	Quaternary[1] structure	Reference
Est-1	*An. stephensi*	autosomal	2	monomer	Bianchi 1968b
Est-6	*An. atroparvus*	autosomal	4	monomer	Bianchi and Rinaldi 1970
α-Est	*An. funestus*	autosomal	2	monomer	Green 1977
Est-2	*An. albimanus*	autosomal	2	monomer	VedBrat and Whitt 1976
Est-4	*An. albimanus*	autosomal	2	monomer	VedBrat and Whitt 1976
Est-6	*An. albimanus*	autosomal	3	monomer	VedBrat and Whitt 1976
Est-8	*An. albimanus*	autosomal	3	monomer	VedBrat and Whitt 1976
Est-A	*An. punctipennis*	autosomal	7	monomer	Narang and Kitzmiller 1971a
Est-B	*An. punctipennis*	autosomal	7	monomer	Narang and Kitzmiller 1971a
Est-C	*An. punctipennis*	autosomal	3	monomer	Narang and Kitzmiller 1971b
Est-F	*An. punctipennis*	autosomal	7	monomer	Narang and Kitzmiller 1973
Pgm	*An. plumbeus*	autosomal	4	monomer	Bullini and Coluzzi 1973
Pgm	*An. stephensi*	autosomal	5	monomer	Bullini et al. 1971a, 1971b
Pgm	*An. albimanus*	autosomal	2	monomer	Bullini and Coluzzi 1973
Pgm	*An. maculipennis* complex	autosomal	3	monomer	Bullini and Coluzzi 1973
Pgm	*An. gambiae* complex	autosomal	4	monomer	Bullini and Coluzzi 1973
Xdh	*An. atroparvus*	autosomal	3	monomer	Bianchi and Chessa 1970
Xdh-1	*An. punctipennis*	autosomal	3	monomer	Narang and Kitzmiller 1972
Xdh-2	*An. punctipennis*	autosomal	2	dimer	Narang and Kitzmiller 1972
Adh	*An. stephensi*	autosomal	3	dimer	Iqbal et al. 1973a, 1973b
Odh	*An. punctipennis*	autosomal	2	monomer	Narang and Kitzmiller 1972
Odh	*An. culicifacies*	autosomal	2	dimer	Ahmed et al. 1978
Acph	*An. culicifacies*	autosomal	2	dimer	Ahmed et al. 1978
Adh	*Cx. tritaeniorhynchus*	autosomal	5	dimer	Sakai et al. 1973
Amy	*Cx. tritaeniorhynchus*	sex linked	5	monomer(?)	Sakai et al. 1976
Mdh	*Cx. p. quinquefasciatus*	autosomal	5	monomer(?)	Narang and Narang 1975
6-pgdh	*Cx. p. quinquefasciatus*	autosomal	2	dimer	Cheng and Hacker 1976
Cbl-Est[2]	*Cx. pipiens* (L)	autosomal	3	monomer	Gargan, T. P. and A. R. Barr 1977
Est	*Cx. pipiens pipiens*		2	monomer(?)	de Stordeur 1976
6-pgdh	*Cx. pipiens* complex (Australia)	autosomal	3	monomer	Miles 1974
α-Gpdh	*Cx. pipiens* complex (Australia)	autosomal	2	dimer	Miles 1974

Table 6. Continued

Enzyme	Species	Linkage	No. of codominant alleles	Quaternary[1] structure	Reference
Me-1	*Cx. pipiens* complex	autosomal	4	dimer	Miles 1974
	(Australia)	autosomal	3	monomer	Miles 1974
Hk	*Cx. pipiens* complex	autosomal	4	monomer	Bullini et al. 1972
Pgm	*Ae. aegypti*	autosomal	14	monomer	Saul et al. 1977
Est-6	*Ae. aegypti*				

[1] In some instances, the quaternary structure has been inferred by us from the locus description by the author or from a published photograph.
[2] Carboxylesterase.

impact assessed on any conclusions drawn in the foregoing studies (see Bernstein et al. 1973).

Electrophoretically detectable gene loci have also proven of great value in mating behavior studies which enable examination of questions concerned with species barriers, gene flow and general reproductive biology. Miles (1977) has shown that positive assortative mating occurs in *Culex* species in the laboratory which apparently stems from discriminatory behavior on the part of the female. Miles (1976) has also observed this to hold in nature as well, suggesting that female mate-recognition behavior may play a strong role in maintaining unique gene pools. Studies by Coluzzi and Bullini (1971) indicate that similar barriers to gene flow exist for anophelines while Bullini et al. (1974, 1976) have shown that *Cx. pipiens* is essentially monogamous with only 4.4% of females showing evidence of multiple insemination (2 male mates). The last studies are particularly valuable because the effective number of times a vector will mate is critical for release programs aimed at genetic control of population density.

The analysis of genetic structure in natural populations is also of value to those interested in applying genetic control methods. Studies are especially lacking here since mostly single populations or single gene loci have been studied to date (Narang 1970, Connor 1972, Bullini et al. 1970a, 1970b, 1972, 1973a, 1973b, Bullini and Coluzzi 1972, Bullini and Coluzzi 1973, Coluzzi and Bullini 1971, Sakai et al. 1973; Saul et al. 1978). Tabachnick and Powell (1976) report that they are conducting a widespread investigation of *Ae. aegypti*, and the results for 4 loci are reported in detail for some African populations (Tabachnick and Powell 1978). Scott and McClelland (1975) have demonstrated the value of studying more than just a single locus. At 3 loci (*Ap, Lap,* and *Cbb*) an indoor ecotype of *Ae. aegypti* could be distinguished from an outdoor ecotype occurring sympatrically in the same area while at 3 other loci (*Pgm, Me*

and *Est*), 2 of which have been most widely studied, no significant differences existed. It is likely that assortative mating of the type demonstrated by Miles (1976) may be at work here, but this suggestion needs verification. Our laboratory has obtained some interesting data on populations of *An. crucians* and some other species which we have been studying for the past few years. Table 7 lists the data for the *Pgm* locus of the 20 loci we have studied. Interestingly, the table demonstrates lower levels of variation in southern populations of mosquitoes, regardless of species, while northern populations show variation levels 3 times higher! In *crucians* there is some evidence of cyclical changes at this locus; a more recent survey (January, 1978) shows a heterozygosity estimate of 2%, consistent with the January, 1976 finding.

Electrophoretic loci are also valuable as genetic markers to follow differences occurring during ontogeny (Simon 1969, VedBrat and Whitt 1974, Briegel and Freyvogel 1971, Briegel 1972), tissue specificity (Bianchi and Rinaldi 1974) and with respect to sex-limited expression (Green 1977, Freyvogel et al. 1968). Taken altogether, these studies enable a deeper understanding of how genes work and are expressed in mosquitoes. Where ontogenetic changes are concerned, they offer a special insight which may prove valuable if coupled with the study of larvicide effects in nature.

Finally, we would interject a word of caution in applying electrophoresis to studying mosquitoes. The interpretations made from electropherograms (i.e., gel slices histochemically treated) must be done cautiously and verified by segrega-

Table 7. A comparison of genetic variation at the *Pgm* locus in Northern versus Southern populations of several Nearctic mosquito species.

Northern Species	Population	Number Analyzed	Allele Frequencies (%)[1] 96	98	100	102	Average Population Variability (%) observed	expected
Culex pipiens	Amherst, Mass.	59	1.7	21.6	67.8	7.6	52.5	46.2
	Rte. 45, Il.	41	—	24.4	74.4	1.2	31.7	38.7
	Brownfield Woods, Il.	38	1.3	13.2	77.6	7.9	44.7	37.4
Anopheles punctipennis	Champaign County, Il.	71		4.2	83.8	9.2	23.9	23.2
						Average =	38.2	36.4
Southern Species								
Anopheles crucians	Vero Beach, Fla.							
	(July, 1975)	106		2.4	90.9	2.8	8.1	9.5
	(Jan., 1976)	88		11.1	89.9	—	1.6	19.9
	(Mar., 1976)	97		2.1	98.9	—	2.1	4.2
	(Apr., 1976)	59		3.4	96.9	—	3.3	6.6
	(July, 1976)	19		5.2	81.6	13.2	7.8	31.4
Anopheles walkeri		64		1.6	91.4	6.7	17.2	17.3
Anopheles quadrimaculatus		39		1.3	98.4	1.3	5.1	5.1
						Average =	8.9	12.2

[1] allele designations (96, 98, 100, 102) are not homologous across species.

tion data wherever possible. There is much evidence available from the studies of proteins in other organisms, primarily rodents, humans and *Drosophila* that not all bands on a gel will represent allelic expression. Called "conformational bands," these may represent partial degradation products or structural modifications of the protein under study, either of which may have no genetic basis. Such conformers are present in mosquitoes as evidenced by the data and speculation of Bullini and Coluzzi (1973) and Narang and Narang (1975). At least one research report has misinterpreted such banding patterns (Kreutzer et al. 1977) where sample 3 of their Figure 1 must be interpreted by their technique to have three alleles (an impossibility in a normal mosquito) and samples 5 and 6 of that same Figure appear to have a slightly faster conformer band present. By these authors' interpretation, every sample they present in Figures 1 and 2 are heterozygous, yet Bullini and Coluzzi (1973) pointed out that *Aedes* species, and some anophelines, may have two-bands representing one allele. Interpretation, then must be done cautiously to obtain data for the meaningful study of mosquitoes (see also the comments of Saul et al. 1977). The naming of these variants should coincide as much as possible with the recommendations of the International Unions of Pure and Applied Chemistry and of Biochemistry (i.e., see enzyme nomenclature revisions, 1972).

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Appendix 1. Apparatus and equipment useful in setting up an electrophoresis laboratory. The laboratory should have gas and running water available as well as 110/220 current.

Item	Capacity	Function
Top-loading Balance (i.e., Mettler model P203)	1 mg–100 gr	weighing out starch for gels; weighing microquantities of biochemicals
Refrigerator (any model)	10 or more cubic feet	storing biochemicals
Freezer (any model)	10 or more cubic feet	storing biochemicals
Refrigerator or Beer Cooler[1] (i.e., 2-door cooler, masterbilt, New Albany, Miss.)	15 or more cubic feet	to run electrophoretic separations at 4°C
pH meter (any model)	normal range, 0–14 with reference electrodes	to determine pH of biochemical buffers
closing door incubator (i.e., Blue M model #200A Blue M electric, Blue Island, IL)	above ambient to 37°C	to hold histochemical stains during staining
Power supplies (i.e., Heathkit model IP-17)	to 150 mA, 500V, AC/DC	to provide power for electrophoretic separations
Still with deionizer (any model)	≈10 liters per day	to provide copius amounts of ddH_2O for buffers
Electrode chambers and gel molds	as described in accompanying text Fig. 1, Table 2	to hold supporting media for electrophoretic separations
Peripheral gel equipment (slot guide, sample homogenizer cutting guide, slicer)	as shown in Fig. 2	see text; to prepare gels and for electrophoresis and staining
10–16 large containers (preferably with spigots)	8 liters or more	to store pre-mixed buffer solutions
1 or more case(s) of 1000 ml erlenmeyer flasks	1000 ml	to mix and cook gel solutions in
50 ml beakers	50 ml	for mixing and pH-ing solutions
250 ml beakers	250 ml	to make stains in; these should be wrapped with tin foil to reduce effect of light on photo-sensitive stains
pipets	1 ml, 5 ml	to measure out standard quantities of stain ingredients; these may be disposable (2 ml syringes may be substituted)
500 ml dark bottles (i.e., Nalgene)	500 ml	to store small amounts of pre-mixed stain ingredients
small electrical motor	¼ hp, high torque	for holding grinding post to homogenize samples
1–2 microliter syringes	1 ml, .10μ1 markings	to add known amounts of buffer to grinding block for homogenizing samples

[1] This should be provided with an automatic cut-off switch which cuts power to the units in the cooler whenever the doors are opened.