IN VITRO
16(4):392-398; 1980

CHARACTERIZATION OF INVERTEBRATE CELL LINES

II. Isozyme Analyses Employing Starch Gel Electrophoresis

W. J. TABACHNICK AND D. L. KNUDSON[1]

Department of Biology, Yale University, New Haven, Connecticut 06510 (W. J. T.) and Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510 (D. L. K.)

(Received August 20, 1979; accepted October 8, 1979)

SUMMARY

The usefulness of isozyme patterns for distinguishing 14 lepidopteran and 2 dipteran cell lines was evaluated. The lepidopteran cell lines used in this study represent eight taxonomic families with one family, Noctuidae, having five representatives. Cell extracts were examined for 18 isozymes using a starch gel electrophoretic system. Ten isozymes proved to be suitable because their isozyme patterns permitted the allocation of the cell lines into distinct groups. Furthermore, four isozymes (isocitrate dehydrogenase, malic enzyme, phosphoglucoisomerase, and phosphoglucomutase) were found to be adequate to distinguish the cell lines. The isozyme patterns observed for the two dipteran and one of the lepidopteran cell lines were analogous to the profiles found using the intact insect. Isozyme analyses differentiated the cell lines and may prove useful for identifications of species of origin. The use of this technique as a criterion for identification of invertebrate cell lines is proposed.

Key words: dipteran cell lines; lepidopteran cell lines; isozymes; electrophoresis; characterization; identification.

INTRODUCTION

Greene and Charney (1) and Greene and coworkers (2) first demonstrated the usefulness of isozyme techniques for distinguishing invertebrate cell lines. In their studies, dipteran cell lines were compared with a lepidopteran cell line. The lines were examined by immunologic, karyologic, and enzymatic procedures in an attempt to characterize the lines. The three experimental approaches demonstrated that cell lines derived from tissues of insects from different orders were distinguishable. These techniques, however, did not make it possible to distinguish between the *Aedes aegypti* and *Ae. albopictus* cell lines.

Although karyologic techniques have proven useful in the characterization of vertebrate cell lines, the use of the technique for discriminating between invertebrate cell lines within the same order of Class Insecta, has not been productive (1-3). Immunologic techniques have been shown to exhibit similar discriminating abilities (1,2). Serologic techniques, however, have been used recently in a limited study to distinguish lepidopteran cell lines at an intrafamilic taxonomic level (4).

In this communication, the usefulness of isozyme profile analyses as criteria for characterization and identification of 14 lepidopteran and 2 dipteran cell lines was examined. Eighteen isozyme systems were evaluated for discriminating ability, including two of the three isozymes reported previously (1,2). The 16 cell lines and the 18 isozyme systems provide a stringent test of the ability of the isozyme profile analyses to discriminate between taxonomic relatives.

MATERIALS AND METHODS

Cell lines. The cell lines used in this study are presented in Table 1. The growth medium for the cell lines was TC100, and its formulation is presented elsewhere (3); the growth, morphology,

[1]To whom enquiries should be addressed.

TABLE 1

INVERTEBRATE CELL LINES[a]

Order	Family	Genus Species	Common Name	Cell Line Designation	Assigned Number[b]
Diptera	Culicidae	*Aedes aegypti*	Yellow fever mosquito	ATC-10	1
		Aedes albopictus	Mosquito	ATC-15	2
Lepidoptera	Arctiidae	*Estigmene acrea*	Salt marsh caterpillar	BTI-EAA	3
	Bombycidae	*Bombyx mori*	Silkworm	BM-N	4
	Lasiocampidae	*Malacosoma disstria*	Forest tent caterpillar	IPRI-MD-108	5
	Lymantriidae	*Lymantria dispar*	Gypsy moth	IPLB-LD-65Z	6
	Noctuidae	*Heliothis zea*	Corn earworm or cotton bollworm	IMC-HZ-1	7
		Heliothis zea	Corn earworm or cotton bollworm	IPLB-HZ-1075	8
		Mamestra brassicae	Cabbage moth	IZD-MB-0503	9
		Spodoptera frugiperda	Fall armyworm	IPLB-SF-21AE	10
		Spodoptera littoralis	Cotton leafworm	UIV-SL-573	11
		Trichoplusia ni	Cabbage looper	TN-368	12
	Olethreutidae	*Laspeyresia pomonella*	Codling moth	CP-1268	13
		Laspeyresia pomonella	Codling moth	CP-169	14
	Sphingidae	*Manduca sexta*	Tobacco hornworm	MRRL-CH-I	15
	Tortricidae	*Choristoneura fumiferana*	Spruce budworm	IPRI-CF-124	16

[a] All cell lines grown in TC100 medium, except ATC-10 and ATC-15, which were grown in Mitsuhashi and Maramorosch medium.

[b] The assigned number represents an arbitrary designation used in Figs. 1 and 2.

and handling characteristics of the cell lines have also been described elsewhere (5,6).

Preparation of cell extracts. The cell lines were grown as suspension cultures in liter spinner flasks (7). The cells were harvested by centrifugation, and the cell pellet was washed three times in a balanced salt solution (8). The final pellet was resuspended in 0.15 *M* NaCl and 1 m*M* EDTA to yield 2×10^8 cells/ml. Two-tenths-milliliter aliquots were prepared, and they were stored at −70° C.

Isozyme analyses. The cell lines were assayed for their enzyme phenotypes using standard techniques of starch gel electrophoresis. The isozyme

TABLE 2

ENZYMES TESTED

Suitability for Test	Enzyme	Enzyme Commission Number	Enzyme Abbreviation
Suitable			
	Esterase	3.1.1.2	EST
	Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH
	Hexokinase	2.7.1.1	HK
	Isocitrate dehydrogenase	1.1.1.42	IDH
	Lactic dehydrogenase	1.1.1.28	LDH
	Leucine amino peptidase	3.4.1.1	LAP
	Malic enzyme	1.1.1.40	ME
	Phosphoglucoisomerase	5.3.1.9	PGI
	Phosphoglucomutase	2.7.5.1	PGM
	Tetrazolium oxidase	—	TO
Unsuitable			
	Acetaldehyde oxidase	1.2.1.3	AO
	Adenylate kinase	2.7.4.3	ADK
	Alcohol dehydrogenase	1.1.1.1	ADH
	Alkaline phosphatase	3.1.3.1	APH
	Fumarase	4.2.1.2	FUM
	α-Glycerophosphate dehydrogenase	1.1.1.8	αGPDH
	Malic dehydrogenase	1.1.1.37	MDH
	Xanthine dehydrogenase	1.2.3.2	XDH

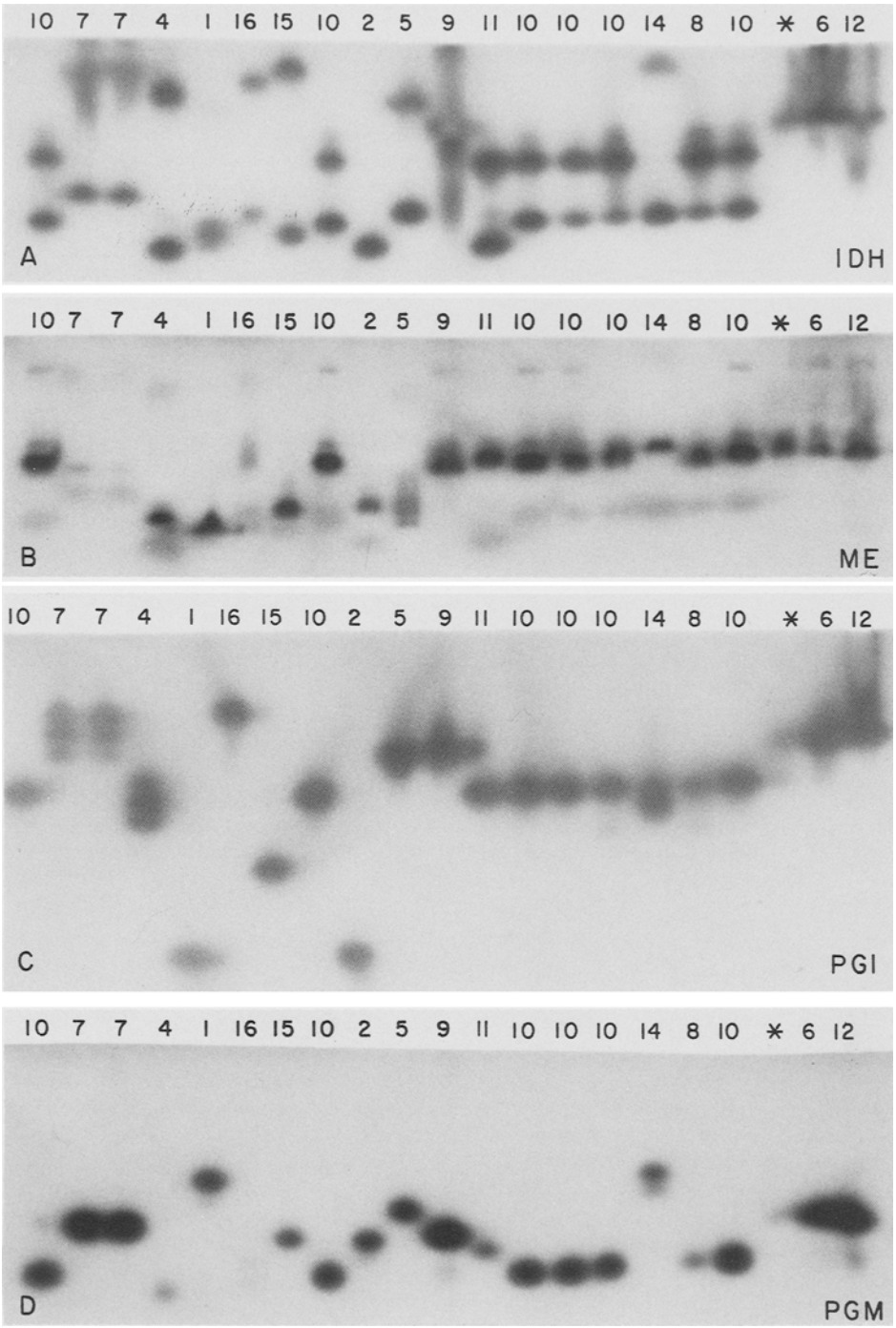

FIG. 1, *A* to *D*. Photomicrograph representing starch gels in which the cell extract samples were electrophoresed from top to bottom, incubated in a reaction buffer appropriate for a given isozyme, and stained. The assigned numbers for the cell lines and the abbreviations for the isozymes are found in Tables 1 and 2, respectively. The *asterisk* in the figure represents the early BTI-EAA sample, which was demonstrated to be the TN-368 cell line, and this is discussed in the text. These data are also discussed in the text.

systems that were employed are presented in Table 2. The details of the electrophoretic procedures including sample preparation, buffer systems, and staining solutions are described elsewhere (9,10). The staining solution for isocitrate dehydrogenase (IDH) was modified by substituting 100 mg $MgCl_2$ for $MnCl_2$ (9), and the C electrophoretic buffer system (9) was used to assay for esterase (EST).

The mobility of the specific enzyme band in the electrophoretic system was recorded relative to an internal standard. The cell line of *Spodoptera frugiperda* (IPLB-SF-21AE) was chosen as the standard because of its wide usage by invertebrate cell culturists. The predominant and most intensely staining band of the IPLB-SF-21AE line was assigned a value of 100 for each electrophoretic system. The enzyme phenotypes of the other cell lines were expressed relative to the mobility of 100. Since genetic studies with field-collected *Ae. aegypti* demonstrated two independent regions of IDH activity (11), this nomenclature designating a slow migrating IDH-1 region and a fast migrating IDH-2 has been maintained for the cell lines used in this study.

RESULTS

Table 1 lists the 16 invertebrate cell lines that were analyzed for enzyme phenotypes. The samples were stored at −70° C, and their enzyme activity was stable under these conditions for 10 of the enzymes tested.

The enzymes tested and their abbreviations are listed in Table 2. Eight enzymes proved unsuitable because the profiles were either poorly resolved or their was a lack of enzymatic activity in the majority of cell lines. Nevertheless, 10 enzymes consistently yielded reproducible profiles. Figures 1 and 2 are representative of the enzyme phenotypes that were observed. Profiles for tetrazolium oxidase (TO) are not represented because the white TO bands faded rapidly, making photographic reproduction difficult. Esterase (EST) profiles contained many bands of varying intensity as evidenced by the profiles of sample 10 (Fig. 2*C*). As a result of this variation, EST proved to be the least useful enzyme for diagnostic comparisons. Although glucose-6-phosphate dehydrogenase (G6PDH) and lactic dehydrogenase (LDH) profiles were recordable, the smeared profiles were suggestive of some enzyme denaturation due to the freeze-thaw treatment of the cell sample. Nevertheless, they were useful for a number of cell lines.

The relative mobilities of the enzyme phenotypes that were observed are recorded in Table 3. These data reveal that the cell lines were distinguishable from each other with respect to the mobility of their enzymes. The IPLB-LD-65Z and IPLB-HZ-1075 lines are exceptions because they were not distinguishable from TN-368 and IPLB-SF-21AE, respectively. Initially, the BTI-EAA was not distinct from TN-368. A second BTI-EAA culture was requested from the original supplier of the line, and it yielded the relative mobilities as recorded (Table 3).

Although the cell lines are distinguishable, only three cell lines were tested and shown to exhibit enzyme mobilities consistent with the species of origin. Adult *Ae. aegypti* and *Ae. albopictus* mosquitoes and *Bombyx mori* larvae yielded enzyme phenotypes analogous to those observed for the ATC-10, ATC-15, and BM-N cell lines, respectively.

DISCUSSION

With the increasing number of established invertebrate cell lines (3,6), the need for criteria for characterization and identity becomes apparent. Criteria that have proven useful for vertebrate cell lines, such as karyology, have limited application to invertebrate cell lines (1-3,12). Cytogenetic studies discriminate between orders or Class Insecta, and likewise, the serologic approach for characterization exhibited a similar taxonomic level of discrimination (1,2). Although a serologic study on five cell lines derived from lepidopteran tissues suggested that intrafamilic distinctions were possible, the extensive serologic cross-reactivity between cellular antigens made interpretations often difficult (4).

Although the initial reports in which the enzyme phenotypes were examined for invertebrate cell lines did not distinguish the *Ae. aegypti* and *Ae. albopictus* cell lines (1,2), only LDH, G6PDH, and malic dehydrogenase (MDH) were employed in the enzyme screening. Two of these enzymes were also tested in this study, and the mosquito cell lines exhibited identical enzyme mobilities. Nevertheless, the mosquito lines were readily distinguished by malic enzyme (ME) and phosphoglucomutase (PGM). Thus, a large number of different enzyme systems should be analyzed before two cell lines may be considered identical.

TABLE 3

RELATIVE ENZYME MOBILITY[a]

	Cell Line Designation	Enzymes										
		EST[b]	G6PDH	HK	IDH-1	IDH-2	LDH	LAP	ME	PGI	PGM	TO
1	ATC-10	73,98,134, 167	285	124	N	100,106,113	100,125	N	149	196	58	100,327
2	ATC-15	80	295	86,124	N	113	125	N	132	196	85	327
3	BTI-EAA	95	225	97	117	N	N	NT	121	115	90,98	NT
4	BM-N	22,83,100, 127	345	146	48	114	N	107	140	91, 103,115	107	273
5	IPRI-MD-108	34,48,76, 171	225	115,136	50	95	80	100	130,137,146	67	71	209
6	IPLB-LD-65Z[c]	71	225	90	69	N	69	85	100	77	80	209
7	IMC-HZ-1	132	100	100	26	83	121	107	100	32,62,77	80	100
8	IPLB-HZ-1075[d]	102	100	100,115	100	100	100	100	100	100	100	100
9	IZD-MB-0503	102,122,135	225	90	69	N	80	85	108	67	85	209
10	IPLB-SF-21AE	100,115	100	100,115	100	100	100	100	100	100	100	100
11	UIV-SL-573	76,88,100, 115,135,185	110	90,128	100	113	114	100	100	100	89	273
12	TN-368	71	225	90	69	N	69	85	100	77	80	209
13	CP-1268	72	170	115	18	100	80	128	92	100	58	NT
14	CP-169	115	170	115	18	100	80	128	92	100,109,116	58	209
15	MRRL-CH-I	80,132,145	100	115	26	106	53	96	132	136	85	300
16	IPRI-CF-124	78,132,145	170	132	36	100	94	N	149	37	96,100	B

[a] Enzyme mobilities were recorded relative to the IPLB-SF-21AE enzyme patterns. B = blurred enzyme pattern; N = nil enzyme activity or no staining reaction observed; NT = enzyme not tested.

[b] For abbreviations of enzymes *see* Table 2.

[c] Probably TN-368.

[d] Probably IPLB-SF-21AE.

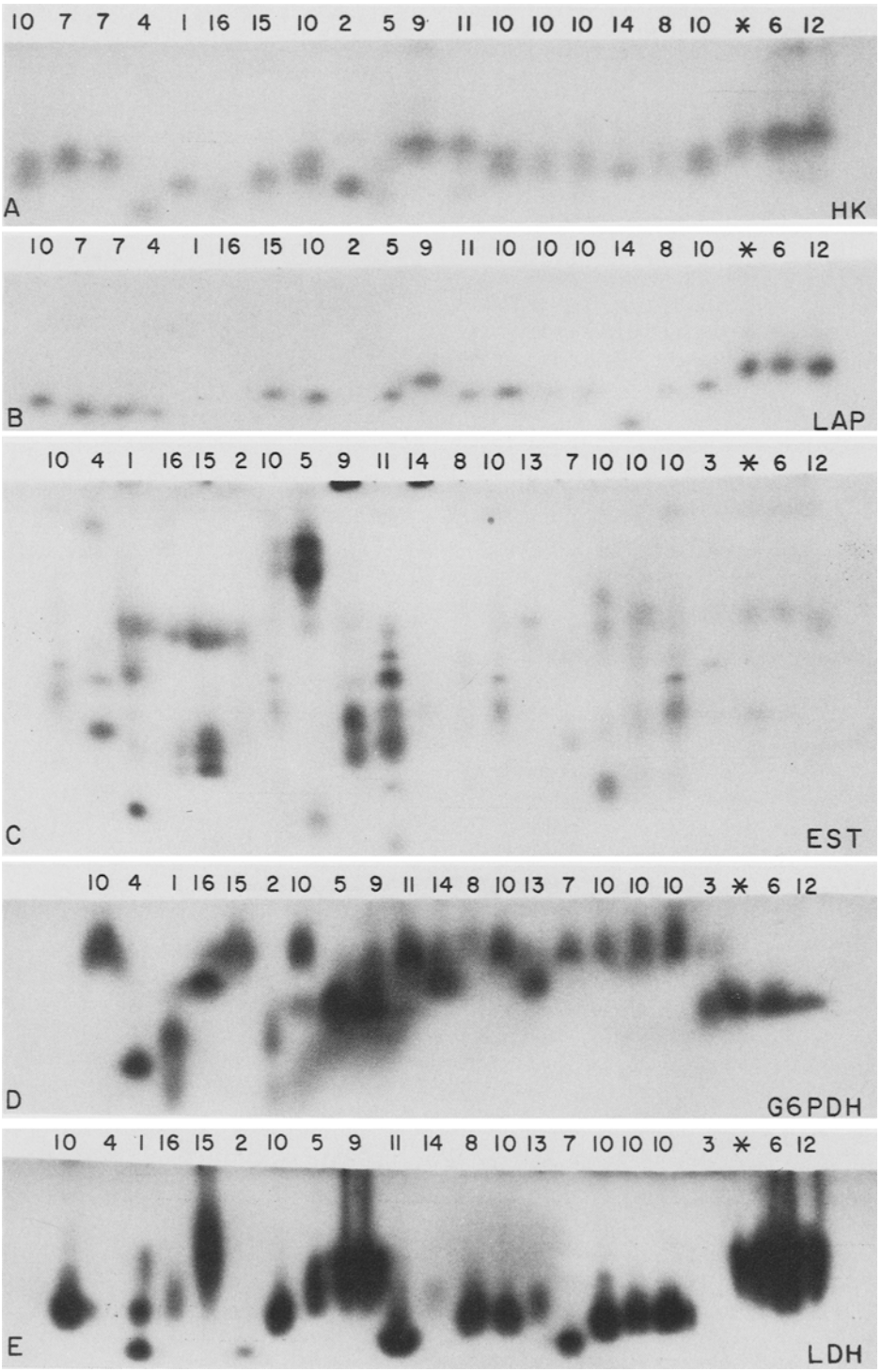

FIG. 2, *A* to *E*. Refer to the legend of Fig. 1.

Alternatively, these data (Table 3) suggest that the screening of a large number of enzymes may not be necessary to distinguish the cell lines. For example, the enzyme systems of IDH, ME, phosphoglucoisomerase (PGI), and PGM discriminate between the cell lines in this study.

Since it was demonstrated that the BTI-EAA cell line was either inadvertently contaminated with TN-368 or a TN-368 flask was mislabeled as BTI-EAA in this laboratory, the usefulness of the isozyme approach for identification is emphasized. Since a number of invertebrate cell lines are maintained in many laboratories, there is clearly the possibility of an error in labeling or in contamination. The isozyme procedure provides criteria by which these mistakes could be detected.

The enzyme phenotype should not be considered as the sole criterion for identity, but rather as an adjunct to cell morphology, growth characteristics, and karyology. The IPLB-LD-65Z and IPLB-HZ-1075 cell lines, for example, exhibit parameters identical to the TN-368 and IPLB-SF-21AE cell lines, and thus they probably resulted from laboratory accidents in handling. The enzyme phenotypes for the true IPLB-LD-65Z and IPLB-HZ-1075 cell lines remain to be demonstrated.

It is suggested that electrophoretic isozyme analyses may prove to be the most useful criterion for cell line identity and that the technique should become routine in laboratories where more than one invertebrate cell line is being handled. The technique is fast, it can be done economically in comparison to other less reliable methods, and it is ideally suited to the screening of a large number of samples.

REFERENCES

1. Greene, A. E.; Charney, J. Characterization and identification of insect cell cultures. Curr. Top. Microbiol. Immunol. 55: 51-61; 1971.
2. Greene, A. E.; Charney, J.; Nichols, W. W.; Coriell, L. L. Species identity of insect cell lines. In Vitro 7: 313-322; 1972.
3. Knudson, D. L.; Buckley, S. M. Invertebrate cell culture methods for the study of invertebrate-associated animal viruses. Maramorosch, K.; Koprowski, H. eds. Methods in virology. vol. 6. New York: Academic Press; 1977: 323-391.
4. Aldridge, C. A.; Knudson, D. L. Characterization of invertebrate cell lines. I. Serologic studies of selected lepidopteran lines. In Vitro 16: 384-391; 1980.
5. Knudson, D. L.; Lescott, T.; Tinsley, T. W. Establishment of a continuous cell line of *Spodoptera littoralis*. In Vitro 16: 369-370; 1980.
6. Hink, W. F. A compilation of invertebrate cell lines and culture media. Maramorosch, K. ed. Invertebrate tissue culture: research applications. New York: Academic Press; 1976: 319-369.
7. Knudson, D. L. Plaque assay of baculoviruses employing an agarose-nutrient overlay. Intervirology 11: 40-46; 1979.
8. Stockdale, H.; Gardiner, G. R. Utilization of some sugars by a line of *Trichoplusia ni* cells. Krustak, E.; Maramorosch, K. eds. Invertebrate tissue culture: applications in medicine, biology, and agriculture. New York: Academic Press; 1976: 267-274.
9. Ayala, F. J.; Powell, J. R.; Tracey, M. L.; Mourao, C. A.; Perez-Salas, S. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics 70: 113-139; 1972.
10. Ayala, F. J.; Tracey, M. L.; Barr, L. G.; McDonald, J. F.; Perez-Salas, S. Genic variation in natural populations of five *Drosophila* species and the hypothesis of the selective neutrality of protein polymorphisms. Genetics 77: 343-384; 1974.
11. Tabachnick, W. J.; Munstermann, L. E.; Powell, J. R. Genetic distinctness of sympatric forms of *Aedes aegypti* in East Africa. Evolution 33: 287-295.
12. Nichols, W. W.; Brandt, C.; Bowne, W. Cytogenetic studies on cells in culture from the Class Insecta. Curr. Top. Microbiol. Immunol. 55: 61-69; 1971.

The many invertebrate cell culturists who kindly supplied their cell lines are gratefully acknowledged. This research was supported in part by the World Health Organization, the Rockefeller Foundation, and U.S. Public Health Service Grants AI-13727 and AI-05118.