Acta entomologica serbica, 1997, 2 (1/2):1-7 UDC 576.312.32:595.773

GENETIC STRUCTURE OF A LABORATORY STRAIN OF *AEDES AEGYPTI* L. (DIPTERA: CULICIDAE)

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Genetic structure of a laboratory strain of *Aedes aegypti* was analyzed by determining isozyme variability on native Polyacrylamid gel electrophoresis (PAGE). Six genetix loci, coding for glucosophosphate isomerase (GPI), phosphoglucomutase (PGM), malic enzyme (ME), phosphogluconate dehydrogenase (PGD), glycerol 3-phosphate dehydrogenase (GPD) and isocitrate dehydrogenase (IDH) were analyzed. Average heterozygosity (H) of 0.123, and proportion of polymorphic loci based on Criteria I and II of 0.667 and 0.500 respectively were determined. Due to the presence of a null allele at *Est-6* locus, electrophoretic data for this locus could not be used for deducing population genetic structure of the species *Ae. aegypti*.

KEY WORDS: *Aedes aegypti*, allelic variation, genetic structure, isozyme variability

INTRODUCTION

Aedes aegypti L. is one of more than 100 mosquito (Diptera: Culicidae) species in the Ethiopian and Oriental subgenus *Stegomyia*. *Ae. aegypti* differs from other species of the subgenus in the pattern on the mesonotum and structure of the hypopigium. The yellow fever mosquito, *Ae. aegypti*, is a complex species in the sense of morphological, physiological, and behavioral variaton, which is greater than the variation found in most insect species (TRPIS & HAUSERMANN, 1975). MATTINGLY (1956) noted a variety and two subspecies of *Ae. aegypti: Ae. aegypti aegypti* (its coloration is brownish and pale in comparison to the African subspecies); *Ae. aegypti formosus* (the sub-Saharan African continent) and *Ae. aegypti* var. *queenslandensis* (pale form with scattering of cream-colored scales; often found mixed with the type form). The probable ancestral habitat of the yellow fever mosquito, *Ae. aegypti* are African forests. Eggs are deposited in suitable tree holes or artificial con-

teiners where larval development takes place (WALLIS & TABACHNICK, 1990). It is distributed in tropics and subtropics of both hemispheres. The species was registered in Southern Europe (France, Spain, Portugal, Italy, Yugoslavia, Albania and Greece).

The genetic structure of *Aedes aegypti* populations has been extensively studied by electrophoretic techniques in defining genetic distance and relationships between populations (TABACHNICK & POWELL, 1978; TABACHNICK *et al.*, 1979; WALLIS *et al.*, 1984; WALLIS & TABACHNICK, 1990); geographic and temporal patterns of genetic variation (TABACHNICK, 1982); complexity and organization of the genome (WARREN & CRAMPTON, 1991) and linkage map (WALLIS & TABACHNICK, 1982; SEVERSON *et al.*, 1993). Esterase isozymes in particular have been extensively studied in *Ae. aegypti* due to their role as indicators of geographical variability (SAUL *et al.*, 1976) and their influence on insecticide resistance (MOCHES *et al.*, 1987).

In this paper, the genetic structure of a laboratory strain of *Aedes aegypti* L. based on allozyme variability of seven loci is presented.

MATERIAL AND METHODS

Laboratory population of *Aedes aegypti* London strain originates from a colony provided from Department of Environmental Biology, University of Manchester and was reared for five years in the laboratories of University of Novi Sad.

Soluble proteins of adult mosquitoes were separated in polyacrylamide gel electrophoresis (PAGE), on 5% polyacrilamide slab gels. Isozyme variability was studied using two buffer systems: (1) TBE buffer: glucosophosphate isomerase (E.C.5.3.1.9), esterase (E.C.3.1.1.?), phosphoglucomutase (E.C.2.7.5.1), malic enzyme (E.C.1.1.1.40) and phosphogluconate dehydrogenase (E.C.1.1.1.43); (2) TC buffer; glycerol 3-phosphate dehydrogenase (E.C.1.1.1.8), isocitrate dehydrogenase (E.C.1.1.1.42), according to MUNSTERMANN (1979). Individual adult specimens were macerated in 25 microliters of a sucrose grinding solution and centrifuged for three minutes at 15,000 g. Two to three microliters of the supernatant were applied to each slab gel slot.

Electrophoresis was performed for 3-4 hours at a constant voltage of 220V (TC buffer) or 330V (TBE buffer). Gels were then placed in the appropriate staining solutions at 37°C with substrates specific for the enzyme being assayed. Gels were fixed in a methanol-water-acetic acid mixture (5:5:1).

Genetic variability was defined by allele and genotype frequencies, number of alleles per locus, percentage of loci polymorphic at 0.99 and 0.95 criteria, mean heterozygosity and Hardy-Weinberg expectations of mean heterozygosity. Expected

heterozygosity at a single locus was calculated by $H_e=1-\Sigma p_i^2$, where p_i is the frequencies of the i^{th} allele out of a total of n alleles at the locus.

RESULTS AND DISCUSSION

Four out of six isozyme loci investigated, showed some electrophoretically scorable variation (Tables I & II; Figure 1). Among six loci, only *Me* and *Gpd* were monomorphic. The mean proportion of loci which were found to be polymorphic was 0.667 and 0.500 (criteria I: $P_{0.99}$; and II: $P_{0.95}$). The average heterozygosity per locus (H) was 0.123 (Table III). In two loci, *Pgm* and *Pgd*, the average observed heterozygosities were lower than expected heterozygosities based on Hardy Weinberg values (Table I).

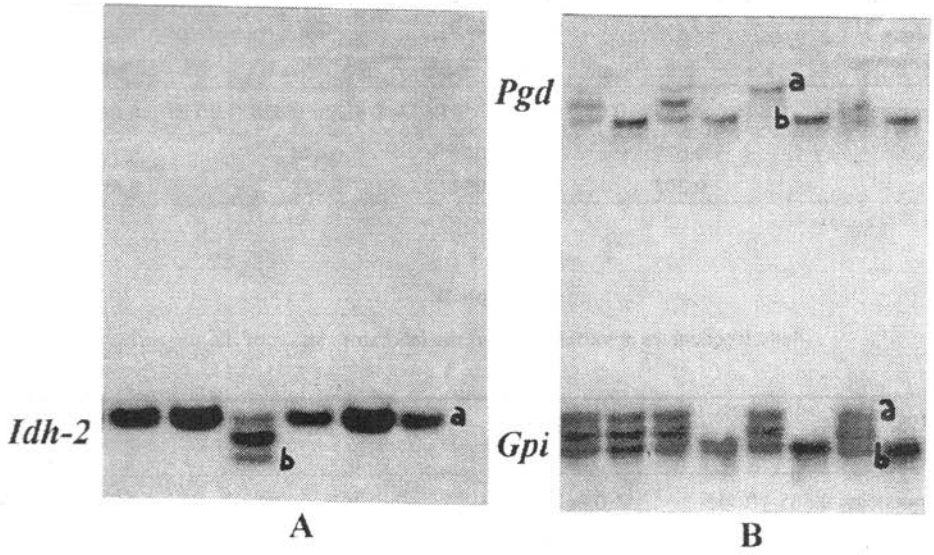

Fig. 1. Zymograms of A) Isocitrate dehydrogenase (IDH) and B) Phosphogluconate dehydrogenase (PGD) and Glucosophospate isomerase (GPI).

Ae. aegypti represents a suitable model-system for studies of evolutionary mechanisms (selection, genetic drift, inbreeding, gene flow) as well as changes in genetic structure of populations. Comparing the mean number of alleles, heterozygosity and polymorphism, it has been shown that those values are always lower in laboratory populations than in natural ones (MUNSTERMANN, 1979). It has also been confirmed that sylvan strain carried more alleles per locus and had a greater average heterozygosity of domestic and island populations (TABACHNICK & POWELL, 1978; TABACHNICK *et al.*, 1979; TABACHNICK, 1982). Explanation of this phenomenon lies

in the fact that natural forest populations probably are exposed to a much more heterogeneous environment than laboratory populations (MUNSTERMANN, 1979). POWELL (1971) has shown that a heterogeneous environment is largely responsible for maintaining or increasing the level of enzyme polymorphism. Therefore steady environment could be responsible for maintaining lower levels of isozyme variability and for the fixation of certain alleles and/or coadapted gene complexes in laboratory strains. Effects of inbreeding, genetic drift and selection, however, will act to increase this disparity, and reduce heterozygosity, as shown for isolated island populations (WALLIS et al., 1984).

Table I

Genotype frequencies at variable loci of the laboratory strain of *Ae. aegypti*.

Locus	*Pgm*			*Idh-2*		*Gpi*		*Pgd*		
Genotype	a/a	b/b	a/b	a/a	a/b	b/b	a/b	a/a	b/b	a/b
Frequencies	0.846	0.077	0.077	0.933	0.067	0.586	0.414	0.179	0.643	0.178
H_0		0.077		0.067		0.414			0.178	
H_e		0.203		0.064		0.328			0.392	

Table II

Allelic frequencies at variable loci of the laboratory strain of *Ae. aegypti*.

Locus	*Pgm*		*Idh-2*		*Gpi*		*Pgd*	
Allele	a	b	a	b	a	b	a	b
Frequencies	0.885	0.115	0.967	0.033	0.207	0.793	0.268	0.732
n	26		30		29		28	

Table III

Estimates of genetic variability of the laboratory strain of *Ae. aegypti*.

Mean No. alleles per locus	Mean Observed heterozygosity	Mean Expected heterozygosity	Percentage of loci polymorphic *
1.667	0.123	0.165	0.667 (0.500)

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99 (0.95)

The esterases include complex group of enzymes that hydrolyze different ester bonds. Different isozymes are coded by at least six gene loci, expression of which is dependent on the substrate of the catalyzed reaction. In addition to three other alleles, a null allele at the *Est-6* locus was registered, when the gels were incubated in a solution containing α-naphtyl acetate (heterozygous with the null allele presumably had single banded phenotype and were scored as homozygoutes). Due to the presence of this allele, electrophoretic studies of this locus alone could not be used to deduce the population genetic structure of the species *Ae. aegypti* (SAUL *et al.*, 1976; TABACHNICK & POWELL, 1978). TREBATOSKI & CRAIG (1969) studied an esterase locus which they called esterase 6 and located on linkage group II, 23,3 cm from the *spot* locus.

The four alleles registered in the survey of electrophoretic variability of *Est-6* locus (Table IV) forms two homo- and four heterozygotes (Table V). This locus shows the highest value of observed heterozygosity (H=0.689, Table V) of all other loci in this study (Table I). Allele number at *Est-6* locus was lower in the laboratory strain, which can be attributed to the bottleneck effect, inbreeding and genetic drift, and depends on the number of generations. Thus, SAUL *et al.* (1976) showed marked decrease in n, from 2 in F_1 to only one allele in F_{11}, as well as a decrease from four to only two alleles after 45 years of rearing a laboratory population. Natural populations are characterized by a higher number of alleles present at *Est-6* locus, from two to seven in domestic and three to seven feral populations and from three to six in peri-domestic populations (SAUL *et al.*, 1976) and 9 allele in feral populations (MUNSTERMANN, 1979). The number of *Est-6* phenotypes in natural populations is also higher (5-10, TABACHNIK & POWELL, 1978).

Table IV
Allelic frequencies at *Est-6* locus of the laboratory strain of *Ae. aegypti*

Allele	a	b	c	d
Frequencies	0.414	0.517	0.017	0.052
n		29		

Table V
Genotype frequencies at *Est-6* locus of the laboratory strain of *Ae. aegypti*

Genotype	a/a	b/b	a/b	b/c	b/d
Frequencies	0.318	0.172	0.552	0.035	0.103
H_o			0.689		
H_e			0.558		

REFERENCES

MATTINGLY, P.F., 1956. Species hybrids in mosquitoes. *Trans. Roy. Entomol. Soc.*, 108:2-36.

MOUCHES, C., M. MAGNIN, J.B. BERGE, M. DE SILVESTRI, V. BEYSSAT, N. PASTEUR & G.P. GEORGHIOU, 1987. Overproduction of detoxifying esterases in organophosphate-resistent Culex mosquitoes and their presence in other insects. *Proc. Natl. Acad. Sci.* USA, 84: 2113-2116.

MUNSTERMANN, L., 1979. *Isozymes of Aedes aegypti: Phenotypes, linkage, and use of genetic analysis of sympatric populations in East Africa*. Ph.D. Dissertation, Univ. Notre Dame.

POWELL J.R., 1971. Genetic polymorphism in varied environments. *Science*, 174: 1035-1036.

SAUL, S., P. GUPTAVANIJ & G. CRAIG, 1976. Genetic Variability at an Esterase Locus in Aedes aegypti. *Ann. Entomol. Soc. Amer.*, 69 (1): 73-79.

SEVERSON, D., A. MORI, Y. ZHANG & B. CHRISTENSEN, 1993. Linkage Map for Aedes aegypti Using Restriction Fragment Length Polymorphism. *J. Heredity*, 84: 241-247.

TABACHNICK, W., L. MUNSTERMANN & J. POWELL, 1979. Genetic Distinctness of Sympatric Forms of Aedes aegypti in East Africa. *Evolution*, 33 (1): 287-295.

TABACHNICK, W.J., 1982. Geographic and temporal patterns of genetic variation of Aedes aegypti in New Orleans. *Am.J.Trop.Med.Hyg.* 31 (4): 849-853.

TABACHNICK, W.J. & J.R. POWELL, 1978. Genetic structure of the East African domestic populations of Aedes aegypti. *Nature*, 272: 535-537.

TREBATOSKI, A.M. & G.B. CRAIG, 1969. Genetics of an esterase in Aedes aegypti. *Biochem. Genet.*, 3: 383-392.

TRPIS, M. & K. HAUSERMANN, 1975. Demonstration of differential domesticity of Aedes aegypti (L.) (Diptera: Culicidae) in Africa by mark-release recapture. *Bull. Entomol. Res.*, 65: 199-208.

WALLIS, G.P. & W.J. TABACHNICK, 1982. Linkage of and IDH locus with sex and two lethals in Aedes aegypti. *J. Heredity*, 73: 291-294.

WALLIS, G.P. & W.J. TABACHNICK, 1990. Genetic analysis of rock hole and domestic Aedes aegypti on the Caribean island of Anguilla. *J. Am. Mosq. Control Assoc.* 6(4): 625-630.

WALLIS, G.P., W.J. TABACHNICK & J.R. POWELL, 1984. Genetic Heterogeneity among Caribbean populations of Aedes aegypti. *Am. J. Trop. Med. Hyg.* 33(3): 492-498.

WARREN, A.M. & J.M. CRAMPTON, 1991. The Aedes aegypti genome: complexity and organization. *Genet. Res. Camb.* 58: 225-232.

ГЕНЕТИЧКА СТРУКТУРА ЛАБОРАТОРИЈСКЕ ПОПУЛАЦИЈЕ ВРСТЕ *AEDES AEGYPTI* L. (DIPTERA: CULICIDAE)

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Извод

Анализирана је генетичка структура лабораторијске популације врсте *Aedes aegypti* методом полиакриламид гел електрофорезе (PAGE) на основу алозимске варијабилности шест локуса који детерминишу синтезу ензима: глукозофосфат изомеразе (GPI), фосфоглукомутазе (PGM), малични ензим (ME), фосфоглуконат дехидрогеназе (PGD), глицерол 3-фосфат дехидрогеназе (GPD) и изоцитрат дехидрогеназе. Утврђено је да је просечна хетерозиготност (X) 0.123, и полиморфност на основу критеријума I и II била 0.667 и 0.50. У присуству α-нафтил ацетата је анализирана алозимска варијабилност Est-6 локуса. Због присуства нултог алела електрофоретска проучавања овог локуса се не могу користити у одређивању генетичке структуре популације врсте *Ae. aegypti*.

Received October 10, 1997
Accepted December 8, 1997