BIOSCIENTIAE
Volume 1, Nomor 1, Januari 2004
Halaman 1-10

CUTICULAR COMPONENT ANALYSIS FOR DISCRIMINATION OF *AEDES AEGYPTI* (LINNAEUS) FROM BANJARMASIN AND YOGYAKARTA

Abdul Gafur

Biology Study Program, Faculty of Mathematics and Natural Sciences,
Lambung Mangkurat University
Jl. A. Yani Km 36 Banjarbaru, Kalimantan Selatan

ABSTRACT

Gas chromatographic analysis of cuticular component was carried out to reveal differences in cuticular hydrocarbon composition among populations of *Aedes aegypti* that are spatially far separated. Females from two populations in Banjarmasin and one in Yogyakarta were compared. Stepwise discriminant analysis selected five discriminator peaks from chromatograms, which, by cross validation, could be used to distinguish between females of the three populations with 100% success rate. This indicates genetic differences between populations of *Aedes aegypti* in Banjarmasin and that in Yogyakarta.

Keywords: cuticular hydrocarbon, chromatography, discriminant analysis, *Aedes aegypti*

INTRODUCTION

Aedes aegypti is a widely distributed species of mosquito and is demonstrated to develop intraspecific variations, such as vectorial capacity (Aitken *et al.*, 1977), degree of anthropophily (Mukwaya, 1977), and response to insecticides and repellent (Rutledge *et al.*, 1978). Certain populations of this species show microgeographic differences, electrophoretic variations, variations in development and oviposition site preferences (Tabachnick and Powell, 1978; Tabachnick, 1993), habitat selection (Trpis and Hausermann, 1978), as well as

morphological differences (Vandehey *et al.*, 1978).

In Indonesia, distribution of this species has spread to all provinces (Djakaria, 1988). With different environmental conditions among localities, it is probable that certain variations have already arisen among populations of this species in this country. This should be depicted by various available methods.

Analysis of cuticular components, particularly hydrocarbons, with gas chromatography has been successfully applied in discrimination, identification, and determination of relationship among members of species complexes, sibling species, and strains of mosquitoes and other insects. In view of usefulness and ease of application of the method, it is desirable to analyze and compare cuticular component profiles of populations of *Aedes aegypti* from different localities in Indonesia. Populations of this species in Banjarmasin and Yogyakarta were selected in consideration of spatially wide distance between the two towns, including Java Sea and approximately 200 kms of mainland crossing North to South Coast of Java Island. That distance may effectively block contact between populations of mosquitoes of the two towns.

MATERIALS AND METHODS

Mosquitoes

Females of *Aedes aegypti* from Banjarmasin were collected from two villages: Karang Mekar and Kuin Cerucuk which are ± 7 kms apart, including a ± 100 m wide Martapura river. *Aedes aegypti* from Yogyakarta were collected from a population at Terban village.

Collections were carried out by collecting larvae from water containers. Larvae were then reared to imagoes. Adult mosquitoes emerging were captured with aspirator and were then kept unnourished in jars to die.

Extraction and Gas Chromatography

Pooled samples of 10 females were submerged in 150 µl of solvent (n-hexane) for 10 minutes. Extracts were then blown with nitrogen to near dryness and resuspended in 2 µl of n-hexane containing an internal standard (10 ppm of pentadecane). Samples were finally injected into a Hewlett-Packard 5890 Series II gas chromatograph with capillary column (Shimadzu CBP-1 nonpolar 50 m, 0.22 mm internal diameter, 0.25 µm phase thickness). Carrier gas (helium) flow rate was 7 ml/minute. Column temperature was programmed 75-310°C (15°C/minute). Injector and detector temperature was 320°C and 340°C, respectively. A Hewlett-Packard 3396 Series II integrator was connected to the chromatograph. Three replications were applied for each population.

Data Analysis

Peak identification was carried out by subtracting retention time of the internal standard (pentadecane) from that of a peak and dividing the result with retention time of the last considered peak, resulting in a corrected relative retention time (CRRT) (Kittayapong *et al.*, 1990). Peaks of the same or almost the same CRRT were considered representing the same cuticular component. Rare peaks were excluded to prevent inclusion of contaminants. Standardization of peak area was performed by dividing a peak area with the peak area of internal standard. The result was log transformed using $X' = \log (X + 1)$ formula (Sokal & Rohlf, 1995).

Stepwise discriminant analysis with Mahalanobis' distance and F-to-enter = 5 was performed with SPSS r.7.5.1. Analysis was carried out using peaks consistently appear on the three replications of at least one population. Degree of successful discrimination/identification was quantified by percentage of correct allocations. Cross validation with leave-one-out method was applied to reduce bias.

RESULTS

Cuticular component profiles of female *Aedes aegypti* show 36-78 peaks. Peaks not consistently appear on every replication of at least one population were excluded from analysis, resulting 31 peaks for comparison of the three populations.

Stepwise discriminant analysis selected five discriminator peaks (Table 1). Two discriminant functions were resulted from two independent comparisons among three groups. Discriminant function 1 could explain nearly 100% of total variance (Table 1), and be consequently considered more important than discriminant function 2 for discrimination among the three populations. This is also reflected by distribution of samples in discriminant function plot (Figure 1). Discriminant function 1 played major role in discriminating KC from KM and TB, while discriminant function 2 helped in discriminating KM from TB (Figure 1).

Based on the chromatographic characteristics, with cross validation, all (100%) samples could be correctly allocated (identified) to their population of origin (Table 2).

Table 1. Summary of discriminant analysis of female *Aedes aegypti* from Banjarmasin and Yogyakarta

	Discriminant function	
	1	2
% variance	100.0	0.0
Canonic correlation	1.000	0.966
Peak scores (standardized discriminant function)		
24	9,882	1,308
56	18,226	1,999
58	-2,640	-0,764
69	-4,311	0,423
79	12,259	-0,136
Centroids (average scores)		
Karang Mekar (Banjarmasin)	-189,399	-3,464
Kuin Cerucuk (Banjarmasin)	312,451	-0,528
Terban (Yogyakarta)	-123,052	3,992

Table 2. Identification of female *Aedes aegypti* from Banjarmasin and Yogyakarta

Original population	Number of sample	Predicted population		
		Karang Mekar	Kuin Cerucuk	Terban
Karang Mekar	3	3 (100%)	0	0
Kuin Cerucuk	3	0	3 (100%)	0
Terban	3	0	0	3 (100%)

Percent of correct identification = 100%

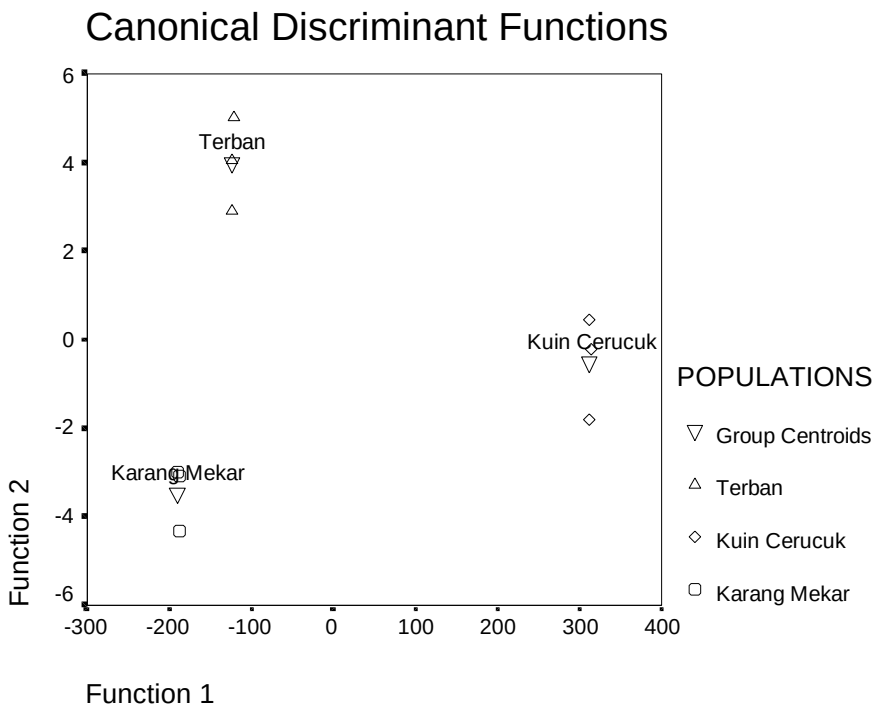

Figure 1. Distribution of discriminant scores of samples of female *Aedes aegypti* from Banjarmasin and Yogyakarta in space of discriminant function 1 and 2.

DISCUSSION

This study has demonstrated that female *Aedes aegypti* from Banjarmasin and Yogyakarta have different profiles of cuticular components. Within population variation is much less than among population variation of this species. Stepwise discriminant analysis has selected five peaks as discriminator variables that will give best separation and most accurate prediction for identification/discrimination of new samples.

Among main components of insect cuticle are hydrocarbons (Hepburn, 1985) located in wax layer. These chemically very stable compounds (Carlson

& Service, 1980) using appropriate technique of extraction can be obtained even from old and dry specimens. Therefore, even without conducting peak identification, such as with GCMS, many authors (Phillips *et al.*, 1990a, 1990b; Kamhawi *et al.*, 1992; Anyanwu *et al.*, 1993) believed that these are compounds extracted from their specimens and formed gas chromatograph profiles of their samples.

Cuticular hydrocarbons may be used by insects in effective recognition of potential mates (Phillips *et al.*, 1990b). These compounds are very useful in early detection of speciation or divergence of insect populations, and are among the first to change to ensure reproductive isolation (Anyanwu *et al.*, 1993). Since cuticular hydrocarbon composition is genetically determined (Anyanwu *et al.*, 1993), differences in cuticular component (hydrocarbon) profiles among populations of *Aedes aegypti* under study may, therefore, indicate ongoing genetic separation or speciation leading to reproductive isolation.

Population of *Aedes aegypti* at Terban (Yogyakarta) is spatially very far separated from those at Karang Mekar and Kuin Cerucuk (Banjarmasin). The separation, mainly by Java Sea, may completely prevents free genetic exchange between Aedes *aegypti* of Yogyakarta and that of Banjarmasin. Meanwhile, populations of *Aedes aegypti* at Karang Mekar and that at Kuin Cerucuk, are separated by a 7 km distance, including a 100 m wide Martapura river. Flying distance of this species that is only about 50 m (Sugito, 1990) is far less than the spatial separation of the two populations. In addition, strong wind blow above wide river will be a potent barrier of contact between the two populations. The block of contact is too much to counter by effect of human migration.

Without free gene exchange, the three populations of *Aedes aegypti* may be considered to be geographically isolated. Every geographic isolate is an incipient species and important evolutionary unit that is frequently sufficiently different to be ranked as subspecies (Mayr & Ashlock, 1991). There is a good possibility that the three populations of *Aedes aegypti* under study are developing genetic separation leading to different subspecies. However, more

studies are needed to depict the degree of separation and the taxonomic rank achieved.

Another implication of differences in cuticular hydrocarbon composition is the possibility of different response to insecticides, as demonstrated among strains of *Anopheles stephensi* (Anyanwu *et al.,* 1993). This is based on the fact that cuticular lipids are among the regulators of insecticide entrance into insect body (Brook, 1976 *cit* Blomquist & Dillwith, 1985). There may also be such difference between Aedes *aegypti* of Banjarmasin and of Yogyakarta that show dissimilarity in cuticular hydrocarbon composition.

Suppose that there are differences in response or sensitivity to insecticides among the three populations of *Aedes aegypti*, gas chromatographic analysis may be a potential effective method in identifying the sensitive target population. This will be of much help in improving effectiveness of chemical control measures against *Aedes aegypti* and, in turn, in controlling dengue hemorrhagic fever.

CONCLUSION

Female *Aedes aegypti* from populations in Banjarmasin and Yogyakarta show differences in their cuticular hydrocarbon composition. The differences can be used in discriminating the populations with very high degree of success.

ACKNOWLEDGEMENTS

The author wishes to thank the DGHE Ministry of National Education, Republic of Indonesia for supporting fund for this study. Dr. J. Situmorang has been of much help with his advice during this research. Mr Sudomo, of Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, was of assistance in GC analyses.

REFERENCES

Aitken THG, Downs WG, & Shope RE. 1977. *Aedes aegypti* strain fitness for yellow fever transmission. *Am. J. Trop. Med. Hyg.* **26,** 985-989.

Anyanwu GI, Davies DH, Molyneux DH, Phillips A, & Milligan PJ. 1993. Cuticular hydrocarbon discrimination/variation among strains of the mosquito, *Anopheles (Cellia) stephensi* Liston. *Ann. Trop Med. Parasit.* **87,** 269-275.

Blomquist GJ & Dillwith JW. 1985. Cuticular Lipids. In *Comparative Insect Physiology, Biochemistry and Pharmacology, Vol 3: Integument, Respiration and Circulation*. Kerkut GA & Gilbert LI (Editors). Pergamon Press, New York. Hlm 117-154.

Carlson DA & Service MW. 1980. Identification of mosquitoes of *Anopheles gambiae* Giles species complex A and B by analysis of cuticular components. *Science* **207,** 1089-1091.

Djakaria S. 1988. Vektor Penyakit, Virus, Riketsia and Bakteri. In *Parasitologi Kedokteran*. Gandahusada S, Pribadi W, & Ilahude H D (Editor). Fakultas Kedokteran Universitas Indonesia, Jakarta. Hlm 175-180.

Hepburn HR. 1985. Structure of The Integument. In *Comparative Insect Physiology, Biochemistry and Pharmacology, Vol 3: Integument, Respiration and Circulation*. Kerkut GA & Gilbert LI (Editor). Pergamon Press, New York. Hlm 1-58.

Kamhawi S, Lane RP, Cameron M, Phillips A, Milligan P, & Molyneux DH. 1992. The cuticular hydrocarbons of *Phlebotomus argentipes* (Diptera: Phlebotominae) from field populations in northern India and Sri Lanka, and their change with laboratory colonization. *Bull. Ent. Res.*, **82,** 209-212.

Kittayapong P, Clark JM, Edman JD, Potter TL, Lavine B K, Marion JR, & Brooks M. 1990. Cuticular lipid differences between the malaria vector and non-vector forms of the *Anopheles maculatus* complex. *Med. vet. Entomol.*, **4**, 405-413.

Mayr E & Ashlock PD. 1991. *Principles of Systematic Zoology.* Second edition. McGraw-Hill, Inc., New York.

Milligan PJM, Phillips A, Molyneux DH, Subbarao SK & White GB. 1986. Differentiation of *Anopheles culicifacies* Giles (Diptera: Culicidae) sibling species by analysis of cuticular components. *Bull. Ent. Res.*, **76,** 529-537.

Mukwaya LG. 1977. Genetic control of feeding preferences in the mosquitoes *Aedes* (*Stegomyia*) *simpsoni* and *aegypti*. *Physiol. Ent.*, **2**, 133-145.

Phillips A, Milligan PJM, Maroli M, Lane RP, Kamhawi S, Broomfield G, & Molyneux DH. 1990*a*. Intraspecific variation in the cuticular hydrocarbons of the sandfly *Phlebotomus perfiliewi* from Italy. *Med. Vet. Entomol.*, **4,** 451-457.

Phillips A, Sabatini A, Milligan PJM, Boccolini D, Broomfield G & Molyneux

DH. 1990*b*. The *Anopheles maculipennis* complex (Diptera: Culicidae): comparison of the cuticular hydrocarbon profiles determined in adults of five Palaearctic species. *Bull. Ent. Res.,* **80,** 459-464.

Rutledge LC, Moussa MA, Lowe CA, & Sofield RK. 1978. Comparative sensitivity of mosquito species and strains to the repellent diethyl toluamide. *J. Med, Entomol.,* **14,** 536-541.

Sokal RR & Rohlf F J. 1995. *Biometry*. Third edition. W.H. Freeman and Company, New York.

Sugito R. 1990. Aspek entomologi demam berdarah dengue. In *Berbagai Aspek Demam Berdarah Dengue dan Penanggulangannya*. Laporan Semiloka, Depok 27-28 November 1989. Haryanto H, Harun SR, Wuryadi S, & Djaja IM (Editor). Hlm 37-48.

Tabachnick WJ & Powell JR. 1978. Genetic Structure of the East African domestic populations of *Aedes aegypti*. *Nature* **272,** 535-537.

Tabachnick WJ. 1993. Patterns of genetic variation and the ecology of the yellow fever mosquito, *Aedes aegypti*. In *Evolution of Insect Pests: Patterns of Variation*. Kim KC & McPheron BA (Editor). John Wiley & Sons, Inc., New York. Hlm 413-422.

Trpis M, & Hausermann W. 1978. Genetics of house-entering behaviour in East African populations of *Aedes aegypti* (L.) (Diptera: Culicidae) and its relevance to speciation. *Bull. Ent. Res.*, **68,** 521-532.

Vandehey RC, Leahy MG, & Booth KS. 1978. Analysis of colour variations in feral, peridomestic and domestic populations of *Aedes aegypti* (L.) (Diptera: Culicidae). *Bull. Ent. Res.,* **68,** 443-453.