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DISCRIMINATION OF FEMALE *AEDES AEGYPTI* (DIPTERA: CULICIDAE) FROM BANJARMASIN AND YOGYAKARTA BASED ON WING MEASUREMENTS

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ABSTRACT

Morphometric analysis of wing venation was performed for discrimination of females of three populations of *Aedes aegypti* previously could only be separated by gas chromatographic analysis of cuticular components. Images of detached wings observed under a microscope of low magnification were saved as bitmap files. Cartesian coordinates of termination and branching points of each wing were digitally recorded and were subsequently used in determination of interpoint distances. Two discriminant analyses using standardized distances selected 7 variables that could distinguish females of the three populations with 86.7% and 85% success rate. The differences in wing measurements gave further evidence of genetic separation of populations of the species.

Keywords: *Aedes aegypti,* discriminant analysis, wing

INTRODUCTION

Aedes aegypti is considered one of the most important mosquito species due to its vectorial capacity for deadly dengue virus and its worldwide distribution. In Indonesia, this species, and the dengue hemorrhagic fever (DHF) it transmits, has spread to all provinces (Djakaria, 1988; Suwasono & Nalim, 1989). Because this species has developed genetic differences among populations in several parts of the world, it may be expected that the genetic separation can also be detected among populations of the species in different parts of Indonesia.

Gafur (2004) has reported genetic divergence reflected from differences in cuticular components among three populations of this species in Banjarmasin and Yogyakarta. However, no morphological comparison so far performed. The difference in cuticular components may imply distinction in mosquito susceptibility to insecticides or other cuticular-governed properties, or even in its vectorial capacity. Therefore correct discrimination among cuticular groups of mosquito may be important in control measures. While identification or discrimination based on chromatographic analysis of cuticular components may provide high success rate, the technique is still relatively complicated and expensive, and therefore unavailable in most laboratories in Indonesia. It will be easier for a field worker or a less-equipped laboratory if discrimination can be achieved using a simple microscope with lower magnifications.

In search for morphological character useful for discrimination of chromatographically different populations of *Aedes aegypti,* morphological comparison was performed focusing on easy-to-observe characters. In the present study wing was chosen as studies on other species of Diptera have discovered within as well as among species differences (Adsavakulchai *et.al.*, 1998; Hole *et al.*, 2003).

MATERIALS AND METHODS

Mosquito collection

Aedes aegypti mosquitoes were collected from Banjarmasin and Yogyakarta, at the same location as Gafur (2004). Two villages in Banjarmasin (Karang Mekar and Kuin Cerucuk) and one in Yogyakarta (Terban) were selected as collection sites.

Collections were made by capturing larvae from indoor water containers. The collected larvae were then reared in glass jars. Emerging imagoes were captured by aspirator and placed unnourished in paper cups to die. Specimens were separated by sex, and only females used in this study.

Wing measurements

Both wings of each specimen were detached and placed between microscope slides and cover slips. Wings were examined under a compound microscope with 20x and 40x magnification and were then photographed. The photographs were scanned to produce digital images and were saved in BMP format.

Wing measurements were conducted by first determining Cartesian coordinates of branching and terminal points of venation. Based on the coordinates, distances between points were calculated using following formula:

$$J_{AB} = \sqrt{(x-p)^2 + (y-q)^2}$$

J_{AB} : distance between point A (x,y) and B (p,q)

The distances were finally standardized using two distances considered representing wing length. The first was the distance between the branching point of mediocubitus (mcu) crossvein from anterior cubitus (CuA) and the termination point of the third radial branch (R3). The second standard was the distance between the starting point of radial sector (Rs) and the termination point of the first radial branch (R1). For convenience, the first standard was coded KG, and the second OI. The standardized distances were used as characters or variables in statistical analyses.

Data analysis

Univariate analysis of variance was applied to select significantly different distances to be used in distinguishing the three *Aedes aegypti* populations. If univariate ANOVA fail to find distinguishing character, stepwise discriminant analysis was performed to obtain character combination best discriminating the three populations.

Jack-knife estimator was applied to reduce bias in estimation of correct identification in discriminant analysis. In this case, one specimen was excluded from analysis, and the resulting discriminant function was used to identify the excluded specimen; this process was repeated with other specimens.

All statistical analyses were applied using SPSS statistical software.

RESULTS

Sixty wings from ten individuals of each of the three populations were examined. Eighteen points (Figure 1) of each wing were observed and their Cartesian coordinates were determined. Point J was excluded because in most specimens it was covered with heavy scales causing coordinate determination impractical.

Interpoint distances were determined based on Cartesian coordinates, resulting 153 distances. For standardization, distances between point O and I (OI) and between point K and G (KG) were selected. Therefore, other distances were divided by these two standardizing distances, and the resulting standardized distances were used in statistical analyses.

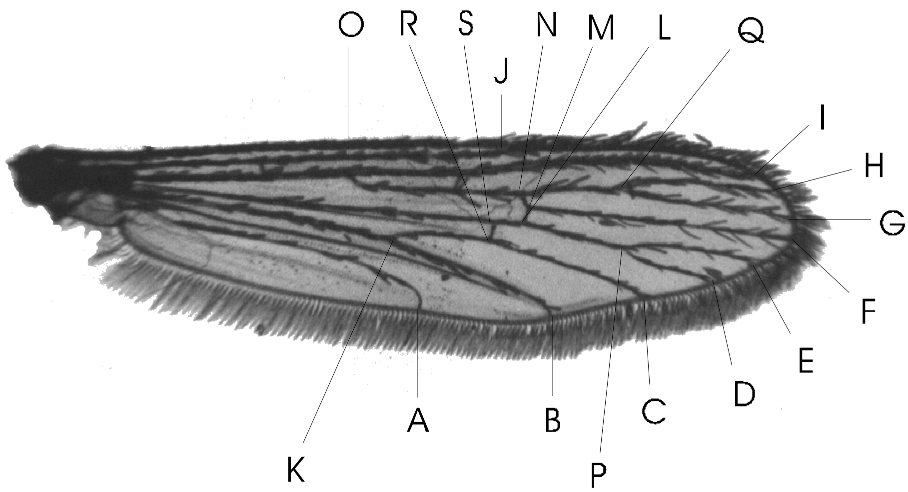

Figure 1. Observed branching and terminal points of female *Aedes aegypti* wing venation

Univariate ANOVA has found significantly different characters (distances). However, multiple comparisons using DMRT could not find any one character showing significant difference between all two-population comparisons. That means that there were not any individual distance could independently discriminate specimens from the three populations. Therefore, discriminant analysis was applied.

KG-Standardized distances

Ninety two normally distributed distances were used in stepwise discriminant analysis with 'F to enter' set to 3.84. The analysis showed highly significant differences among the three populations. The discriminant analysis produced two discriminant functions (DF) and selected 7 variables as discriminators (Table 1). DF 1 could explain 70.7% of total variance, while DF 2 could explain the rest 29.3%. Therefore, DF 1 could be considered more important than DF 2. This was also reflected by distribution of discriminant function scores of samples in a discriminant function plot (Figure 2), showing that DF 1 played major role in discriminating TB from KC and KM, while DF 2 helped in further discriminating KM from KC.

In identification of samples using identification equation developed from the analysis, with jack-knife estimator, 86.7% of specimens could be correctly identified to their original populations (Table 2).

Table 1. Summary of canonical discriminant function based on KG-standardized distances for wings of female *Aedes aegypti* from Banjarmasin and Yogyakarta

	Discriminant function	
	1	2
% of variance	70,7	29,3
Canonical correlation	0,845	0,714
Unstandardized discriminant function coefficient:		
CN	-1,232	49,278
DP	38,458	6,538
LM	-125,193	68,850
MS	123,583	13,386
NQ	-27,944	-10,004
BE	-23,567	-20,622
BN	23,555	53,909
Constant	-8,368	-31,286
Function at group centroid:		
Kuin Cerucuk	-1,797	0,796
Karang Mekar	-0,171	-1,400
Terban	1,969	0,605

Table 2. Identification of wing specimens of female *Aedes aegypti* based on seven KG-standardized distances on venation

Original population	Predicted population			Total
	Kuin Cerucuk	Karang Mekar	Terban	
Kuin Cerucuk	19	1	0	20
Karang Mekar	2	15	3	20
Terban	0	2	18	20

Correct identification = 86.7%

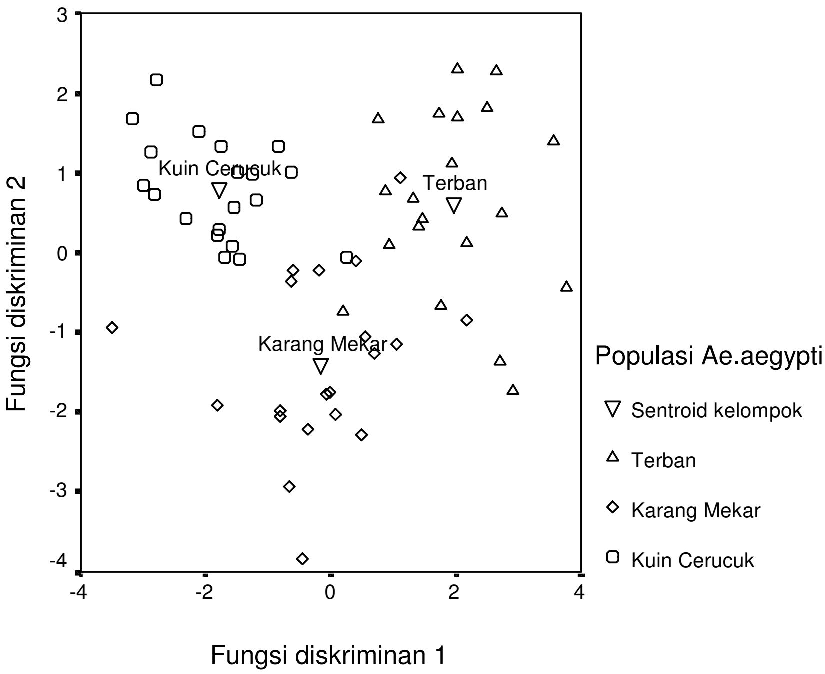

Figure 2. Distributions of female *Aedes aegypti* from Banjarmasin and Yogyakarta in discriminant function space 1 and 2 based on seven KG-standardized distances on wing venation.

OI-Standardized distances

Ninety seven normal-selected distances were used in discriminant analysis with 'F to enter' = 3.84. Among them, seven were selected as discriminator variables (Table 3). Multivariate ANOVA using the seven discriminators showed that the three populations of *Aedes aegypti* were highly significantly different from each other.

DF 1 could explain 67.9%, while DF 2 could explain 32.1%, of total variance (Table 3). DF 1 was of major role in discriminating KC from TB and KM, while DF 2 helped in distinguishing TB from KM (Figure 3).

Classification equation and criteria resulting from the discriminant analysis could be used to identify specimens to their original populations with 85% degree of success (Table 4). Discriminant function plot (Figure 3) showed that specimens are also clustered according to their populations.

Table 3. Summary of canonical discriminant function based on OI-standardized distances for wings of female *Aedes aegypti* from Banjarmasin and Yogyakarta

	Discriminant function	
	1	2
% of variance	67,9	32,1
Canonical correlation	0,827	0,711
Unstandardized discriminant function coefficient:		
BS	9,837	25,509
CN	8,420	68,457
DE	52,991	9,209
FO	-21,545	-33,168
LS	-86,961	28,442
MP	39,774	-28,002
NQ	30,610	-7,647
Constant	1,732	6,559
Functions at group centroid:		
Kuin Cerucuk	1,825	0,603
Karang Mekar	-1,152	-1,388
Terban	-1,672	0,785

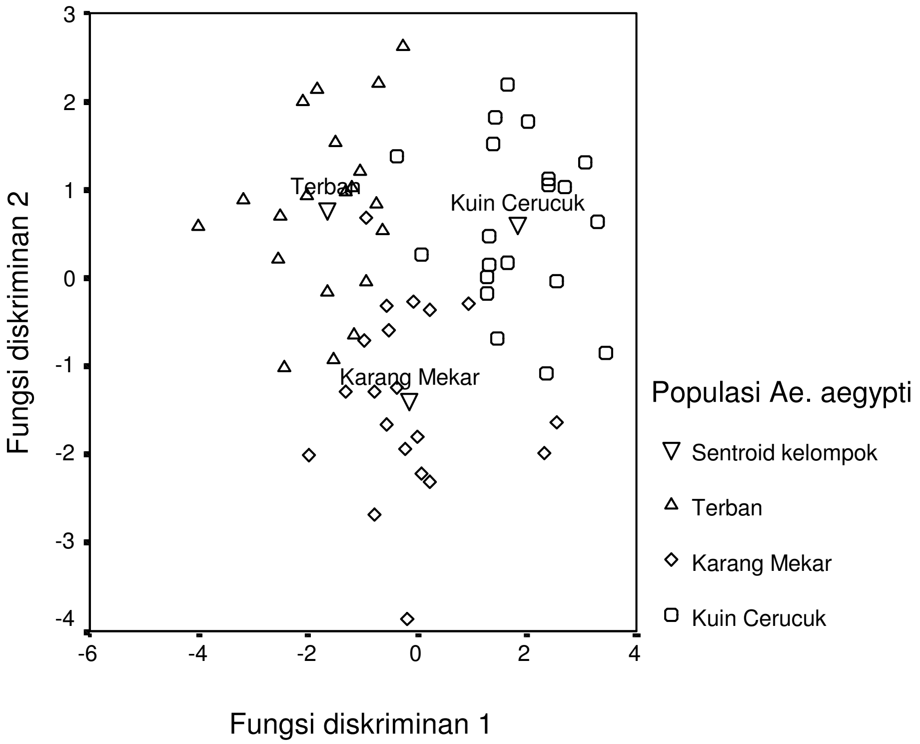

Figure 3. Distributions of female *Aedes aegypti* from Banjarmasin and Yogyakarta in discriminant function space 1 and 2 based on seven OI-standardized distances on wing venation.

Table 4. Identification of wing specimens of female *Aedes aegypti* based on seven OI-standardized distances on venation

Original population	Predicted population			Total
	Kuin Cerucuk	Karang Mekar	Terban	
Kuin Cerucuk	18	1	1	20
Karang Mekar	3	15	2	20
Terban	0	2	18	20

Correct identification = 85%

DISCUSSION

This study focused on discrimination of populations of *Aedes aegypti* using wing measurements. This was based on considerations that mosquito wings offer three advantages. Firstly, wings are among the most durable parts of mosquito body. Many times when pests, e.g. mites, attack specimens of mosquitoes, wings are the only parts left undisturbed. Secondly, wings are more easily observed than other body parts. Finally, wings comprise many quantitative characters. At least, there are 18 branching and terminal points to produce 153 interpoint distances.

Because wing measurements may be influenced by factors, such as larval nutrition (Strickman & Kittayapong, 2003), larval population density (Renshaw *et al.,* 1994; Mercer, 1999), and temperature (Lanciani & Le, 1995; Reisen, 1995), some standardization must be applied. In this study, that was achieved by comparing every distances of wing venation with an 'internal standard', e.g. the wing length. However, wing length used in the present study was not that of Siegel *et al.* (1992): the distance between base of alular notch and the most distal part of wing membrane. It was based on the fact that many times alular notch ruptured during wing preparation. Two other, more robust, easy to observe, lengths were therefore used.

It was demonstrated that *Aedes aegypti* from Banjarmasin and Yogyakarta, previously could only be distinguished using cuticular hydrocarbon composition (Gafur, 2004), also showed differences in morphological characters of wing venation. Quantitative characters in terms of interpoint distances in wing venation could be useful in identification or discrimination of female *Aedes aegypti* from populations under study. Two combinations of interpoint distances might be used: (1) seven KG-standardized distances, namely CN, DP, LM, MS, NQ, BE, and BN; or (2) seven OI-standardized distances, namely BS, CN, DE, FO, LS, MP, and NQ.

Because wing characters are genetically determined (Uppal *et al.*, 1976; Tadano, 1978, 1979), morphological differences in wing characters suggested that there has been genetic divergence among populations *Aedes aegypti* under study.

This finding supported cuticular hydrocarbon analysis (Gafur 2004) and further confirmed genetic divergence of this species in different parts of Indonesia.

Difference in ratios of interpoint distances in wing venation was concordant with a general fact that in almost all organisms there has been changes in ratios of certain body parts during evolutionary changes (Sokal and Rohlf, 1995). In *Aedes aegypti* the ratios of interpoint distances of the wing might be among the first to change during evolutionary divergence of this species. Mating behaviour of this species involves a mechanical communication through wing beat. Females are attracted to 'swarming' males, while wing beat of a female stimulates a male to mate her (Roth, 1948 cited by Matthews & Matthews, 1978). Recognition of a conspecific mate must be well-performed on this stage, and sounds from wing beat must be species specific. Wing venation may play important role in the specificity.

In addition to mating behaviour, wing measurements also reflect reproductive and vectorial capacity of mosquitoes. Body size, as indicated by wing length, has been demonstrated to positively related to higher fecundity (Blackmore & Lord, 2000; Armbruster & Hutchinson, 2002), longevity (Renshaw, 1994; Ameneshewa & Service, 1996), host-finding success (Renshaw et al., 1994), bloodfeeding success (Nasci, 1986; Lyimo & Takken, 1993), and vectorial capacity (Ameneshewa & Service, 1996). There is a possibility that the populations of *Aedes aegypti* in the present study has also diverged in those characteristics, and that needs to be further studied. If so, knowledge of these properties through wing measurements may be useful in determination of urgency and method of control measures against *Aedes aegypti* populations.

The present approach of discrimination based on interpoint distances of wing venation has several advantages. It can be applied with either left or right wing of an individual mosquito. Therefore, identification can always be made even for specimens with only one wing left. Furthermore, this technique is relatively easy and simple. We need only simple microscope with 40x enlargement, beside simple and easy to find equipments for wing slide preparation. All these may allow field observations by one with mid-level of microscopic skill. Lastly, the method is inexpensive due to simple equipments needed, and can be applied in laboratories with limited budget and facilities.

However, the technique so far can only be applied with removed wings. This may hinder applications on type specimens of taxonomic collections. Further studies for improvements are needed for applications on intact wings. In addition, success rate of discrimination is less than 100%. This should be understandable due to probability nature of statistical methods. More samples will produce more reliable discriminant functions which, in turn, result in higher success rate.

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