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The Use of Female Wing Measurements for Discrimination of *Aedes aegypti* (L.) (Diptera: Culicidae) Populations from South Kalimantan

ABDUL GAFUR[1*], AULIA AJIZAH[2]

[1]*Study Program of Biology, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Jalan A. Yani Km. 36, Banjarbaru 70700, Indonesia*
[2]*Study Program of Biology Education, Faculty of Teachers Tranning and Education, Lambung Mangkurat University, Jalan Brigjen H. Hasan Basry, Banjarmasin 70123, Indonesia*

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Populations of *Aedes aegypti* in South Kalimantan, which have been discriminated by their cuticular components, were further studied in search for differences in their wing morphometry. Female mosquitoes were collected from five towns in the province of South Kalimantan, and Cartesian coordinates of terminal and branching points of individual wing were determined. Relative interpoint Euclidean distances were used as variables (characters) in statistical analyses. One-way ANOVA found significantly several different characters (P < 0.01). Stepwise discriminant analysis using these characters selected five discriminators which, by cross validation, could identify female *A. aegypti* from Barabai and Marabahan with 75 and 77.8% of success rate, respectively. On average, 57.7% of wing specimens were successfully allocated to their original populations. The study revealed differences in wing measurements among populations of *A. aegypti* in South Kalimantan and confirmed genetic divergence of the species in the province.

Key words: *Aedes aegypti*, wing morphometry, discriminant analysis, identification, discrimination

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INTRODUCTION

A number of studies on *Aedes aegypti* have been conducted, particularly on their vectorial capacity and on control measures against them. However, only few biosystematics studies on this species have been conducted so far, unexceptionally in Indonesia. Data on the intraspecific variation of this species in Indonesia is scarcely available. In the mean time, their nation-wide distribution in Indonesia (Djakaria 1988), has possibly allowed genetic variation within the range of the species across the country.

Gafur (2004a) detected the genetic differences between populations of *A. aegypti* from Banjarmasin and Yogyakarta based on gas chromatographic analyses of their cuticular hydrocarbons. However, despite the high sensitivity of gas chromatography –in particular the Gas Chromatography Mass Spectrometry (GCMS)– is relatively expensive and is not available in most Indonesian laboratories. Due to the wing measurements in separating strains of several mosquito species (Nasci 1990; Siegel *et al.* 1994) and other insects (Yu *et al.* 1992; Weeks *et al.* 1999), and the advantages availabllity and the relatively cheap equipments, Gafur (2004b) applied morphometric analysis of *A. aegypti*. It was revealed that the GC-separated Banjarmasin and Yogyakarta populations could also be discriminated by measurements of wing venation.

In order to examine further the sensitivity and usefulness of the wing morphometric analysis, as well as its possible development, in the present study the analysis was applied to discriminate the populations of *A. aegypti* from South Kalimantan. In addition, the present study would also to confirm the genetic divergence of the species within the province by Gafur (2004a,b). Since previous studies have demonstrated differences in wing venation between sexes (Eritja 1996; Gafur 2005), and the fact that the female that directly cause human health problems, the present study focused on differences among the females *A. aegypti*.

MATERIALS AND METHODS

Mosquito Collection. Mosquitoes were collected from five towns in South Kalimantan (Figure 1). Adult mosquitoes were captured with aspirator from indoor resting sites, mainly hanging clothes, as well as by collecting larvae from water containers, e.g. concrete tanks, drums, clay jars, buckets, and aquariums, all during daytime. Larvae were reared and fed with goldfish pellet in 200 ml glass jars. Adult mosquitoes were kept unfed in paper cups to death. Sexes of adults were screened by morphological characters of antennae, mouthparts, and external genitalia. Only female mosquitoes were used. Seventy eight adult female mosquitoes were collected, consisting of those from Banjarmasin (42), Marabahan (9), Barabai (8), Pelaihari (10), and Kotabaru (9).

Wing Measurements. Both left and right wings of individual mosquito were removed and placed between microscopic object and cover glasses. The wing were freed from scales by carefully sliding the cover glass over the object glass. In total, 156 wing slides were made. However, only half was examined since only one wing was used from each

*Corresponding author. Phone/Fax: +62-511-4773868,
E-mail: agafur@fmipa.unlam.ac.id

Figure 1. The map of South Kalimantan showing five towns which populations of *A. aegypti* were sampled.

individual mosquito, in consideration of wing asymmetry in mosquitoes (Mpho *et al.* 2000). Random selection between left and right wings was performed based on random numbers generated by Microsoft Excel 2003. This would ensure the results of this study be applicable to both wings.

Wings were observed under a compound microscope (ChindoN) with an eyepiece connected to PC Vision 300 USB digital camera (MediaForte 1998) directly sending images to a personal computer. The magnification was the highest one that allowed full image of wing to be observed in the field of microscope view. Most of the time 20x magnification was used, however, for some wing specimens the highest magnification was 40x. Digital images were captured and saved in BMP format by TrueView software (MediaForte 1999). The image files were then imported by Scion Image 3b software (Scion 1998) which determined the Cartesian coordinates of terminal and branching points on wing venation (Figure 2) as described by Rattanarithikul and Panthusiri (1994).

Twenty two points (a – v) were observed (Figure 2), but only 21 (a – u) were used in determination of interpoint distances. Point *v* was only used as wing length (v – g).

Based on the Cartesian coordinates of points, interpoint Euclidean distances were calculated using the following formula:

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

D: Euclidean distance between point I and II; x, y: Cartesian coordinate of point I on x- and y-axes; p, q: Cartesian coordinate of point II on x- and y-axes.

One distance was selected as a standard and the others were divided by the standard, resulting in relative distances. The procedure was then repeated using another distance as the standard, and so forth until all distances were once used as standard. Calculations were made using Microsoft Excel© 2003. The relative distances constituted the data for statistical analyses and were preferable to absolute values as different environmental condition during larval stages might affect

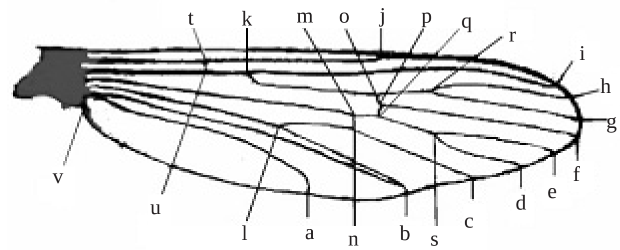

Figure 2. Branching and terminal points on the wing of *A. aegypti*. Anatomical terms follows Rattanarithikul and Panthusiri (1994); v-g: wing length.

absolute distances (Siegel *et al.* 1994; Lanciani & Le 1995; Mercer 1999; Strickman & Kittayapong 2003). Relative distances were also more robust to differences in magnification.

Statistical Analysis. From 211 absolute distances, (210 interpoint distances plus 1 wing length) 22,155 relative distances were obtained as the characters of each wing. Statistical analyses were performed: (1) to find character(s) showing different values among populations, and (2) to examine the usefulness of the difference(s), if any, in the allocation of wing specimens to their original populations.

Univariate analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) were used in step (1). In step (2), stepwise discriminant analysis using Mahalanobis' distance was performed on characters showing highly significant difference ($P < 0.01$) and forming homogeneous subsets in DMRT. Only selected characters were used in discriminant analysis because using all characters would require much better computer facility.

Discriminant analysis produces discriminant functions as linear combinations of characters that could discriminate groups under study (Dillon & Goldstein 1984). Stepwise discriminant analysis apply a selection procedure – e.g. Mahalanobis' distance – on characters to select the best discriminating characters. In the present study score of each specimen based on the discriminant functions was used to allocate the specimen to its original population. Percentage of correct allocation reflected the reliability of the discriminant functions. Bias in the estimation of correct allocations was reduced by cross validation. Here one specimen was excluded from analysis, and the resulting discriminant functions were used to allocate the specimen; the procedure was then repeated using another specimens, and so forth until all specimens were finally allocated. The Mahalanobis' distances were also used in a cluster analysis applying Average Linkage (Between Groups) algorithm. SPSS v.7.0 (Norusis 1996) was used in the statistical analyses.

RESULTS

Seventy eight female wings were examined. Univariate ANOVA found 1,193 characters that showed highly significant differences among populations ($P < 0.01$, Table 1). DMRT ($\alpha = 0.01$) showed that 131 of them formed homogenous subset of Barabai, and were considered as distinguishing characters of Barabai population; 26 characters formed homogenous subset of Marabahan; two characters could not distinguish Banjarmasin from Kotabaru, but separated both from all other populations; one character combined Pelaihari and Marabahan in one subset, but separated the two from other populations. These characters were then used in a discriminant analysis.

Stepwise discriminant analysis produced four discriminant functions and found five discriminators (Table 2). Based on the functions, by cross validation, allocation of wing specimens to their original populations could be achieved with 57.7% average success rate (Table 3). Table 4 represents a matrix of Mahalanobis' distances of population pairwise. Banjarmasin, Kotabaru, and Pelaihari showed higher similarities to each other as reflected by small values of Mahalanobis' distance. The greatest difference was found between Banjarmasin and Barabai as well as between Banjarmasin and Marabahan. A dendrogram based on Average Linkage clustering using the distance matrix is displayed as Figure 3.

DISCUSSION

Our results revealed differences in wing measurements among populations of *A. aegypti* in South Kalimantan. On average, only 57.7% of wing specimens could be correctly allocated to their original populations. However, regarding individual populations, 77.8% specimens from Marabahan and 75% from Barabai could be correctly allocated. With these degree of accuracy, based on Anyanwu *et al.* (1993) who claimed their highly successful of 75% accuracy, our identification of specimens from the two populations were also considered reasonably successful. The low average success rate (57.7%) was due to lower rates of the other three populations.

Since wing venation is genetically determined (Uppal *et al.* 1976; Tadano 1979), differences in wing venation among populations of *A. aegypti* from South Kalimantan indicates genetic divergence among them. In this case, the divergence is reflected by differences in the ratio of interpoint distances on the wing venation. This is in line with Sokal and Rohlf (1995) that during the course of evolution, changes in the ratios of certain parts of the body probably occurred.

The values of Mahalanobis' distance and the corresponding dendrogram (Figure 3) suggested the degree of separation among populations of *A. aegypti* in South Kalimantan. However, the relative degrees of divergence were not consistent with geographic distances. For example, according to the wing measurements the highest separation was between Banjarmasin and Barabai and the lowest was between Banjarmasin and Kotabaru. By contrast, the longest spatial distance is between Banjarmasin and Kotabaru (Figure 1) while Banjarmasin and Barabai is less spatially separated. The spatial distance between Banjarmasin and Marabahan is the shortest; nonetheless the Mahalanobis' distance based on wing measurements between the two was next to the highest.

Table 1. Summary of anova results on relative distances on wing venation of *A. aegypti*

Character	Quantity
Total examined	22,155
Highly significantly different (P < 0.01)	1,193
Forming homogenous subset in DMRT (P < 0.01) for:	
Barabai	131
Marabahan	26
Banjarmasin + Kotabaru	2
Marabahan + Pelaihari	1

Table 2. Discriminant function coefficients and five discriminator characters resulted from stepwise discriminant analysis on relative distances on wing venation of *A. aegypti*

Characters	Discriminant functions			
	1	2	3	4
aq/vg	27.889	4.278	-29.512	0.843
mn/ku	1.760	6.389	0.828	-5.637
mp/gs	10.468	-25.368	-3.694	15.216
pr/ku	1.270	1.519	1.937	2.540
pu/hr	5.067	1.762	4.138	-3.811
Constant	-17.374	-2.002	-2.385	0.718

Each character is a ratio between two interpoint distances. VG: wing length. See Figure 2 for points represented by letters.

Table 4. F values indicating Mahalanobis' distances between five populations of *A. aegypti* from South Kalimantan based on five discriminator characters on wing venation

	Marabahan	Barabai	Pelaihari	Kotabaru
Banjarmasin	11.021	11.979	3.566	2.401
Marabahan		7.856	4.522	9.539
Barabai			9.584	6.758
Pelaihari				4.194

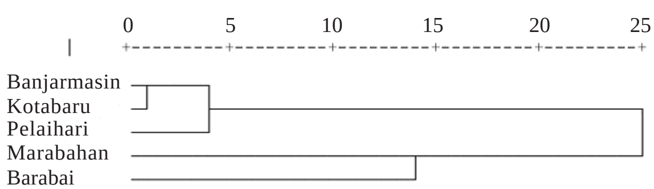

Figure 3. A dendrogram based on Mahalanobis' distances among five populations of *A. aegypti* from South Kalimantan (Table 4). The distances have been rescaled to 0-25.

Table 3. Summary of allocation of wing specimens of *A. aegypti* to their original populations based on discriminant function scores

Original population	Predicted population				
	Banjarmasin	Marabahan	Barabai	Pelaihari	Kotabaru
Banjarmasin	21 (50.0%)	2 (4.8%)	3 (7.1%)	9 (21.4%)	7 (16.7%)
Marabahan	1 (11.1%)	7 (77.8%)	0 (0%)	1 (11.1%)	0 (0%)
Barabai	1 (12.5%)	1 (12.5%)	6 (75.0%)	0 (0%)	0 (0%)
Pelaihari	3 (30.0%)	1 (10%)	0 (0%)	5 (50.0%)	1 (10.0%)
Kotabaru	2 (22.2%)	0 (0%)	0 (0%)	1 (11.1%)	6 (66.7%)

Numbers indicate quantity and percentage (in brackets) of specimens allocated to a predicted population. Average correct allocation = 57.7%.

The inconsistencies were probably due to the preselection of characters applied in the present study. Due to computer memory limitation, discriminant analysis could only be performed with a limited number of characters. Only those characters showing highly significant difference (P < 0.01) in ANOVA and forming homogenous subset in DMRT were used in the analysis. Consequently, selection of discriminator characters in the discriminant analysis was only among the preselected characters. Despite that the preselection procedure would expectedly maximize separation, there was a possibility that an insignificantly different character become a good discriminator in combination with other character(s) (Milligan *et al.* 1986). Further study in search for better on preselection procedure is needed. A better computer, with more memory and higher speed will allow the use of more characters in multivariate analysis and reduce the necessity of character preselection, so that more diagnostic combination of characters will hopefully be found and higher success rate in identification could be achieved.

The difference in wing venation among populations of *A. aegypti* in South Kalimantan suggested that wings of female could be useful for discrimination or identification of populations, as it has been demonstrated in the separation between *A. aegypti* from Banjarmasin and Yogyakarta (Gafur 2004a,b). This study contributed new informationn *A. aegypti* in South Kalimantan. Nevertheless, since only populations from Barabai and Marabahan that could be successfully identified, further studies are required in search for characters or character combinations that will perform better discrimination of all *A. aegypti* populations in South Kalimantan and even in other localities.

Genetic divergence among populations of *A. aegypti* by means of wing measurements may bring important implication. In some species of mosquitoes it has been found that body size in terms of wing length positively correlated with fecundity (Blackmore & Lord 2000; Armbruster & Hutchinson 2002), life span (Renshaw *et al.* 1994; Ameneshewa & Service 1996), host-seeking (Renshaw *et al.* 1994) and blood-sucking (Nasci 1986; Lyimo & Takken 1993) success, and vectorial capacity (Ameneshewa & Service 1996). It is possible that among populations of *A. aegypti* in South Kalimantan revealed changes in one or more aforementioned reproductive and vectorial traits may hinder control measures against the mosquitoes. As a result, these characteristics, will put constraint on dengue hemorrhagic fever eradication program. The possibility may extend to other parts of Indonesia where populations of *A. aegypti* have probably developed genetic divergence relating to the above traits. Further studies will be wide open.

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