Morphometric studies on *Culex quinquefasciatus* and *Mansonia africana* (Diptera: Culicidae) in Abeokuta, south-western Nigeria

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Abstract: Some of the important mosquito species are generally sympatric in terms of their geographical distributions, and are difficult to identify based solely on examination of adult females using the available morphological keys. In this study, adult mosquitoes were collected indoors in Abeokuta in south-western Nigeria between August 2005 and July 2006 using Center for Disease Control light traps. The objective was to investigate possible morphological differences in populations of *Culex quinquefasciatus* and *Mansonia africana*. Six morphological characters namely, wing length, antennal length, proboscis length, foreleg length, mid leg length and hind leg length were measured in the two species. A total of 868 *Cx quinquefasciatus* and 962 *M. africana* were collected during the study period. The mean length was observed to be higher in most characters during the wet season than the dry season but the variation was not statistically significant ($P > 0.005$). In *M. africana*, the antennal length, proboscis length, foreleg length and midleg length showed one peak each. The wing length exhibited three peaks while hind leg length showed two peaks. For *Cx quinquefasciatus*, the antennal length, proboscis length, foreleg length, mid leg length and hind leg length indicated one peak. The wing length however showed three peaks. Each of the peaks observed in wing length and hind leg length of *M. africana* and wing length of *Cx quinquefasciatus* was assumed to be a specific population. The coefficient of differences (CDs) for each population indicated the presence of three populations in *M. africana* (CD > 1.28). However, the hind leg length showed that the two populations were the same (CD< 1.28). Three populations were also obtained from CD for wing leg length of *Cx quinquefasciatus* (CD>1.28). Other characters indicated the presence of only one population. The results therefore underscore the need to investigate the status of the two species in relation to species complex.

Keywords: Morphometric analysis, *Culex quinquefasciatus, Mansonia africana*, Nigeria

Introduction

Some of the mosquito vectors of public health importance are generally sympatric in terms of their geographical distributions. Such vectors generally present high degree of morphological similarity at adult stage and many times difficult to identify based solely on examination of adult females using the available morphological keys (Calle *et al.,* 2002). This problem is common with *Anopheles gambiae* and *An. funestus* species complexes. In epidemiological studies (on disease transmission or evaluation of control measures) it is necessary to identify the species of adult females which are found near humans. Traditionally, morphometric studies have contributed substantially in resolving taxonomic problems in mosquito identification (Delgado & Rubio-Palis, 1993; Petraca *et al*., 1998; Calle *et al*., 2002).

Culex quinquefasciatus and *Mansonia africana* are among the most abundant mosquitoes in Africa. The two species are also vectors of a number of human and animal pathogens and parasites including viruses and nematodes. *Cx quinquefasciatus* is one of the major vectors of *Wuchereria bancrofti* infection in the world (White, 1971; Subra, 1981) while *M. africana* is known to transmit Rift Valley fever (Meegan & Bailey, 1988; Fontenille *et al*., 1998), and yellow fever.

Observations had long been made on the existence of species complex in many dipteran insects (Gillett, 1972). These observations were based on inconsistent behavioural habits of many species in different geographical zones. Most importantly, member of the family Culicidae (mosquitoes) have been known to consist of many closely related siblings (Lehane, 1991). Most of these siblings had however been separated either through molecular characterization or multivariate analysis (morphometrics) (Adesiyan *et al.,* 1998). The use of morphometric technique relies mainly on variation in size and shape of the insects (Daly, 1985).

In tropical region of the world, *Culex pipiens* complex, a group in which *Cx quinquefasciatus* belongs, has been a subject of many taxonomic and genetic studies after the discovery of closely related interbreed siblings in the complex (Knight, 1978; Pryor & Daly,

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1991). Though, attempts had been made to separate siblings of this complex by measuring the distance between the tip of the genital arm to its intersection with the dorsal (Sundararaman, 1949; Barr & Kartman, 1951; Pryor & Daly, 1991), there is paucity of information on the use of other morphological characters in discriminating siblings of this species. Apart from the differences in ecotypes which could render the result from one geographical zone inapplicable in others, morphometric techniques have not been widely applied on *Cx quinquefasciatus* in Africa. However, no attempts have been made in investigating *M. africana* species status. It is against this background that the present work was designed to investigate the possible morphological differences in *Cx quinquefasciatus* and *M. africana* in Abeokuta, south-western Nigeria.

Materials and Methods

Study area and sampling of adult mosquitoes

The research was conducted in Abeokuta Metropolis located on approximately latitude $7^{o}10N$ and longitude $3^{o}21E$ in the transitional zone between tropical rainforest and derived savannah zone in the south-western, Nigeria. Abeokuta usually experiences two seasons; the dry season (November to March) and the wet season (April to October). Five stratified locations were selected for the study namely Ago-Ika, Ijaye, Kugba, Ibara and Obantoko.

Adult mosquitoes were collected in three randomly selected houses in each of the study locations once a week using Center for Disease Control (CDC) light traps (Model 512, J.W. Hock Ltd, Gainesville, Florida, USA), between August 2005 and July, 2006. Each trap was suspended from the roof about 1.8m above the floor and 0.5m from a bed occupied by an adult sleeper. The trap was operated with 6.0 volt rechargeable battery every week. The sleeper in each room was instructed to switch on the trap at 20:00 hr and switch it off at 5:00 hr after the neck of the collection bag has been properly tied. All mosquitoes collected in the traps were removed and kept in labelled EDTA bottles for laboratory analysis. *Cx quinquefasciatus* and *M. africana* were sorted out of the other mosquitoes using keys described by Gillett (1972). Six characters, antennal length, proboscis length, wing length, foreleg length, mid leg length and hind leg length were carefully detached and measured in each mosquito using calibrated microscope.

Statistical analysis

Monthly means of all measured characters in each of the two species were subjected to regression analysis. Co-efficient of difference (CD) was calculated for each character having at least two peaks after the frequency distribution of the grouped data has been plotted. The CD was calculated according to Mayr (1969a)

$$CD = \frac{M_b - M_a}{SD_a + SD_b}$$

Where CD = Co-efficient of difference
M_b = Population with the large mean
M_a = Population with the smaller mean
SD_b = Standard deviation of the population with the larger mean
SD_a = Standard deviation of the population with the smaller mean.

Results

A total of 868 *Cx quinquefasciatus* and 962 *M. africana* were collected during the study period. The results of morphometric analysis on *Cx quinquefasciatus* and *M. africana* revealed that there were variations in the characters. The mean length was observed to be higher in most characters during the wet season than the dry season but the variation was not statistically significant ($P > 0.005$) (Table 1).

Table 1: Mean length of the characters in *M. africana* and *Cx quinquefasciatus* in Abeokuta

Characters (mm)	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Mansonia africana												
Antennal length	1.14	1.13	1.13	1.13	1.15	1.12	1.11	1.12	1.15	1.14	1.16	1.13
Wing length	2.95	2.91	2.98	2.9	2.84	2.79	2.84	2.79	2.9	2.88	2.84	2.85
Proboscis length	1.36	1.37	1.36	1.35	1.34	1.36	1.32	1.37	1.34	1.3	1.34	1.41
Fore leg length	5.14	5.19	5.19	5.24	5.25	5.13	5.08	5.12	5.16	5.16	5.19	5.22
Mid leg length	6.4	6.41	6.38	6.36	6.4	6.35	6.33	6.33	6.33	6.28	6.35	6.4
Hind leg length	7.85	7.92	7.91	7.89	7.87	7.82	7.8	7.77	7.85	7.82	7.71	7.85
Culex quinquefasciatus												
Antennal length	1.33	1.29	1.33	1.25	1.26	1.25	1.32	1.32	1.34	1.36	1.3	1.34
Wing length	3.08	3.08	3.06	2.95	2.85	2.89	3.01	3.02	3.05	3.07	2.84	2.97
Proboscis length	1.64	1.73	1.78	1.65	1.64	1.63	1.66	1.66	1.62	1.65	1.6	1.65
Fore leg length	5.31	5.32	5.33	5.31	5.21	5.27	5.28	5.3	5.32	5.36	5.35	5.33
Mid leg length	6.83	6.83	6.85	6.76	6.63	6.6	6.79	6.84	6.65	6.74	6.67	6.7
Hind leg length	7.86	7.93	7.94	7.9	7.92	7.84	7.85	7.83	7.89	7.87	7.82	7.87

In *M. africana*, the antennal length, proboscis length, foreleg length and midleg length showed one peak each. The wing length exhibited three peaks while hind leg length showed two peaks. For *Cx quinquefasciatus*, the antennal length, proboscis length, foreleg length, mid leg length and hind leg length indicated one peak. The wing length however showed three peaks. Each of the peaks observed in wing length and hind leg length of *M. africana* and wing length of *Cx. quinquefasciatus* was assumed to be a specific population, and in order to determine the uniqueness of each of the populations, coefficient of differences (CDs) were calculated for each population. The results obtained from CD also indicate the presence of three populations in *M. africana* (CD > 1.28) but the hind leg length showed that two populations were the same (CD< 1.28) (Table 2). Three populations were also obtained from CD for wing leg length of *Cx quinquefasciatus* (CD>1.28) (Table 2).

Table 2: Morphometric analysis of the populations in (a) *Mansonia africana* and (b) *Culex quinquefasciatus* in the study locations in Abeokuta

Character	Population	Mean (mm)	Standard Deviation	Coefficient of Difference
a) Mansonia Africana				
Wing length	A	2.76	0.04	AC=3.46
	B	2.84	0.013	AB=1.63
	C	2.93	0.009	BC=4.09
Hind leg	A	7.83	0.01	AB=0.67
	B	7.95	0.07	
b) Culex quinquefasciatus				
Wing length	A	2.71	0.120	AC=2.50
	B	2.91	0.013	AB=1.50
	C	3.04	0.011	BC=5.41

CD value >1.28 is significant

Discussion

Morphological variation was observed in the measured characters in *Cx quinquefasciatus* and *M. africana* which was not season specific. The variation may be associated with the physiological conditions of individual or may be genetically controlled. According to Mayr (1969b), most of the morphological variation is clinal and thus, the variation observed in some characters as revealed by CD may be clinal.

The indication of three populations by wing length of *Cx quinquefasciatus* may probably be possible. Until recently, *Cx pipiens pipiens*, *Cx pipiens quinquefasciatus* and *Cx. pipiens fatigans* have been regarded as subspecies of *Cx pipiens* complex and they have been taken as three taxonomically distinct populations (Jakob *et al.,* 1980; Pryor & Daly, 1991). *Cx pipiens* complex were later observed to consist of hybrid and intermediate forms of *Cx. pipiens pipiens* and *Cx pipiens quinquefasciatus* as reported in various morphometric studies, genetic and molecular assays using polymerase chain reaction (Cornell *et al*. 2003; Smith & Fonseca, 2004). The wing length could also be another variation in their morphological composition and may be used in sorting members of the complex.

The wing length of *M. africana* indicated three probable populations but the hind leg length showed that two populations are the same. Though, the complex of this species has not been reported in any part of the world, its irregular biting behaviour (anthropophilic and zoophilic) in different geographical zones (Gillett, 1972; Amusan, 2004) underscores the need to investigate its species status.

Further studies are therefore recommended on molecular and morphometric analysis of these two species, most importantly on *M. africana*, so as to shed light on its species status.

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References

Adesiyan, S.A, Jerris, M.A. & Hollander, J.D. (1998) Morphological variation in *Glossina pallidipes* Austen (Diptera: Glossinidae) over its geographical range. *Nigerian Journal of Entomology* 15, 122-135.

Amusan, A.A.S. (2004) *Distribution of mosquitoes* (*Diptera:Culicidae*) *and disease transmission patterns in Ogun State*., Ph D Thesis, University of Agriculture, Abeokuta, Ogun State pp 336.

Barr, A.R & Kartman, L. (1951) Biometrical notes on the hybridization of *Culex pipiens L.* and *Culex quinquefasciatus* Say. *Journal of Parasitology* 37, 418 -420.

Calle, D.A., Quinones, M.L., Erazo, H.F. & Jaramillo, N. (2002) Morphometric discrimination of females of five species of Anopheles of the subgenus Nyssorhynchus from southern and northwest Colombia. *Memórias do Institudo Oswaldo Cruz* 97, 1191-1195.

Cornell, A.J., Mcabee, R., Rasgon, J., Stanich, M., Scott,T. & Coetzee, M. (2003) Difference in extent of genetic introgression between sympatric *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* in California and South Africa *Journal of Medical Entomology* 40, 36-57.

Daly, H.V. (1985) Insect morphometrics. *Annual Review of Zoology* 30, 415 -438.

Delgado, N. & Rubio-Palis, Y. (1993) Identification of *Anopheles (Nyssorhyncus)* (Diptera: Culicidae) occurring in Western Venezuela. *Mosquito Systematics* 25, 222-230

Fontenille, D., Traore-Laminzana, M., Diallo, M., Thonnon, J., Digoutte, J.P. & Zeller, H.G. (1998) New vectors of Rift Valley Fever in West Africa. *Emerging Infectious Diseases* 4, 289-293.

Gillett, J.D. (1972) *Common African Mosquitoes and their Medical Importance*. William Heinemann Medical Books Ltd; London p 236.

Jakob, W.L., Francy, B., Mullinex, J. & Taylor, S.A (1980) Further studies on the *Culex pipiens* complex in Nemplis, Tennessee. *Mosquito Systematics* 12, 371 -376.

Knight, K.L. (1978) *Supplement to Catalogue of the Mosquitoes of the World* (*Diptera: Culicidae*) Thomas Say Foundation, supplement to volume V1

Lehane, M.J. (1991) *Biology of Blood Sucking Insects*. Herpercollins Academic London, 288 pp.

Mayr, E. (1969a) *Principle of Systematic Zoology.* MC Graw-Hill, New York 428 pp.

Mayr, E. (1969b) *Population, Species and Evolution* Harvard University Press, Cambridge 453pp.

Meegan, J.M. & Bailey, C.H. (1988) Rift Valley Fever, Monath, T.P. ed. *The Arboviruses: Epidemiology and Ecology*, Volume 4. Boca Raton, FL, CRC Press, 51-76.

Petrarca, V., Sabatelli, G., Toure, Y.T., Dideco, M.A. (1998) Morphometric multivariate analysis in *Anopheles gambiae* s.s. (Diptera: Culicidae). *Journal of Medical Entomology* 35, 16-25.

Pryor, S.C. & Daly, J. (1991) Temporal variation in morphological and genetic characteristics within a hybrid population of *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology* 25, 481-486.

Smith, J.L. & Forseca, D.M. (2004) Rapid assays for identification of members of the *Culex* (*Culex*) *pipiens* complex, their hybrid and other sibling species (Diptera: Culicidae). *America Journal of Tropical Medicine and Hygiene* 70, 339-345.

Subra, R. (1981) Biology and control of *Culex pipiens quinquefasciatus*, Say, 1823 (Diptera: Culicidae) with special reference to Africa. *Insect Science and its Application* 4, 319-338.

Sundararaman, S. (1949) Biometrical studies on integration in the genitalia of certain populations of *Culex pipiens* and *Culex quinquefasciatus* in the United States America. *Journal of Tropical Medicine and Hygiene* 50, 307-314.

White, G.B. (1981) Studies on transmission of bancroftian filariasis in north-eastern Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 65, 819-829.