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**Annual Report**

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# Genetic Differentiation in the Yellow Fever Vector *Aedes simpsoni* complex.


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**Executive summary:**

*Aedes simpsoni* complex is widely distributed in Africa South of the Sahara and at least one member of the complex plays a leading role in the sylvatic transmission of yellow fever virus on the continent. Of the members, there is only one which can be readily differentiated from the rest. This component member is found only in South Africa and is technically referred to as *Ae. simpsoni* sensu stricto. At least two other species, *Ae. lilii* and *Ae. bromeliae* are found in the rest of Africa. *Ae. lilii* is non-human-biting and *Ae. bromeliae* is human-biting. Within the two species are other differentiating components. Except *Ae. simpsoni* sensu stricto of South Africa, all the others are not morphologically separable. The purpose of this study, is to find a character or a tool which can differentiate each component member.

It was reported last year that egg size from different populations could only be quantitatively differentiated. Further extensive morphological measurements of the hind tibia, mid-tibia, mid-tarsomere, front femur and wing size between Bwamba and Entebbe populations showed significant differences between the two populations. All these differences, however are polymorphic and cannot be used to differentiate individuals between species.

It has been reported previously that an enzyme Supra Oxidase dismutase at locus 2 differentiates between the Human-biting populations and the non-human-biting populations. The alleles for human biting are fast and those for the non-human biting are slow. At a molecular level, sequence divergence has been demonstrated: The sequence divergence in the ribosomal ITS clearly separates Bwamba and Entebbe populations into distinct clades, thus confirming the electrphoretic findings that the two behavioural forms are distinct species. However, within these components, are other differentiating components on the continent such as the Nigeria population which is quite different from the Entebbe one although both are non-human biting. Further studies using sensitive molecular techniques are required to sort out all the components. Each of these components need to be tested for susceptibility to yellow fever virus so as to understand the epidemiology of the disease in Africa.

A) **Research objectives:**

i- To find morphological markers for the different species in the complex

ii) To find biochemical markers using electrophoretic and molecular techniques.

iii) To determine phylegenetic relationships of the different species within the complex.

B) **Research accomplishments:**

1) Morphology.

Two populations Bwamba and Entebbe were selected for a detailed morphological and molecular study. Bwamba population is human-biting and Entebbe population is non-human-biting. Measurements were taken for 16 characters using 20 mosquitoes; namely: Hind tibia, hind tarsa, mid femur, mid tibia, mid tarsomere, front femur, front tibia, front tarsomere, hind claw, mid claw, front claw, antenna, palps, proboscis and wings.

Significant differences between the two populations were found in the following characters:

a) Hind tibia - There was a significant difference in the lengths between the two populations. The mean length for the left hind tibia for Bwamba mosquitoes was 35.9 while those for Entebbe was 37.6. The mean length for the right hind tibia for Bwamba was 35.4; while for Entebbe it was 40.5. Thus Entebbe *Ae. simpsoni* generally has longer hind tibia compared to Bwamba *Ae. simpsoni* s.l.

b) Mid femur -There was also a significant difference in the mean length of the left mid femur ( $P < 0.05$ ) while no difference was detected in the right mid femur mean length. The mean length of the left mid femur was 28.2 for Bwamba and 36 for Entebbe. However, when sex was put in consideration, the female mosquitoes showed a significant difference in the left mid femur ( $P < 0.05$ ) while there was no difference in the right mid femur. The mean length for the left mid femur in female mosquitoes was 28.2 for Bwamba and

36.3 for Entebbe. For male mosquitoes, there was a significant difference in the mean length of the right mid femur and no difference in the left mid femur. The mean length for the right mid femur was 33.3 for Bwamba and 23.5 for Entebbe.

c) Mid tibia - There was a difference in both the left ( $P < 0.05$ ) and the tight ( $P < 0.05$ ) mean length of the mid tibia. The mean length for the left mid tibia was 30.4 for Bwamba and 39 for Entebbe. The mean length for the right mid tibia was 32 for Bwamba and 36.3 for Entebbe. Considering sex, the mean length for the left mid tibia in male mosquitoes, was 30 for Bwamba and 36 for Entebbe while for female mosquitoes it was 30.7 for Bwamba and 39.7 for Entebbe. The mean length for the right mid tibia in male mosquitoes was 31.6 for Bwamba and 36 for Entebbe while for female mosquitoes it was 32.2 for Bwamba and 36.4 for Entebbe.

d) Mid tarsomere -The mean length for the left mid tarsomere was 41.7 for Bwamba and 46.3 for Entebbe. However, among female mosquitoes, there was a significant difference ( $P < 0.05$ ) in the mean length of the left mid tarsomere of the two populations: the mean in the female mosquitoes was 42.4 for Bwamba and 47.3 for Entebbe. There was no difference in the right tarsomere for both female and male mosquitoes.

e) Front femur - There was a significant difference in both the mean length of the left and the right front femur. The mean length for the left front femur was 31.4 for Bwamba and 38.3 for Entebbe. The mean length for the right front femur was 30.9 for Bwamba and 35.9 for Entebbe. However, with regard to sex, there was a difference among the male mosquitoes in both the left and in the right front femur, but among the female mosquitoes a difference was only seen in the left front femur. The mean length for the left front femur among male mosquitoes was 29.4 for Bwamba and 37.3 for Entebbe. The mean length for right front femur among male mosquitoes was 29 .8 for Bwamba and 38.3 for Entebbe. The mean length for the left front femur in female mosquitoes was 32.6 for Bwamba and 38.7 for Entebbe.

f) Front tibia - There was a significant difference in the mean length of the left front tibia ( $P < 0.05$ ) while no difference was realised in the mean length of the right front tibia. The mean length of the left front tibia was 29.6 for Bwamba and 33.9 for Entebbe. Considering sex , a difference was only

realised in the mean length of the left front tibia among female mosquitoes and not in male mosquitoes. The mean length of the left front tibia among female mosquitoes was 28.8 for Bwamba and 33.8 for Entebbe.

g) Wings - There was a significant difference in the mean length of both the left and the right wings between the two populations. The mean length of the left wing was 53.7 for Bwamba and 58.8 for Entebbe. The mean length of the right wing was 52.9 for Bwamba and 56.9 for Entebbe, regardless of sex. With regard to sex, there was no difference realised in the male mosquitoes between the two populations. However, in the female population, there was a significant difference realised in the mean length of both the right and left wings ( $P < 0.05$ ). The mean length among the female mosquitoes of the left wing was 55.3 for Bwamba and 59.1 for Entebbe. The mean length of the right wing was 52.6 for Bwamba and 58.6 for Entebbe.

**Electrophoretic studies:**

These results demonstrate that Entebbe non-human-biting population of *Ae. simpsoni* s.l. and Bwamba human-biting population are not mixtures of sympatric species. Like the morphological data, these populations contain a range of polymorphic expression. The results also show that the anthropophilic and the non-anthropophilic populations are clearly distinguishable by superoxide dismutase ( SOD-2 ). Bukasa island population in Lake Victoria differed from other Ugandan populations by a high ratio of polymorphic loci. Virtually all populations studied differ in one form or another showing a high degree of variability in *Ae. simpsoni* complex.

**Molecular biology:**

Sequence divergence in the ribosomal ITS clearly separates the populations into two clades. Data for RAPD are still being analysed.

C) **Collaboration:**

Dr. Barry R. Miller of CDC is closely working with us in sorting out the different components in the species complex. At the same time, he is training one of my staff in molecular biology techniques. It is hoped that Dr. Miller will find time in future to visit our laboratory in Entebbe and help in the setting up a molecular

biology laboratory.

D) **Project Impact:**

The project results are used by the Ministry of Health in Uganda to understand the pattern of distribution of yellow fever in Uganda. It is hoped that other Ministries of heath elsewhere especially in Africa will eventually use them.

E) **Strengthening of Developing Country Institutions:**

During the year, we have not bought any new equipment, but have maintained those we have already. After the training of one of our staff, mr. Jonathan Kayondo in molecular techniques, we will then be in a better position to buy more equipment for molecular biology. We greatly appreciate the support given by USAID/ PSTC for this training.

F) **Future Work:**

We plan to do more sequencing of other populations of *Ae. simpsoni* like some from South Africa for *Ae. simpsoni* s.s., Nigeria, Bukasa island in Lake Victoria and some populations along the East coast which have shorn some degree of differentiation. We also plan to study polymorphisms in microsatillite DNA of these populations to compare their phylogenies with the phylogenies obtained from electrophoresis. Work on vector competence will be continued.

## SECTION 2

A) **Managerial issues:**

During the year the Financial Controller of USAID Mission checked our records of expenditure and no adverse report was made.

B) **Budget:**

No changes in the budget were made.

C) **Special Concerns:**

No change.

D) **Collaboration, Travel, Training and Publications:**

We have collaborated with Dr. Raphael Harbach of the British Museum, U.K. in the study of the ecology of *Anopheles bwambae*. A manuscript has been submitted to the journal of Veterinary and Medical Entomology. Plans are underway for a student to be given place in our laboratory to work for a Ph.D. of the University of London under the supervision of Dr. Harbach and Prof. Curtis of the London School of Hygiene.

4 papers are under preparation: 1- Genetic differentiation in *Aedes simpsoni* complex in East Africa. 2- Electrophoretic studies on *Ae. simpsoni* complex in Africa. 3- A new species in the *Aedes africanus* group. 4- Genetic differentiation and phylogenetic relationships in *Aedes africanus* populations.

A preproposal on genetic differentiation in Aedes africanus is to be submitted to USAID-CDR program in November.

E) **Request for USAID or BOSTID Actions.**

I plan to put in a request for a no-cost extension of this project for an extra period of one year to allow for a completion of the molecular part and the write- up for publications. I will be grateful if permission can be granted.

***Addendum*:**

During the course of these investigations morphological and electrophoretic investigations were carried out on some Ugandan and Nigerian populations of *Aedes africanus*. It was found that the species which was responsible for the yellow fever outbreak in Nigeria in 1987, was actually not *Ae. africanus* but some new species; differing from all the rest known in the *africanus* group. Fig. 1 shows the phylogenetic relationships. Bussi and Bukasa are islands in Lake Victoria. Botanical gardens are on Entebbe peninsula. Zika and Mpanga are on the main land near lake Victoria.

Cluster analysis using unweighted pair group method
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Coefficient used: Nei (1978) unbiased genetic identity

| Population or cluster numbers joined | | Clustering level | Cycle |
|---|---|---|---|
| 2 | 3 | .76955 | 1 |
| 4 | 6 | .73941 | 1 |
| 1 | 2 | .71653 | 2 |
| 1 | 4 | .43858 | 3 |
| 1 | 5 | .31750 | 4 |

Cophenetic matrix
**********

| Population | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 ZIKA, ZK | 1.000 | | | | | |
| 2 BUSSI, BS | .717 | 1.000 | | | | |
| 3 BOTANICAL, BT | .717 | .770 | 1.000 | | | |
| 4 BUKASA, BK | .439 | .439 | .439 | 1.000 | | |
| 5 NIGERIA, NG | .318 | .318 | .318 | .318 | 1.000 | |
| 6 MPANGA, MG | .439 | .439 | .439 | .739 | .318 | 1.000 |

Goodness of fit statistics
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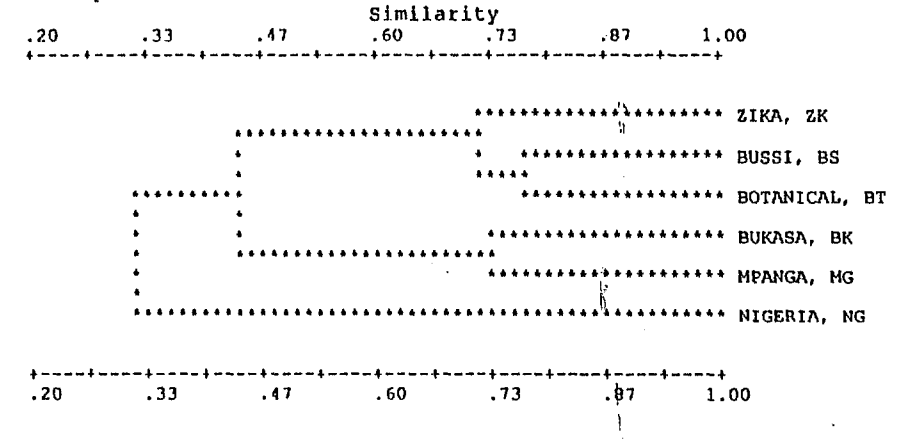
Farris (1972) "f" = 1.054

Prager and Wilson (1976) "F" = 14.714

Percent standard deviation (Fitch and Margoliash,1967) = 26.665

Cophenetic correlation = .870




Cluster analysis using unweighted pair group method
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Coefficient used: Modified Rogers distance (Wright,1978)

Fig. 1 – Dendrogram of the African populations.

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USAID FUNDS 94/95

ID FUNDS 9

| | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|
| 1 | ITEM | BUDGETED | PREVIOUS EXP | PERIOD EXP | TOTAL EXP | BALANCE |
| 2 | SALARIES | 55072 | 10800 | 10800 | 21600 | 3347 |
| 3 | EQUIPMENT | 29949 | 0 | 11035.05 | 11035.05 | 18913.9 |
| 4 | EQUIP SERVICE | 4500 | 204.3 | 0 | 204.3 | 4295 |
| 5 | MATERIALS&SUPPLIES | 11921 | 1873.4 | 3597.28 | 5470.68 | 6450.3 |
| 6 | TRAINING | 5800 | 0 | 0 | 0 | 580 |
| 7 | TRAVEL INTNL | 19000 | 0 | 0 | 0 | 1900 |
| 8 | GASOLINE | 3600 | 0 | 0 | 0 | 360 |
| 9 | LABOUR | 6000 | 0 | 1500 | 1500 | 450 |
| 10 | JOURNALS | 3584 | 0 | 0 | 0 | 358 |
| 11 | PUBLICATIONS | 2000 | 0 | 0 | 0 | 200 |
| 12 | AIR&SEA FRGHT | 7124 | 362.07 | 2298.18 | 2660.25 | 4463.7 |
| 13 | INFLATION | 1200 | 0 | 0 | 0 | 120 |
| 14 | | | | | | |
| 15 | TOTAL | 149750 | 13239.77 | 29230.51 | 42470.28 | 107279.7 |
| 16 | | | | | | |
| 17 | | | | | | |
| 18 | | | | | | |
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| 20 | | | | | | |

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DURING THE FINANCIALYEAR THE PROJECT HAD ABALANCE WITH SIGMA

NG THE