THE EFFECT OF TEMPERATURE ON THE VECTOR COMPETENCE OF CULEX UNIVITTATUS THEOBALD (DIPTERA: CULICIDAE) FOR WEST NILE AND SINDBIS VIRUSES

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ABSTRACT

The mosquito Culex univittatus is the primary vector of West Nile (WN) and Sindbis (SIN) viruses on the inland plateau in South Africa. The influence of different temperatures on the vector competence of this mosquito for both viruses and on the longevity of uninfected mosquitoes was investigated. The replication and distribution of each virus within the mosquito was studied by the fluorescent antibody test and additionally, the salivary glands from infected and uninfected insects were compared histologically.

Orally infected mosquitoes were held at 14°C, 18°C, 26°C and 30°C and also at fluctuating outside summer ambient temperature for the vector competence studies. At regular intervals, a sample of the insects were fed individually by a capillary method to demonstrate virus transmission. The capillary fluid with salivary secretions was titrated in Vero cells as was an extract of the whole mosquito. This enabled three graphs to be constructed showing variations in the titre of the mosquito, the titre of virus transmitted and the transmission rate against time. Infection rates and titres of virus in individual mosquitoes for both these viruses were unaffected by temperature. However, both WN and SIN viruses multiplied slower at lower temperatures, and both the extrinsic incubation period and the period before maximum transmission rates were attained increased as incubation temperature decreased. Incubation temperatures as high as 30°C, however, appeared to adversely affect the vector capability of Cx. univittatus for SIN virus by reducing the transmission rate. Vector competence of WN-infected Cx. univittatus at outside summer temperatures was similar to that obtained at constant 26°C. This was not the case with SIN virus, as the
result at outside temperatures differed greatly from that obtained at any of the constant temperatures. It showed that *Cx. univittatus*, under natural temperature conditions, is not such an efficient vector of SIN virus as originally proposed from past studies conducted at constant temperatures.

Both viruses reduced the average mean life expectancy of orally infected *Cx. univittatus* when held at 26°C, but SIN virus appeared to be pathogenic by disrupting salivary gland cells, which considerably reduced the life span of the mosquito at 14°C.

Fluorescent antibody studies confirmed that WN and SIN viruses, first multiplied in the posterior midgut region of the mosquito, and thereafter spread to other organs. SIN virus also spread to other organs such as the ventral diverticulum and abdominal ganglia.

According to the longevity and vector competence studies, transmission of WN and SIN viruses by *Cx. univittatus* was most favoured at temperatures of 30°C and 26°C respectively, and epidemics of these viruses in South Africa would most likely be expected when mean summer temperatures approximate these respective temperatures.
Declaration

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

D.S. Corre

2 day of November, 1987.
This study was undertaken as an MSc under the supervision of Dr. P.G. Jupp and Dr. R. Swanepoel, and I would like to express my gratitude for their guidance and support.

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ABBREVIATIONS USED IN THE TEXT.

Ae......Aedes
CE......California encephalitis
CHIK....Chikungunya
CPD50...50% cytopathic dose
Cx......Culex
DEN......Dengue
E.......Eosin
EEE......Eastern equine encephalitis
EI......Extrinsic incubation
FA......Fluorescent antibody
H.......Haematoxylin
HAF.....Hyperimmune ascitic fluid
HI......Haemagglutination inhibition
IR......Infection rate
JBE......Japanese B encephalitis
PBS......Phosphate buffered saline
PI......Post infection
RH......Relative humidity
RVF......Rift Valley fever
SF......Semliki Forest
SIN......Sindbis
SLE......St.Louis encephalitis
TAH......Tahyna
TOT......Transovarial transmission
TR......Transmission rate
WEE......Western equine encephalitis
WHA......Whataroa
WN......West Nile
WR......Resistant to infection with Western equine encephalitis
WS......Susceptible to infection with Western equine encephalitis
YF......Yellow fever
CONTENTS

CHAPTER 1. INTRODUCTION

1.1. Factors influencing transmission of arboviruses......................... 2
1.1.1. Extrinsic factors........................................ 2
   A. Rainfall.................................................... 3
   B. Susceptible hosts......................................... 3
   C. Temperature............................................... 3
   D. Seasonal source of virus.................................. 4
1.1.2. Intrinsic factors......................................... 6
   A. Virus replication.......................................... 7
   B. Genetics as a basis for controlling vector competence of mosquitoes. 10
   C. Barriers to replication and dissemination of arbovirus in mosquito bodies............................... 12
   D. Temperature............................................... 12

1. Viruses occurring in the cooler regions of the world....................... 13
2. Viruses from warm regions (Tropical to sub-tropical)..................... 14
3. Viruses from temperate areas.................................. 15

1.2. Epidemiology of WN and SIN viruses in South Africa......................... 21

CHAPTER 2. MATERIALS AND METHODS

2.1. Mosquito colony and rearing........................................ 29
2.2. Virus.......................................................... 30
2.3. Oral infection of Culex univittatus
   2.3.1. Membrane feeding....................................... 31
   2.3.2. Viraemic chicks....................................... 31
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4. Temperature conditions</td>
<td>32</td>
</tr>
<tr>
<td>2.5. Transmission</td>
<td>33</td>
</tr>
<tr>
<td>2.5.1. Definitions of infection and transmission rates</td>
<td>33</td>
</tr>
<tr>
<td>2.5.2. Determination of method for transmission tests</td>
<td>33</td>
</tr>
<tr>
<td>2.5.3. Capillary method</td>
<td>38</td>
</tr>
<tr>
<td>2.6. Mosquito infection rates</td>
<td>38</td>
</tr>
<tr>
<td>2.7. Longevity studies</td>
<td>39</td>
</tr>
<tr>
<td>2.8. Multiplication and distribution of WN and SIN viruses in mosquito organs</td>
<td>41</td>
</tr>
<tr>
<td>2.8.1. Conjugate and antibodies</td>
<td>41</td>
</tr>
<tr>
<td>2.8.2. Fluorescent antibody staining</td>
<td>41</td>
</tr>
<tr>
<td>2.9. Staining of salivary glands</td>
<td>42</td>
</tr>
</tbody>
</table>

**CHAPTER 3. RESULTS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1. Evaluation of artificial feeding</td>
<td>44</td>
</tr>
<tr>
<td>3.2. Evaluation of methods for transmission tests</td>
<td>44</td>
</tr>
<tr>
<td>3.3. Temperature vector competence studies</td>
<td>46</td>
</tr>
<tr>
<td>3.3.1. Effect of temperature on the competence of <em>Cx.univittatus</em> as a vector of WN virus</td>
<td>47</td>
</tr>
<tr>
<td>3.3.2. Effect of temperature on the competence of <em>Cx.univittatus</em> as a vector of SIN virus</td>
<td>54</td>
</tr>
<tr>
<td>3.4. Histological examination of infected salivary glands of <em>Cx.univittatus</em></td>
<td>76</td>
</tr>
<tr>
<td>3.5. Longevity studies</td>
<td>79</td>
</tr>
<tr>
<td>3.6. Replication and sequential distribution of virus in the organs of <em>Cx.univittatus</em></td>
<td>87</td>
</tr>
</tbody>
</table>
## CHAPTER 4. DISCUSSION

4.1. Replication and distribution of WN and SIN viruses in *Cx.univittatus* ........................................ 102

4.2. Temperature and competence of *Cx.univittatus* as a vector of WN virus........................................ 103

4.3. Temperature and competence of *Cx.univittatus* as a vector of SIN virus........................................ 107

4.4. Longevity of *Cx.univittatus* infected with WN and SIN viruses respectively........................................ 109

## CHAPTER 5.

5. GENERAL CONCLUSIONS............................................ 112

REFERENCES........................................................ 113

APPENDIX............................................................ 131
### TABLES

<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Summary of results obtained from temperature vector competence studies on viruses occurring in the tropics.</td>
<td>18</td>
</tr>
<tr>
<td>2.</td>
<td>Feeding rates of <em>Cx.univittatus</em> through four types of membranes on (A) stirred blood and (B) unstirred blood.</td>
<td>44</td>
</tr>
<tr>
<td>3.</td>
<td>Comparative transmission rates.</td>
<td>46</td>
</tr>
<tr>
<td>4.</td>
<td>Replication of West Nile virus in various organs of <em>Cx.univittatus</em> at 14°C.</td>
<td>89</td>
</tr>
<tr>
<td>5.</td>
<td>Replication of West Nile virus in various organs of <em>Cx.univittatus</em> at 18°C.</td>
<td>90</td>
</tr>
<tr>
<td>6.</td>
<td>Replication of Sindbis virus in various organs of <em>Cx.univittatus</em> at 14°C and 18°C.</td>
<td>91</td>
</tr>
</tbody>
</table>
FIGURES

Fig. 1. External structure of a salivary gland from Cx.univittatus. 9

Fig. 2. Total monthly rainfall figures in mm during the summer months from 1970-1980 in Upington. 25

Fig. 3a. Average maximum and minimum temperature in °C experienced during the summer months in Johannesburg from 1976-1986. 28

Fig. 3b. Total monthly rainfall in mm in Johannesburg during the summer months of 1978-1985. 28

Fig. 4. Droplet method: mosquito feeding. 37

Fig. 5. Capillary method: mosquito feeding. 37

Fig. 6. Daily maximum and minimum ambient temperature recorded when the WN-infected mosquitoes were exposed to in the outside walk-in cage. 49

Fig. 7. Daily maximum and minimum ambient temperature recorded when the mosquitoes infected with SIN virus were exposed to in the outside walk-in cage. 51

Fig. 8. Cx.univittatus held at 30±1°C and 60-70% RH and infected with WN virus. 58
Fig. 9. *Cx. univittatus* held at 26±1°C and 75-80% RH and infected with WN virus. 58

Fig. 10. *Cx. univittatus* incubated at outside fluctuating summer temperature and 30-80% RH and infected with WN virus. 60

Fig. 11. *Cx. univittatus* held at 18±1°C and 60-75% RH and infected with WN virus. 62

Fig. 12. *Cx. univittatus* held at 14±0.5°C and 50-75% RH and infected with WN virus. 64

Fig. 13. *Cx. univittatus* held at 30±0°C and 80-70% RH and infected with SIN virus. 67

Fig. 14. *Cx. univittatus* held at 26±1°C and 75-80% RH and infected with SIN virus. 69

Fig. 15. *Cx. univittatus* incubated at outside fluctuating summer temperature and 30-80% RH and infected with SIN virus. 71

Fig. 16. *Cx. univittatus* held at 18±1°C and 60-75% RH and infected with SIN virus. 73

Fig. 17. *Cx. univittatus* held at 14±0.5°C and 50-75% RH and infected with SIN virus. 75

Fig. 18. Section through salivary gland lobes of female *Cx. univittatus*,
a. uninfected
b. infected with WN virus
c. infected with SIN virus. 78
Fig. 19. Percentage longevity of uninfected *Cx.univittatus* from 0-70 days shown in relation daily maximum and minimum ambient temperatures in °C. 82

Fig. 20. Percentage longevity of uninfected *Cx.univittatus* held at:
   a) constant 30±1°C
   b) constant 26±1°C
   c) constant 18±1°C. 84

Fig. 21a. Percentage longevity of *Cx.univittatus* infected with WN virus and incubated at 26±1°C. 86

Fig. 21b. Percentage longevity of *Cx.univittatus* infected with SIN virus and incubated at 26±1°C. 86

Fig. 22. Fluorescent antibody studies on the midgut region of
   a) uninfected *Cx.univittatus*
   b) SIN-infected *Cx.univittatus*. Mag. 200X. 93

Fig. 23. Fluorescent antibody studies on the salivary glands of
   a) uninfected *Cx.univittatus*
   b) *Cx.univittatus* infected with SIN virus
   c) *Cx.univittatus* infected with WN virus.
   Mag. 100X. 95

Fig. 24. Fluorescent antibody studies on the foregut region of
   a) uninfected *Cx.univittatus*
   b) *Cx.univittatus* infected with SIN virus.
   Mag. 200X. 97
Fig. 25. Fluorescent antibody studies on the abdominal ganglia from
a) uninfected *Cx.univittatus*
b) *Cx.univittatus* infected with SIN virus. Mag. 200X.

Fig. 26. Fluorescent antibody studies on the ventral diverticulum of
a) uninfected *Cx.univittatus*
b) *Cx.univittatus* infected with SIN virus. Mag. 200X.
CHAPTER 1.

INTRODUCTION.

An aspect of prime importance in the epidemiology of arthropod-borne viruses (arboviruses) is an understanding of the ecological conditions and circumstances which favour the occurrence of human epidemics due to a particular virus. Several field studies have enhanced our understanding as to the reasons for the development of epidemics of certain arboviruses. However, the evidence provided by some field observations tends to be too circumstantial so that additional evidence is needed from parallel laboratory studies. One such environmental factor recorded in the field which needs assessment in the laboratory is environmental temperature and the role it has on influencing the competence of several arthropod vectors to transmit associated viruses. Field observations conducted on West Nile (WN) and Sindbis (SIN) viruses in South Africa (McIntosh et al., 1976; Jupp et al., 1986) have suggested that ambient temperature might be important in favouring epidemics of these two viruses. The present study further investigates the effect of environmental temperature on the longevity and vector competence of the vector *Culex univittatus* Theobald for both WN and SIN viruses respectively by laboratory experimentation. These experimental observations are then correlated with field observations to strengthen our understanding of the importance of ambient temperature in the epidemiology of WN and SIN viruses.

Temperature is not the only environmental factor influencing transmission of mosquito-borne viruses and the other factors are also discussed in this chapter. A detailed review of the epidemiology of WN and SIN
viruses in South Africa is also given as this is necessary for an interpretation of the results obtained from the temperature vector competence studies.

1.1. Factors influencing transmission of arboviruses.

Several factors influence the occurrence of arboviruses and Hardy et al (1975) divided these factors into two groups, namely, extrinsic and intrinsic factors. Extrinsic factors influence the exposure of mosquitoes to viraemic and clinically susceptible hosts (Hardy et al, 1979) and include such concepts as distribution of vertebrate hosts and arthropod vectors and feeding preferences of the arthropod vectors. Intrinsic factors, on the other hand, are those that determine if, once virus has been ingested by an arthropod, it can initiate an infection, reach the salivary glands and be transmitted (Hardy et al, 1979). It is beyond the scope of this thesis to discuss all these extrinsic and intrinsic factors in detail and therefore only a brief review is given. Temperature as an intrinsic factor is however discussed in more detail for several viruses.

1.1.1. Extrinsic factors.

A study conducted on western equine encephalitis (WEE) virus in Kern County, California, revealed those extrinsic factors which favoured epidemics of the virus there, both in 1952 and 1958 (Reeves et al, 1984). These factors were:

1) An unusually large population of the mosquito *Cx. tarsalis*, the major vector of WEE virus, brought about by unusually heavy rains,

2) An abundance of susceptible avian hosts and
3) the consistent occurrence of high densities of *Cx. tarsalis* during the summer in Kern County. These extrinsic factors have also been associated with epidemics of other mosquito-borne viruses and are discussed below.

**A. Rainfall.**

Epidemics of several arboviruses have been correlated with high rainfall which brought about much larger populations of mosquito vectors. Examples of this correlation between rainfall and arbovirus epidemics include those of Rift Valley fever (RVF) virus in Kenya (Davies et al., 1985) and in South Africa (McIntosh and Jupp, 1981); WN virus in South Africa (McIntosh et al., 1976); SIN virus in South Africa (McIntosh et al., 1976; Jupp et al., 1986) and dengue (DEN) virus in Malaysia (Foo et al., 1985). Because of the breeding habits of mosquitoes this correlation between large numbers of mosquitoes and epidemics of arboviruses is not difficult to understand, and more such correlations between high rainfall and epidemics of arboviruses will most likely be found.

**B. Susceptible hosts.**

A sufficiency of susceptible vertebrate hosts is also critical in transmission of arboviruses and is always included as a variable in models that predict epidemics of arboviruses (Scott et al., 1983; and De Moor et al., 1970), where the larger the number of susceptible hosts the larger the epidemic predicted.

**C. Temperature.**

Coupled with the global distribution of arboviruses is the distribution of the natural arthropod vectors. Many experiments have proved that arthropods (including mosquitoes) have temperature tolerances
and therefore are only found in certain types of climatic conditions. For example, some tropical mosquitoes belonging to the genus *Haemagogus* (vectors of yellow fever (YF) virus) live optimally at temperature ranges higher than most other species of mosquito, with greater longevity at 30°C (Bates and Roca-Garcia, 1945). Mosquitoes living in temperate regions have lower optimal temperature ranges and *Aedes triseriatus*, a vector of eastern equine encephalitis (EEE) virus, lives longer at 21°C (32-42 days) than at higher temperatures of 32°C (16 days) (Chamberlain and Sudia, 1955). Some mosquitoes such as *Cx. quinquefasciatus*, collected in even colder climatic regions, are able to live up to 110 days at 10°C but only 45 days at 26°C (Lamotte, 1963). Increased breeding and longevity of mosquito vectors in their natural environments would therefore help to maintain a consistent population of mosquitoes during summer periods and thus favour transmission of arboviruses and increase the chances of epidemics. The situation as regards temperature is, however, complicated as temperatures are not constant but differ between seasons and from year to year. Some years are more favourable for flight range, growth, reproduction and certain other functions that affect the survival of mosquitoes. Furthermore, levels of metabolism of mosquitoes are largely controlled by ambient temperatures so that mosquito activities fundamental to virus transmission, such as blood feeding, would also be affected and vary between seasons.

**D. Seasonal source of virus.**

Seasonal climatic changes cause fluctuations in arthropod numbers, and when their numbers drop to low levels in response to adverse conditions (i.e., winter months in temperate regions and dry seasons in the tropics), transmission cycles between man, arthropod
vectors and vertebrate hosts become interrupted. Associated with this interruption in transmission, other pathways of virus maintenance, and in particular overwintering, have had to be investigated to explain how viruses remain endemic with continued seasonal appearance (Tesh, 1984). Studies have shown that such overwintering pathways for arboviruses do exist. These include maintenance of virus in vertebrate hosts that develop chronic latent infections, long-living and persistently infected primary arthropod vectors and through infected arthropods capable of vertical transmission (transovarian transmission—TOT) of virus to progeny (Reeves, 1974). Definitive examples of TOT in mosquitoes are relatively few and those that have been firmly established have originated from both field and laboratory investigations. These field studies have involved recoveries of arboviruses from naturally infected immature mosquitoes such as larvae (Andrews et al, 1977) and eggs (Chamberlain et al, 1964). Laboratory studies successfully demonstrating TOT of several mosquito-borne viruses (Watts et al, 1973; Berry et al, 1977; Tesh, 1984) are increasing as an understanding of the mechanisms of TOT and laboratory methods improve so that the importance of TOT as an overwintering mechanism is rapidly being realized.

Field studies in North America and Europe have confirmed that some species of mosquitoes are capable of hibernating through the winter as adults in a state of diapause (Bennington et al, 1958; Schaefer and Washino, 1969; Buffington, 1972; Sulaiman and Service, 1983). Laboratory studies have also demonstrated hibernation of mosquitoes, and the environmental conditions which induce the physiological changes to trigger diapause are reasonably well understood for some species of mosquitoes (Tekle, 1968; Bellamy and Reeves, 1981; Spielman et al, 1973; Washino, 1977; Mitchell, 1981; Bailey
et al, 1982; Wilton and Smith, 1985). In South Africa where winter temperatures are not as cold as those in North America and Europe, adult mosquitoes have been collected during the winter season in a quiescent state (Jupp, 1969). As opposed to diapause, which is true hibernation viz. a genetically determined state of suspended activity and suppressed development (Wilton and Smith, 1985), quiescence is a more temporary suspension of activity in direct response to an unfavourable influence, the removal of which will promptly permit the mosquito to return to normal activity (Bellamy and Reeves, 1963). Since adult mosquitoes are capable of surviving cold winter months, the question arises as to whether infected overwintering mosquitoes could serve as another overwintering reservoir of mosquito-borne viruses. Few isolations of arboviruses have been made from field collected diapausing or quiescent mosquitoes (Bailey et al, 1978). However, several laboratories have subjected infected adult mosquitoes to simulated winter conditions to demonstrate that diapausing and quiescent mosquitoes can serve as overwintering reservoirs (Hurlbrut, 1950; Aspock and Kunz, 1970; Hayashi et al, 1976), and transmit virus to vertebrate hosts in the spring. Finally, the endemic persistence of mosquito-borne viruses has also been explained by the repetitive arrival at the beginning of each summer season of infected migratory hosts, such as birds. This hypothesis has been supported by the isolation of several mosquito-borne viruses from migratory birds worldwide (Calisher, 1971; Taylor et al, 1958; Scherer et al, 1958; Work, 1958).

1.1.2. **Intrinsic factors.**

Mosquitoes ingest virus particles via infected blood meals, and the sequential infection and multiplication of the virus in the organs of the mosquito determine whether it becomes a competent vector or not (Hardy et
al.1979). Such sequential multiplication of virus is reviewed, followed by the effects of various intrinsic factors upon it.

A. Virus replication.

The sequence of viral replication within competent mosquito vectors was summarized by Hardy et al. (1983) as follows:

a) The virus must initiate an infection in the mesenteron of the mosquito,

b) multiply productively in the mesenteronal epithelial cells,

c) escape from the mesenteron and finally

d) infect and multiply in the salivary glands and ovaries whence it can be transmitted orally to vertebrate hosts and perhaps transovarially to progeny.

Furthermore, Hardy et al. (1983) summarized and divided the replication of arboviruses within mosquitoes into three major amplification stages. These stages when the virus actually replicates within mosquito cells resulting in an overall increase in titre. These amplification phases are as follows:

1) Infection first occurs in the mesenteron, thereafter it spreads or disseminates to other tissues via the haemocoel.

2) Infection in cells and tissues associated with the haemocoel.

3) Infection of the salivary glands.

There is much evidence supporting the above summary for the sequence of infection in the mosquito and some of the more convincing studies are discussed below.

Pioneering work on the sequence of viral replication was done by LaMotte (1860) with Japanese B encephalitis (JBE) virus in Cx. pipiens. La Mottte's results have since been confirmed and extended by immunofluorescence studies on JBE virus in other
species of mosquitoes (Doi, 1970), DEN-2 virus in *Ae. albopictus* (Kuberski, 1979), electron microscopy of Venezuelan equine encephalitis virus in *Ae. aegypti* (Larsen et al, 1971), California encephalitis in *Ae. aegypti* and *Culiseta inornata* (McLean et al, 1975) and St. Louis encephalitis (SLE) in *Cx. pipiens pipiens* (Whitfield et al, 1973).

To be more specific, after virus particles have entered the mosquito via an infective blood meal, the virus moves to the midgut where virus penetration of host parenchyma and active infection of epithelial cells occurs (Chamberlain and Sudia, 1961; Kuberski, 1979). The virus then gains access to other cells of the mosquito through the haemolymph (LaMotte, 1960; Danielova, 1962), such cells being the fat body cells, nerve ganglia, ovarian cells, malpighian tubules and the salivary gland cells. Salivary glands produce saliva which is secreted as the mosquito imbibes blood and, if the salivary gland is infected, virus is secreted into the host. Therefore, transmission of virus occurs when the salivary glands are persistently infected with virus (Thomas, 1963; Chamberlain, 1968; Miles et al, 1973; Kramer et al, 1981). This transmission of virus via infected salivary fluid is termed biological transmission and should be clearly distinguished from mechanical transmission. Arthropods mechanically transmitting virus only act as carriers and transmit virus from infected to uninfected hosts via contaminated mouthparts (Chamberlain and Sudia, 1981). For biological transmission, however, a period must elapse to allow imbibed virus (ingested in an infected blood meal) to reach the salivary glands of the mosquito before virus can be biologically transmitted. This time period is known as the extrinsic incubation (EI) period (Hardy et al, 1983). The EI period may therefore be defined as the interval between ingestion of virus and subsequent ability to transmit virus by the mosquito (Turrell et al, 1985).
are, however, some exceptions to this where transmission of virus did not correlate precisely with the presence of virus in the salivary glands. In a study done on Cx.thieleri infected with SIN virus (Jupp, 1985), it was found that even though its salivary glands were infected, the mosquito did not always transmit virus. Therefore one should be careful not to assume that transmission rates will equal salivary gland infection rates. Considerable viral replication occurs in the salivary glands and it is of interest to mention the areas in the salivary gland where viral replication occurs. Each salivary gland of a female Ae.aegypti is made up of three lobes, two lateral and a smaller median or middle lobe. The lateral lobes are further divided into proximal, intermediate and distal portions, and the middle lobe into intermediate and distal parts. Salivary glands of Cx.univittatus appear to have a similar structure and are shown in Figure 1.

Fig.1. External structure of a salivary gland from Cx.univittatus.MAG.100X.
A study on the replication of CHIK virus in salivary glands of *Ae.aegypti* (Janzen et al, 1970) showed that the site of greatest potential for virus replication was in the proximal portion of the lateral lobes. Immunofluorescent studies (Kuberski, 1979) also demonstrated that DEN-2 viral antigen appeared initially in the proximal portions in *Ae.albopictus* salivary glands. Perhaps of more significance from the transmission point of view, was that the appearance of fluorescence in the salivary glands was more granular than in any of the other parts of the mosquito. Kuberski suggested that this type of fluorescence confirms that salivary glands play an integral part in secreting infectious virions necessary for effective transmission of virus.

B. Genetics as a basis for controlling vector competences of mosquitoes.

Variation in susceptibility to oral infection of a mosquito-borne virus commonly occurs between populations of the same species of mosquito. For instance, geographic strains of *Ae.albopictus* vary in susceptibility to infection with DEN virus (Gubler and Rosen, 1976), strains of *Ae.aegypti* vary in their susceptibility to and transmissibility of Tahyna (TAH) virus (Labuda et al, 1985) and infection and transmission rates for La Crosse (LAC) virus differ quite considerably between different strains of *Ae.triseriatus* (Grimstad et al, 1977). Cross-breeding between strains with different vector competence has been done and some factors controlling the competence seem to be inheritable. Different strains of *Ae.albopictus* also vary in susceptibility and in the quantity of virus present in infected individuals after a standard incubation period with CHIK virus. Crossing strains with high and low CHIK virus thresholds resulted in offspring with infection rates and mean viral titers intermediate between those shown
by the parent colonies (Tesh et al, 1976). Strains of Cx. tarsalis resistant (WR) and susceptible (WS) to oral infection with WEE virus have also been selected. Hardy et al (1978) crossed these strains and the F1 progeny were almost 100% susceptible indicating susceptibility may have been dominant. However, backcrossing with WR and WS strains and F2 progeny gave inconclusive results, which led Hardy et al (1983) to suggest that inheritance of susceptibility to WEE virus was probably multifactorial.

More recent cross-mating experiments with strains of Cx. tritaeniorynchus (Giles) also showed that the susceptibility to WN virus could be inherited (Hayes et al, 1984). These experiments indicated that the trait of oral susceptibility is dominant over resistance to viral infection, and both male and female mosquitoes of the susceptible strains are carrying factors for increased susceptibility. Backcrossing however showed that increased susceptibility is partially dominant, since the F1 progeny developed intermediate titres of WN virus when compared to titres of the parental strains.

Due to this variation in vector competence, and because factors affecting vector capability seem to be inheritable traits, genetics must play a vital role in controlling the vector competence for arboviruses. Still further evidence for this comes from vector competence studies of colonized mosquitoes of different filial generations. It has been reported that mosquitoes, like many other arthropods, once having been removed from the field, undergo drastic genetic selection with rapid alteration of the gene pool due to colonization in response to the conditions in the laboratory. Several studies have demonstrated that vector capability of many mosquitoes gradually changes with increase in filial generation, eg, with RVF virus (Gargan et al, 1983) and YF virus (Lorenz et al, 1984). These changes have even been correlated with loci of specific isoenzymes (Lorenz et al, 1984).
C. Barriers to replication and dissemination of arboviruses in mosquito bodies.

A further set of intrinsic factors affecting interrelationships between arboviruses and mosquitoes is the "barrier" effect. These barriers are also genetically determined physiological phenomena involving membranes, enzymes and cell surfaces. Due to their properties they may either prevent viral replication or prevent virus particles from leaving one area and entering another (dissemination) within the mosquito body. Since these barriers are genetically determined traits, their properties and the subsequent effects they have on viral replication are species specific. This helps explain why some species of mosquitoes are better vectors for some viruses than others. This whole concept of barriers has received much attention in the last few years and has been reviewed by Hardy et al (1983).

D. Temperature.

Mosquitoes are cold-blooded animals so that their level of metabolic activity is directly proportional to ambient temperature. As a consequence of this, it follows that a virus within a mosquito would also be affected and multiply best in mosquitoes within certain temperature ranges. Studies on the effect of temperature on vector potential of mosquitoes with various viruses do indeed support this hypothesis and have demonstrated that short EI periods and maximum transmission and infection rates are only attained at certain temperatures. This optimal temperature range is, however, not only favourable in terms of viral multiplication but also in terms of mosquito longevity and activities such as blood feeding. When all these factors combine in a certain manner, optimal conditions for transmission of viruses are created. For instance, lengthened EI periods expected at lower