# STUDIES ON THE *CYTOSPECIES AND* DISTRIBUTION OF *Simulium damnosum* COMPLEX (DIPTERA: SIMULIIDAE) IN PARTS OF NIGERIA, CAMEROON AND EQUATORIAL GUINEA

**RV**

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# CERTIFICATION

This dissertation entitled *studies on the cytospecies and distribution of Simulium damnosum complex (Diptera: Similiidae) in parts of Nigeria, Cameroon and Equatorial Guinea* by Stella Ame Ekpenyong Bassey (Ph.D/SCI/5897/1988-89) meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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# DECLARATION

I hereby declare that this thesis is a record of my own research work. It has not been presented by me in any previous application for a higher degree.

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# ABSTRACT

Cytotoxanomic studies of members of the *Simulium damnosum* complex was carried out in parts of Nigeria, Cameroon and Equatorial Guinea to determine cytospecies status and distribution across different bio-climatic zones and across country boundaries. These three neighbouring West African countries were studied in view of the importance of the knowledge of vector species abundance and distribution in onchocerciasis endemic areas and how their migratory activities affect control programmes.

Sampling sites for the study was chosen within a broad transect from the northern extremes of distribution in Nigeria through its southern bio-climatic zones into south west Cameroon near Mount Cameroon and further south to parts of the island of Bioko in Equatorial Guinea. Sites in Nigeria cut across both savannah and forest zones while in Cameroon and Equatorial Guinea all sites fell within the forest zone. Larval samples were collected from these sites and cytospecies status determined by the observation of the inversion pattern of their polytene giant chromosomes.

In Nigeria four cytospecies were observed, *S. squamosum, S. sirbanum, S. damnosum s.s. and* S. *yahense* with frequency distribution of 58%, 21.3%, 17.3% and 3.3% respectively. Cytospecies observed in Cameroon were *S. squamosum* (84%) and *S. mengense* (16%). I n the island of Bioko, there was observed the existence of a single cytospecies which is confined to the *S. squamosum* sub-species and closely related to *S. yahense.* This cytospecies was found to have no fixed inversion differences from either *S. squamosum* or *S. yahense* but differed from both in the pattern of sex-linkage of inversion 2-L18 which here was Y-linked . Distribution of the Nigerian cytospecies in relation to the different bio-climatic zones showed *S. squamosum* and *S. yahense* to be more restricted to the forest with frequencies of 98% and 100% respectively. *Simulium damnosum*

exhibited a wide distributional range, spanning through forest (57.7%) and savannah (42.3%) areas. *Simulium sirbanum* occurred more in the savannah ( 98.4%) zones. Further more, in the forest zones of Nigeria, *S. squamosum* was found to consist of two different populations, one with a sex-linked chromosome variation on the centromere of chromosome one, and the other population did not have this variation. Measurement of physical and chemical parameters of the water in the some breeding sites in Nigeria showed slight differences between the features for the forest and savannah zones.

It is apparent from results of this study that in Nigeria *S. squamosum* and *S. yahense* are restricted to the forest zones whereas *S. damnosum s.s.* and *S. sirbanum*, typical savannah cytopecies are becoming significant in the forest zones. This may be a reflection of a change in environmental conditions, a factor which may partly explain why cytospecies like *S. sanctipauli* and *S. soubrense* reported to occur in Nigeria by other researchers were not observed. Interestingly, *S. mengense* was not observed in Nigeria and no sex-linked chromosomal variation was observed in any of the *S. squamosum* samples from Cameroon. The above observations together with the uniqueness of *S. mengense* to Cameroon and Bioko type *S. yahense* to Bioko indicate that in spite of the proximity of these countries, there is still some geographic limit to the movement of the *S. damnosum* cytospecies.

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# CHAPTER ONE

# INTRODUCTION

The blackfly *Simulium* (*Edwardsellum*) *damnosum* Theobald (Diptera: Simuliidae) was first described in East Africa in 1903. Later in 1926, Blacklock identified biting females of *S. damnosum* in West Africa to be the vectors of the parasitic nematode, *Onchocerca volvulus*. These worms cause the disease onchocerciasis or "river blindness" in Man. It has also been observed that the epidemiology and clinical manifestations of human onchocerciasis in the forest zones of West Africa differ from those of the savannah region – for example sclerosing keratitis is much higher in the savannah than forest area (Le Berre, 1966; Anderson *et al.*, 1974). These differences have been attributed to the existence of "forest" and "savannah" strains of *O. volvulus* (Duke *et al.*, 1966; Duke, 1967). Similarly, the *S. damnosum* fly has been found to be extremely variable in its biology and ecology. All these observations led entomologists to suspect strongly that *S. damnosum* consists of a number of sibling species.

*Simulium damnosum* has been confirmed to be a species complex (Dunbar 1966; 1969) and can only be reliably distinguished by non-morphological means. Chromosomes provide criteria for diagnosing and discriminating between species (Davies and Crosskey, 1991). This cytotaxonomic method of Simuliid identification is considered the most reliable (Le Berre & Fiasorgbor, 1985) and is dependent upon the polytene chromosomes of the larval life stage. It has been used as a reference point in various fields of research, especially in the development of new identification techniques for adult flies, and in control operations such as the Onchocerciasis Control Programme (OCP). The use of these cytological parameters in larval identification in Uganda initially revealed four and later nine cytological segregates (Dunbar, 1966; 1969) of *S. damnosum* s.l. In West Africa, seven cytological segregates

were originally recognised from the *S. damnosum* complex; six of them were reported to represent three pairs of closely related entities (Dunbar and Vajime, 1972).

In 1975, Vajime and Dunbar described the following eight cytospecies from West Africa: *S. damnosum* s.s.; *S. sirbanum* & *S. sudanense*; *S. squamosum*; *S. soubrense*; *S. sanctipauli*; *S. yahense* and *S. dieguerense*. Cytogenetic analysis of *S. damnosum* complex is feasible due to the fact that their salivary gland polytene chromosomes are easily identifiable and they have a low chromosome number $2n=6$ ($n=3$). Details of identification features for these three chromosomes were given by Vajime and Dunbar (1975) and are included in the Appendix (Section 8.3).

Polytene or giant chromosomes are found primarily in well differentiated organs that are engaged in vigorous (often secretory) metabolic activities e.g. salivary glands, intestines and malphigian tubules. These tissues grow by an increase in cell size rather than number. As they expand, the chromosomes also expand and increase in length as a result of the replication of DNA, without separation. Along individual chromosome fibres, there are numerous dense spots where presumably the structure is drawn into tight folds. These locations are called chromomeres, and contain much-folded deoxyribonucleic acid (DNA). Each chromomere is fastened to the homologous or matching chromomere of the neighbouring filaments and these locations become the bands. Chromosomes that result from this growth process are said to be "polytene" having a multi-strand structure resembling a rope (White, 1973).

The bands vary in thickness and contain high concentrations of DNA and histones (proteins associated with DNA). Spaces between the bands are interbands and these contain low concentrations of DNA and histones. The DNA here is usually in an extended state. Each giant chromosome in a set within a cell has its own characteristic sequence or pattern of

banding. This pattern appears with utmost precision in the homologous giant chromosomes of every individual of the species.

At particular stages of development, some of the genetic loci (*i.e.* bands) in the polytene chromosomes may undergo a spectacular change in appearance, by becoming converted into swellings or puffs. The fine structure of individual bands can differ with respect to puffs that are in one location on a chromosome in one tissue and in another location on the same chromosome in another tissue. In some cases, specialised puffs called balbiani rings (named after Balbiani, the scientist who described them) exist. The formation of a puff involves the accumulation of acidic protein, despirilization of DNA within the region (where the puff is to be formed), the synthesis of RNA and the storage of newly synthesised RNA (Beerman, 1965).

Sibling species are usually recognised by certain cytological features such as inversions and sex-differentiation. An inversion is a $180^0$ turn in the banding sequence. It is therefore the reversal of the banding pattern of a section of a particular polytene chromosome. A single chromosome can be inverted (I) or not inverted (S) and an individual diploid fly can be homozygous inverted (II), heterozygous (IS) or homozygous un-inverted (SS), that is, exhibiting the standard sequence. Thus "I" represents the inverted status and "S" the standard status. Fixed inversions are entirely homozygous (II or SS) and the existence of fixed inversions allows absolute recognition of species. In a population subdivided as "A" and "B", if all individuals in population "A" were homozygous un-inverted (SS) whereas all individuals of population "B" have an inverted sequence (II), then the absence of a heterozygote (IS) between the two kinds of homozygotes would show no interbreeding and attest to two distinct reproductive units (*i.e.* species). Population (species) "B" would differ from population (species) "A" by one fixed inversion.

Floating inversions can be in the homozygous (SS, II) or heterozygous (IS) state. According to simple Mendelian inheritance, a floating inversion in a population will occur in Hardy-Weinberg equilibrium (Dobzhansky, 1951; Mayr, 1970). A floating inversion at 50% frequency on the chromosome produces one quarter homozygote each for the standard and inverted sequence and half heterozygote ($^1/_4$ SS, $^1/_2$ IS, $^1/_4$ II). However, for a floating inversion to occur naturally in Hardy-Weinberg equilibrium, the traits must be under normal Mendelian inheritance and the population freely interbreeding.

In most animal species (as in Man) chromosomes in the sperm and egg determine the sex of offspring. Males are known to have an X and Y chromosome, whereas females have two X-chromosomes. Most species within the groups are found to show some form of sex chromosome differentiation by nature of the banding pattern of their sex chromosomes. Sex chromosome differentiation may involve inversions and/or band polymorphism. Band polymorphisms are distinguishing features found on one or both arms of a chromosome. They are differences that may be observed on one or more bands and are stable and inherited. They could appear homozygously or heterozygously and in some cases they have been found to be linked to sex chromosomes and as such used as a sex-determining factor. The band polymorphisms are sometimes associated with a local area of non-pairing (split) of the polytene chromosome arm, especially when they are sex-linked.

Since the initial description of the West African cytospecies, our understanding of their distribution has confirmed the same broad regions; hot humid coastal belt, the two central Guinea savannah zones and the almost arid northern Sudan savannah region. Some cytospecies show preference for savannah, forest or forest-mosaic habitat. The principal savannah cytospecies include; *S. sirbanum* and *S. damnosum* s.s., whereas, the main forest cytospecies are *S. yahense* and *S. sanctipauli* as well as *S. soubrense* and *S. squamosum*. The latter two cytospecies are known to also extend into savannah territory. The scope of the study of members of the *S. damnosum* complex existing in West Africa was initially in

the seven original countries of the Onchocerciasis Control Programme (OCP) - Benin, Ghana, Côte d'Ivore, Mali, Niger, Togo, and Burkina Faso. The list of countries now also includes Sierra Leone, Guinea, Guinea-Bissau and Senegal.

In planning any control programme, it is necessary to first acquire precise knowledge of the distribution of different species (Le Berre and Fiasorgbor, 1985). This could lead to the correlation of the different forms with epidemiologically significant factors such as anthropophily and the tracing of migration patterns or insecticide resistance distribution (Post, 1986). The importance of species identification in any control programme can not be over-emphasised. Distributional data have little significance in the understanding of disease epidemiology unless they can be related to reliably identifiable taxa within the *S. damnosum* complex (Crosskey, 1987). Onchocerciasis vector species seldom occur in isolation from other Simuliids and onchocerciasis zones have been found to consist of many species of Simuliid faunas, of which the majority are completely harmless to Man (Davies and Crosskey, 1991). It is therefore essential to differentiate vector species from innocuous ones. Distantly related species (from other sub-genera for example) can be easily distinguished morphologically, but for closely related (sibling) species the cytotaxonomic method of Simuliid identification is considered the most reliable (Le Berre and Fiasorgbor, 1985). It has been used as a reference point in various fields of research, especially in the development of new identification techniques, and in control operations such as OCP.

Information exists on variations in the original cytotaxonomic studies of the West African *S. damnosum* complex (Vajime & Dunbar, 1975; Quillévéré, 1975). Also, information on re-classifications of some inversions of earlier work abound (Meredith *et al.*, 1983; Post, 1982; 1986; Boakye and Mosha, 1988; Boakye *et al.*, 1993; Vajime, 1989). The result of all this is an array of information in different journals and a proliferation of different modes of naming inversions and their interpretation (Boakye, 1993). The work of Boakye (1993) is an effort towards the summary description of inversions used in the identification of

different cytotypes of *S. damnosum* s.l. in West Africa with particular reference to the OCP area. Here pictures of larval polytene chromosomes showing important inversions in the different cytospecies were presented to serve as training guide for *S. damnosum* s.l. cytotaxonomy.

Knowledge of cytotaxonomic separation of the different cytospecies is important in all onchocerciasis endemic areas and especially where there is an established onchocerciasis control programme or where the control programme is being planned. In Nigeria, a National Onchocerciasis Control Programme (NOCP) was set up in 1985 and control of the disease is being carried out through ivermectin distribution. However, there is a lack of essential baseline data on the role of different sibling species and their role in onchocerciasis transmission (Edungbola, 1991).

In 1989, a control programme was established in Cameroon within the framework of a Primary Healthcare project of the country's Ministry of Health and together with the German Agency for Technical Co-operation (GTZ), onchocerciasis control is being carried out using ivermectin in two divisions of Littoral province (Mendoza *et al.*, 1997). Since 1990, control of onchocerciasis has been carried out on the island of Bioko, Equatorial Guinea through the administration of ivermectin once a year to the entire population of the island (Wilson *et al.*, 1997). On the island of Bioko, vector eradication is proposed by the WHO African programme for Onchocerciasis Control (APOC), and an understanding of the taxonomic nature of the vector will directly assist control strategy and the assessment and detection of potential re-invasion.

It is unlikely that vector control will form a significant part of onchocerciasis control in Nigeria and Cameroon, but the selection of communities for ivermectin treatment depends upon Rapid Epidemiological Survey (detection of symptoms in the endemic human populations) and this is best understood in terms of transmission epidemiology which depends (in part) on the nature of the vector. A Rapid Epidemiological Survey can only

assess the current status, but under changing environmental conditions (for example, deforestation and desertification) the vector populations can change and hence so can the epidemiology. This will not be detected for many years by Rapid Epidemiological Survey, but vector monitoring can give early warning.

The broad objective of the present research work therefore is to study the distribution of *S. damnosum* complex in parts of Nigeria, Cameroon and the island of Bioko in Equatorial Guinea.

## 1.1 OBJECTIVES OF STUDY

Specifically, the study is designed to:

- Determine the cytotaxonomy and distribution of *S. damnosum* s.l. cytospecies in different bioclimatic zones of Nigeria, in a broad north-south transect from Sudan savannah to rainforest, embracing parts of Kano, Kaduna, Plateau, Benue, Enugu, Imo and Cross River States.

- Investigate the cytotaxonomy and distribution of *S. damnosum* s.l. in south-western Cameroon and Bioko (Equatorial Guinea).

- Provide a standard step-wise guide for the specific identification of members of the *Simulium damnosum* complex in Nigeria towards the effective control of onchocerciasis.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 LIFE CYCLE OF BLACKFLY VECTOR OF ONCHOCERCIASIS

Female *S. damnosum* s.l. flies deposit their eggs in masses or chains on submerged rocks and stems of trailing vegetation in swift rocky rivers and at points where water currents are swift. All eggs are coated with a sticky gelatinous outer layer which readily adheres to any substrate with which it comes in contact (Gambrell, 1933). Kyorku (1982) has described the process of hatching in *S. damnosum* s.l.

The number of instars in the family Simuliidae is known to vary between six and nine in different species, and sometimes even in the same species (Craig, 1975; Ruhm and Sanders, 1975; Freden, 1976; Elouard, 1978; Ross and Meritt, 1978). Seven larval instars occur in *S. damnosum* s.l. (Davies and Crosskey, 1991). The mature final instar larvae have a characteristic blackened gill-spot (Crosskey, 1962) and it usually spins a discrete cocoon and pupates inside it. Masses of pupae are commonly seen in patches on rocks or leaves/stems of trailing vegetation at the breeding sites. Mature pupae from which adults are ready to emerge are darker than young ones. These pupae split dorsally and the adult fly rises rapidly to the water surface and flies off. The hatching process takes about one minute (Wenk, 1981).

Mating usually takes place quite close to the emergence site and the females proceed from there to obtain their first blood meals through biting Man or vertebrates, and return to the vegetation near the breeding place. They rest here until the eggs mature, in approximately four days (Lewis, 1956). Two modes of feeding are recognised in blackfly larvae, *viz.* browsing over substrates and filter feeding (Colbo and Wotton, 1981).

## 2.2 VECTOR TAXONOMY

### 2.2.1 Cytological Methods Using Polytene (giant) Chromosome Banding Patterns (Cytotaxonomy)

Based on the analysis of the banding pattern of polytene chromosomes from larval salivary glands, eight species of the *S. damnosum* complex were formally described from West Africa (Vajime and Dunbar, 1975). These were *S. squamosum, S. yahense, S. sanctipauli, S. soubrense, S. damnosum* s.s., *S. sirbanum, S. sudanense* and *S. dieguerense*.

The West African cytospecies all occur on a single phylogenetic lineage leading from Nyamagasani. *Simulium squamosum* has a basal position, in that it is closest to the other Central and East African species. The closest species to *S. squamosum* is *S. yahense* on one line of development. The remaining cytospecies all share the inversion 3L-2. *Simulium soubrense* and *S. sanctipauli* are on a distinct phylogenetic line of their own. *Simulium damnosum* s.s., *S. sirbanum,* and *S. sudanense* are on a third line of development, whilst *S. dieguerense* is separate from them (Vajime and Dunbar, 1975). Since Nyamagasani is designated as the complex standard (Vajime and Dunbar, 1975), it therefore, by definition, has no fixed inversion. It is an arbitrary choice and does not imply that Nyamagasani is the most primitive.

The first two siblings *S. squamosum* and *S. yahense* differ from each other in their mode of sex-chromosome differentiation. That of *S. squamosum* is based on the centromere region of chromosome 1. Here there is a non-pairing altered centric segment (1Ca) found mainly in males. The differential segment differs from the standard arrangement by its possession of a slightly larger expanded region and by lacking certain dark- staining bands characteristic of the standard sequence (Vajime, 1973; Vajime and Dunbar, 1975). In *S. yahense*, sex differentiation is due to an inversion in the 2L arm, 2L-18.

The descriptions of *S. sanctipauli* and *S soubrense* (Vajime and Dunbar, 1975) have been revised by Post (1986) and Boakye *et al.*, (1993). *Simulium sanctipauli* is now described as a sub-complex comprising *S. sanctipauli, S. soubrense, S. leonense* and *S. konkourense* (Boakye *et al.*, 1993).

In the initial description of *S. sanctipauli* and *S. soubrense* they were separated from each other on the basis of the chromosomal inversion 2L-7 (Vajime and Dunbar, 1975). This inversion was not regarded as strictly diagnostic due to the fact that though fixed in some mixed populations, it is polymorphic at other sites and distributed in Hardy-Weinberg equilibrium. Post (1986) has shown that a new inversion 2L-A yields better diagnosis than 2L-7 and so *S. sanctipauli* is differentiated from other members of the sub-complex by this inversion. *Simulium sanctipauli* "Djodji form" is a variant of this species with no fixed inversion differences from other populations of *S. sanctipauli*, but, sex chromosome differentiation here is associated with the inversion 1S-21, males are heterozygous for this inversion and it is absent in females (Surtees *et al.*, 1988). *Simulium leonense* was originally described by Post (1986) as *S. soubrense* "B" and is characterised by the inversion 1L-A. The inversion 2L-A (present in *S. sanctipauli*) is absent from this species. Sex linkage is found to be associated with 3L-4.17 in most populations (Boakye, 1993).

All members of the *S. sanctipauli* sub-complex lacking the inversions 1L-A and 2L-A are *S soubrense* (Post, 1986). Recent observations however show lots of fixed inversion differences between populations of *S soubrense* sometimes found breeding in sympatric situations. Some of these inversion differences have been raised to specific status and others are considered geographic variants and are designated as forms (Boakye, 1993). Those regarded as different species are included in *S. konkourense* (Boakye *et al.*, 1993). The *S. soubrense* forms are, Beffa form (Meredith *et al.*, 1983), Farmington and St Paul forms (Kashan and Garms, 1987), and Chutes Milo form (Boakye *et al.*, 1993). All these forms lack the inversions 1L-A and 2L-A.

*Simulium konkourense* includes two cytospecies, konkouré form (Quillévéré *et al.*, 1982) and menankaya form (Boakye *et al.*, 1993) with a fixed inversion 2L-D.7. Konkouré form also has the inversion 2L-4.X/4.X or 2L-4.X/4.6.D.7. These were formerly considered as forms of *S. soubrense* because they lacked the inversion 1L-A and 2L-A (Boakye, 1993).

*Simulium damnosum* s.s. and *S. sirbanum* are distinguished primarily by rearrangement of the 2L-arm (Vajime, 1973). This is a complex rearrangement of at least three steps, 2L-C, fixed in *S. damnosum* s.s., *S. sirbanum* and *S. dieguerense*. The sequence, 2L-C is a feature of *S. damnosum* s.s. The distinction between *S. damnosum* s.s. and *S. sirbanum* is the inversion 2L-8 (Vajime and Dunbar, 1975). This inversion is characteristically sex-linked in *S. damnosum* s.s. Males are heterozygous and females homozygous standard (without 2L-8 – Vajime and Dunbar, 1975). This inversion is fixed diagnostically in *S. sirbanum* (Vajime and Dunbar, 1975; Vajime, 1989). The sex-determining mechanism in *S. sirbanum* is based on a rearrangement on the IS arm, IS-3. Up to 95% of the females are homozygous standard and 62% of males are heterozygous, IS/3.

*Simulium sirbanum* and *S. sudanense* were originally considered by Vajime and Dunbar (1975) to be different species but the specific status of *S. sudanense* is not universally accepted (Bedo, 1977) and it has recently been synonymised with *S. sirbanum* (Vajime, 1989).

*Simulium damnosum* s.s. and *S. sirbanum* also differ in the proportions of 3L-6. 3L-2 is fixed in both cytospecies, whilst 3L-6 is polymorphic in both species but rare in *S. damnosum* s.s. and much more common in *S. sirbanum* (Vajime, 1973; Vajime and Dunbar, 1975).

The fixed inversions 1L-3.12 (Vajime and Dunbar, 1975) and 1L-35 (Post, 1982) remain unique to *S. dieguerense* and confirms its distinct nature. It is closest to *S. damnosum / S. sirbanum*, sharing inversion 3L-2 and the common basic 2L sequence (Vajime, 1973; Post,

1982). Boakye (1988) observed chromosomal morphology similar to that of Vajime and Dunbar (1975) and Post (1982) in *S. dieguerense* collected in Mali, Guinea and Senegal. The major difference observed by Boakye however was a sex-linked determining system on chromosome 1, an altered region found in 90% of males and 83% of females had normal pairing homologues. This was probably missed by earlier workers due to their small sample sizes.

The precise epidemiological importance of all the different West African sibling species of the *S. damnosum* complex in the transmission of onchocerciasis still remains unclear after many years of larval cytotaxonomic research (WHO, 1985).

The cytotaxonomic identification of adult vector females using malphigian tubule polytene chromosomes may yet prove to be useful under certain conditions (Procunier and Post, 1986). Through this method, it has become possible to demonstrate that the polytene chromosome banding pattern is conserved between larval and adult tissues, and it allows direct identification of man biting species. The identification of adult biting flies, which is an essential factor for vector incrimination would contribute greatly to a better understanding of the epidemiological importance of the different sibling species. However, adult cytotaxonomic identification is successful for only a small proportion of flies, and can only be carried out on blood-fed females.

### 2.2.2 Non-cytological Methods

There is an obvious requirement for the accurate routine identification of adult female flies. It is the adult female which transmits *O. volvulus*, and hence an understanding of transmission requires their identification. Unfortunately, adult flies do not possess routinely workable polytene chromosomes (Procunier and Post, 1986), and hence studies have sought alternative characteristics for examination.

One such method is the extraction and chromatographic analysis of the cuticular components. Carlson and Walsh (1981) applied the method in the separation of members of the *S. damnosum* complex and *S. squamosum* and *S. sirbanum* were separated according to quantitative differences measured in their cuticular hydrocarbons. Further studies by Phillips *et al.* (1985) followed, where the separation of four populations of *S. damnosum* complex from West Africa were carried out. These were *S. sirbanum, S. damnosum* s.s., *S. yahense* and *S. squamosum*. They found that quantitative differences in the amount of cuticular hydrocarbons in the siblings revealed both interspecific and intraspecific differences which allowed good identifications between all four populations of the sibling species. The important hydrocarbons in the differentiation process were determined by gas chromatography and mass spectrometry and were found to lie in the range $C_{22} - C_{35}$, branched and straight chained hydrocarbons.

Electrophoretic examination of enzymes is another method that is employed in species identification. In *Drosophila* it has proved to be not only a straight forward method for identifying species but has allowed for detailed examination of the genetic and evolutionary relationships between sibling species (Meredith and Townson, 1981). Two enzymes, phosphoglucomutase (PGM) and trehalase (TRE) have been found to be of value in species diagnosis of the *S. damnosum* complex. However, these two enzymes permit the identification of only two species, *S. yahense* and *S. squamosum* in samples collected from Mali, Cote d'Ivoire and Ghana. *Simulium yahense* has a unique PGM variant and *S. yahense* and *S. squamosum* have a different trehalase variant from *S. sirbanum*, *S. damnosum* s.s., *S. sanctipauli* and *S. soubrense* (Meredith and Townson, 1981).

Thompson *et al.* (1990) found that changing the electrophoretic conditions revealed a new PGM variant specific to *S. squamosum* from Sierra Leone and Togo. Morphologically identified *S. squamosum* from Cameroon has TRE and PGM bands indistinguishable from

those of *S. sanctipauli* and is therefore genetically distinct from *S. squamosum* from West Africa.

Very high separation of the major species groups of the complex, i.e. *S. damnosum* sub-complex, *S. squamosum*, *S. sanctipauli* sub-complex and *S. yahense* has been achieved through the use of multivariate morphotaxonomy (Wilson *et al.*, 1993). Two approaches were used, the first involved discriminate function analysis of the species groups using all the characters (qualitative and quantitative). The second approach sought colour character combinations unique to the *S. damnosum* sub-complex and to *S. yahense* to separate them from the rest. It also progressively applied discriminant analysis in a dichotomous stepwise manner to within the sub-complex level.

The use of DNA probes for the identification of members of the *S. damnosum* complex from West Africa has been attempted. Three cloned repetitive sequences, which show variation in copy number between sibling species have been isolated (Post and Flook, 1992). These clones can be used as probes for the dot-blot identification of larvae, pupae or adults into the three main West African sub-complexes-*damnosum*, *squamosum* and *sanctipauli*. Whilst the technique was applied successfully in Sierra Leone and Guinea, specimens from Ghana and Togo could not be identified.

## 2.2.3 Species Hybridisation

Natural hybridisation has been observed between species from different *S. damnosum* s.l. sub-complexes. Though these are rare occurrences, they are usually easily recognisable from heterozygous diagnostic isozymes or polytene chromosome inversions. A few cases of interspecific hybridisation have been recorded (Vajime and Dunbar, 1975; Vajime and Quillévéré, 1978; Boakye and Mosha, 1988).

A natural hybrid between *S. sanctipauli* and *S. sirbanum* (frequency of hybridisation, 0.0009) has been observed from larvae collected from the river Sankarani in Eastern Guinea (Boakye and Mosha, 1988). A hybrid was observed by Vajime and Dunbar, (1975) between *S. yahense* and *S. sanctipauli* from Zozola stream in Cote d'Ivoire. Hybrids of *squamosum/sanctipauli, yahense/soubrense* and *yahense/sanctipauli* were observed in Cote d'Ivoire (Vajime and Quillévéré, 1978). Meredith *et al.*, (1987), have successfully crossed males of the Beffa form of *S. soubrense* with female S. *squamosum* experimentally.

Although the degree of fertility and relative viability of hybrids and back cross progeny are not yet known (Post, 1984), the occurrence of these hybrids would create opportunity for gene flow (introgression) between species of the *S. damnosum* complex. The possibility would then exist for the movement (e.g. genes responsible for insecticide resistance) from one cytotype to another and this exchange of genes between populations could have important implications for the control of onchocerciasis (Meredith *et al.*, 1987; Boakye and Mosha, 1988).

## 2.3 Breeding Site Characteristics

All natural fresh waters contain dissolved and particulate inorganic and organic compounds, and gases which vary depending on place, season, time of day, depth and biota. These compounds are vitally important to the existence of aquatic organisms (Grunewald, 1981). Their physico-chemical environment influences the immature stages of *Simulium* that inhabit these various water types. Not much documentation exists on the correlation between physico-chemical parameters of the aquatic environment and its blackfly fauna. Results so far available however have been mostly obtained through field observations.

Generally, temperature of the water is one of the major aspects of the breeding site that affect the distribution of immature stages of *Simulium* larvae in natural streams. In the savannah and tropical rain forest zones of West Africa, *S. damnosum* / *S. sirbanum* and *S.*

*sanctipauli / S. soubrense* have been found to develop at water temperatures ranging from 24°C to 33°C, with a mean value of 27°C (Grunewald, 1981). *Simulium yahense* in the rain forest zone has been found in environments with lower temperatures (23-30°C), mean value, 25°C (Grunewald, 1976a, 1976b; Quillévéré *et al.*, 1976, 1977).

Water current is a physical parameter known to affect organisms in lotic habitats. Although examples of Simuliid larvae completing development in totally stagnant water are known (Crosskey, 1973), the majority of species require flowing water for larval development (Colbo & Watton, 1981). Blackfly larvae are known to depend upon a particular current velocity, not only for their feeding activities, but also for preventing the accumulation of oxygen depleted water around them (Grunewald, 1981). Members of the *S. damnosum* complex have been recorded in sites with current velocity ranging from 0·4m/s to 2·4m/s (Elsen & Hebrard, 1977).

Hydrogen ion concentration and conductivity (related to the overall concentration of dissolved solids) are equally important. *Simulium damnosum* s.s., *S. sirbanum*, *S. squamosum*, *S. soubrense*, Sanje, Nkusi, Nyamagasani and Jovi have been found to prefer habitats characterised by weak acidity to weak alkalinity (Grunewald, 1976a,b; Quillévéré *et al.*, 1976; 1977). Kibwezi and Kisiwani larval habitats were characterised by high alkalinity (pH 7.7- 10) and conductivity values from 400 - 950 µS due to high content of dissolved solids.

The habitats of *S. yahense* and *S. sanctipauli* larvae on the other hand were characterised by acidity (pH 5.7 - 6.2) and conductivity below 50 µS (Grunewald, 1976a, 1976b). However, Quillévéré *et al.*, (1976; 1977) found *S. sanctipauli* in Cote d'Ivoire in alkaline water with conductivity values higher than 100 µS.

Breeding site characteristics of *S. damnosum* s.l. in Nigeria were observed by Mafuyai *et al.* (1996). The observations here showed a pH of 6·9 – 7·7, 7·1 – 7·9 and 6·5 – 6·9 for savannah, montane and forest sites respectively. Most of the *S. damnosum* s.s. and *S. sirbanum* were recorded in sites with pH above 7·2. *Simulium yahense* was found at a single locality where the pH measured between 6·5 and 6·8 and *S. squamosum* was found over a broad range of pH (6·9 – 7·9). The Nigerian sites studied were found to have a rather low conductivity (Mafuyai *et al.*, 1996) compared to others reported in West Africa (Grunewald, 1981). Water velocity range for *S. damnosum* s.l. in Nigeria was 0·99 – 2·124m/s, but with no obvious prefernce for individual cytospecies.

## 2.4 BLACKFLIES AND DISEASE TRANSMISSION

Onchocerciasis is a parasitic disease resulting from infection with the filarial worm *Onchocerca volvulus*. The parasite is transmitted through the bite of some species of blackfly belonging to the genus *Simulium*, which breed in fast-flowing streams and rivers. The adult worms live in nodules in the connective tissues of the body, some of these nodules are visible and palpable in the subcutaneous tissue (Duke, 1990). The male worms are small and mobile (5 cm x 0.1 - 0.2 mm) and the females very long and thin (20 - 50 cm x 0.25 - 0.45 mm). These females can live up to about 12 - 15 years, during which time they bring forth many millions of living larvae known as microfilariae which invade the skin of the host and await ingestion by a suitable female *Simulium* vector when it comes to take a blood meal from that host (Duke, 1971).

A great number of the microfilariae in the skin of the host never get ingested by the *Simulium* fly and eventually die in the tissue causing scarring and disease. Their presence and death initially causes itching and papular rashes. With prolonged infection, lesions, fibroses, atrophy and pigmentary changes usually develop. Some microfilariae may invade the eyes where they can damage the delicate ocular tissues (cornea, iris, ciliary body, retina

and choroid and optical nerve) resulting in severe ocular disease and eventually to ("river") blindness (Duke, 1990). Some microfilariae may invade the lymph glands, causing inflammation, enlargement and scaring. In severe infections, the microfilariae may enter the blood stream and invade the deep organs of the body (Meryers *et al.*, 1977) bringing about a wide variety of clinical manifestations such as, epilepsy, wasting and dwarfism (Duke, 1990). Since the vectors have an aquatic larva requiring flowing water, the disease is usually associated with watercourses, hence its name, "river blindness".

The transmission of *O. volvulus* occurs when a *Simulium* female is obtaining blood meal from the host. Blackflies biting an infected person ingest microfilariae along with the blood meal. Microfilariae usually invade the skin and lie in the sub-epidermal layer awaiting ingestion by the feeding *Simulium*. When ingested with the blood meal, they penetrate the fly's gut, enter the flight muscles and grow through several moults to become infective larvae. This infective larva is referred to as the third (and final) stage larva and is usually found in the proboscis and thorax of the vector. The infective larva of the fly may enter and infect a new host through the laceration caused by the fly in the process of obtaining another blood meal (Blacklock, 1926; Duke, 1973; 1990).

## 2.5 VECTOR AND DISEASE PREVALENCE OF ONCHOCERCIASIS

Crosskey (1981a) has described the distribution of Simuliidae as being cosmopolitan. Blackfly fauna exist in every part of the world where there is running water of sufficient permanence to provide the biological requirements of the developmental stages. Blackflies are absent only from regions that are wholly hostile to them because of total absence of flowing water as in the polar regions and barren deserts. Simuliid vectors of human onchocerciasis are known in both the Old and New World (Crosskey, 1981b; Procunier,

1989). Whenever the vectors are members of the African *S. damnosum* species complex, the foci tend to extend along rivers, coalescing into larger areas where there are many contiguous breeding sites, as for example, in the Volta River Basin. Other African foci in which *S. neavei* group species are vectors tend to be highly localised near small mountain streams containing suitable host crabs for larval blackflies (Jamnback, 1976).

In terms of the frequency of occurrence of these species, a generalisation was made, that six are dominant - *S. squamosum, S. yahense, S. soubrense, S. sanctipauli, S. damnosum* s.s., *S. sirbanum* (Vajime and Dunbar, 1975; Vajime and Quillévéré, 1978). The rare species were said to be *S. mengense / S. dieguerense*. The cytotaxonomy of all the above species have been described by Vajime and Dunbar (1975) except *S. mengense* from the River Menge in western Cameroon and found in Tanzania (Vajime and Dunbar, 1977). This species and *S. damnosum* s.s. are the only two members of the *S. damnosum* complex reported from both East and West Africa (Dunbar and Vajime, 1981). A summary of the distribution of *S. damnosum* in West Africa is given in Table 2.1.

Post (1982), Quillévéré (1975), Quillévéré *et al.* (1976) and Boakye (1988) have found more samples of *S. dieguerense* in Mali. It was an initial paucity of material that gave the impression that this species was very rare and therefore of little vectorial importance in the transmission of human onchocerciasis (Dunbar and Vajime, 1981). The distribution of *S. dieguerense* has been found to be limited to the western part of the Onchocerciasis Control Programme (OCP) Area (Guinea, Mali and Senegal) since no identification has been recorded in the east (Boakye, 1988). The species has been found to have seasonal movements similar to those recorded for the other savannah species (Boakye, 1988).

**Table 2.1** - *Simulium damnosum* complex in West Africa. *(Modified from WHO, 1995a).*

| *S. damnosum* Complex | Principle Area | Habitat |
|---|---|---|
| ***S. squamosum*** **Sub-Complex** | | |
| *S. squamosum s.s.* | Cameroon, Togo, Ghana, Cote d'Ivoire, Bukina Faso, Nigeria | Forest, Guinea Savannah |
| *S. yahense* | Togo, Ghana, Cote d'Ivoire, Guinea, Sierra Leone, Liberia, Nigeria | Forest, Montane Forest, Guinea Savannah |
| *S. mengense* | Cameroon | Forest |
| ***S. damnosum*** **Sub-Complex** | | |
| *S. damnosum* s.s. | Cameroon, Nigeria, Togo, Ghana, Cote d'Ivoire, Liberia, Guinea, Burkina Faso, Mali, Niger, Senegal | Forest, Guinea Savannah, Sudan Savannah |
| *S. sirbanum* | Nigeria, Togo, Ghana, Cote d'Ivoire, Guinea, Gambia, Burkina Faso, Mali, Niger | Forest, Guinea Savannah, Sudan Savannah |
| *S. dieguerense* | Mali | Sudan Savannah |
| ***S. sanctipauli*** **Sub-Complex** | | |
| *S. sanctipauli s.s.* | Ghana, Cote d'Ivoire, Liberia, Sierra Leone | Forest |
| *S. soubrense* | Togo, Cote d'Ivoire, Liberia, Guinea, Burkina Faso, Benin, Nigeria | Forest, Guinea Savannah |
| *S. leonense* | Sierra Leone | Savannah |
| *S. konkourense* | Sierra Leone, Guinea | Savannah |

*Simulium dieguerense* occurs in onchocerciasis endemic areas and therefore could be a vector. Its vectorial role however has been difficult to assess due to its sympatry with *S. sirbanum*, a savannah cytospecies of great vectorial importance. Also, adult populations of *S. dieguerense* have not been morphologically studied to determine whether differences exist between them.

In viewing the vegetational zones where dominant species sub-complexes occur, irregularities are observed in their distribution within the different types of vegetation. In nature, these zones are not absolutely demarcated and forest/savannah mosaic areas occur with islands of forest within the savannah and vice-versa. These irregularities in the

distribution of the vegetational zones have brought about slight differences in reports of forest and savannah species (Vajime and Quillévéré, 1978; Vajime, 1976; 1981).

*Simulium sanctipauli* seems to be the only forest species that has not been found outside the forest zone (Garms and Vajime, 1975). The Djodji form of *S. sanctipauli* is found in Togo and Ghana. The distribution of the remaining forest species (*S. yahense* and *S. squamosum*) shows *S. yahense* to be more restricted to the forest zone than *S. squamosum*, which appears to be more opportunistic and has been found to extend into both Guinea and Sudan savannah. It has been found as far north as Bobo Dioulasso, in Burkina Faso, Tamale in Ghana and Lama-Kara in Togo (Garms and Vajime, 1975; Vajime, 1976).

The typical savannah species, *S. damnosum* and *S. sirbanum* have the widest ranges with extensive overlapping distribution in a north-south direction. *Simulium sirbanum* predominates in the Sudan savannah belt especially in the arid areas of this region which lie towards the northern limits of the *S. damnosum* complex in Mali, Bukina Faso and Niger. On the other hand, *S. damnosum* predominates in the Guinea savannah and transition zones in which the species is sympatric with smaller populations of *S. sirbanum* (Garms and Vajime, 1975; Vajime, 1976).

In the OCP area, *S. damnosum* s.s. extends north into Mali and is essentially a savannah dwelling species, but adapts itself to more wooded biotypes. *S. sirbanum* predominates in the northern part of the control area. It shows a strong preference for extreme savannah conditions. In Benin and Togo, *S. damnosum* has been observed in many rivers except in mountainous streams. *S. squamosum* was breeding mostly in mountainous and hilly areas of Togo, *S. soubrense* and *S. sanctipauli* were found in most rivers in both countries, though most common in southern areas and this was suspected to be the source of re-invasion (Garms *et al.*, 1982). The Beffa form of *S. soubrense* was also found in Togo / Benin (Meredith *et al.*, 1983).

Similarly, in Liberia and Guinea, outside the OCP, *S. damnosum* complex species were also found to exhibit marked differences in their ecology and distribution. The species common in the rain forest zone were mainly *S. sanctipauli* and *S. yahense*. *Simulium sanctipauli* was found inhabiting large rivers and *S. yahense* showed preference for small water courses (Garms and Vajime, 1975). *S. soubrense* and *S. damnosum* were found breeding in both forest and savannah zones while *S. sirbanum* appeared to be a pure savannah species confined to the northern Guinea savannah zone and the periphery of the Fouta Djallon mountain. The habitat preference of the different sibling species may also be partly dependent on their hydro-chemical requirements (Grunewald, 1976a) and differences in water temperature (Ocran *et al.* 1982).

The Nile and Sanje complexes of the East African species of the *S. damnosum* complex consist of the forms, Kagera, Kulfo, Kaku, Ketaketa, *S. damnosum* s.s. sub-complex and Nyamagasani, Sanje, Nkusi, Kisiwani, Sebwe, Kibwezi and Mutonga in the Sanje sub-complex (Dunbar, 1967; Dunbar and Vajime, 1971). Several species of the Sanje sub-complex have been observed breeding in the same river, though at different locations. The controlling factor has been found to be the hydrochemistry of the water/environment with pH as the best single indicator. Sebwe form occurs in the higher reaches at pH 6.4, with increasing hydrogen ion content, it is replaced by Sanje and Nkusi forms at pH 7.8 (Dunbar and Grunewald, 1974). The eastern Kagera and Kulfo forms prefer coming from upland areas. Ketaketa subgroup is found in rivers through lowland country like the *S. damnosum* s.s. sub-complexes in the west (Dunbar and Vajime, 1981)

The non-*S. damnosum* complex vectors of the *S. neavei* group are found in Zaire and East African streams, breeding on the carapaces of crabs (genus *Potamonautes*). The group contains about nine described species and some forms of uncertain taxonomic status (WHO, 1987).

*Simulium neavei* group occurs widely but sporadically in Africa, from Ethiopia southwards to Malawi and westwards through the Zaire river basin. *S. neavei* s.l. and *S. damnosum* s.l. have been seen to occur together in many foci and act in conjunction as vectors, for instance, in the foci of eastern Zaire, the central highlands of Tanzania, south-western Ethiopia and some areas in Uganda (WHO, 1987). Foci that are maintained entirely by *S. neavei* group vectors include the Mount Elgon and Bugoma foci of Uganda (*S. neavei* s.s.) and the Usambara (Amani) focus in Tanzania (*S. woodi*).

The distribution of *S. neavei* group in relation to the spread of onchocerciasis shows *S. ethiopiense* maintaining a moderate level of transmission in Ethiopia, *S. nyasalandicum* is the vector in Malawi and is also found in Tanzania though *S. woodi* is the vector in Tanzania's Usambara mountains. *Simulium neavei* s.s. is the well known vector in Uganda and Zaire but in Kenya, it has been eradicated (WHO, 1987). *S. neavei* vectors have a restricted flight range and are usually confined to forest or bush-lined rivers protected from the direct rays of the sun and from wind (Jamnback, 1976).

The second World Health Organisation (WHO) Expert Committee on Onchocerciasis held in 1966 estimated a global total of 20 million persons infected based on a 1947 estimate. Global figures collected in 1985 and collated in the third report of the WHO Expert Committee on Onchocerciasis (1987) gave the global estimate of persons living in endemic areas and exposed to the risk of infection to be 85.6 million. Out of these, 17.8 million were infected, 336,400 were blind from ocular onchocerciasis and a similar number were thought to be suffering severe visual impairment. Africa (and the Yemen) accounted for 17.7 million infected and 335,000 blind. The remainder (<0.6%) were in Latin America. Most recent estimates of the number of people infected has been provided by WHO (1995a). They estimate that 17.5 million people in Africa and Yemen and 140 455 people in the Americas have the disease. The decrease in prevalence and near zero incidence of new infection since

1975 in the area of successful Onchocerciasis Control Programme (OCP) in West Africa is a contributing factor to the current decreasing global estimate.

The epidemiology of onchocerciasis is broadly distributed over the wide savannah areas, extending from the southern margins of the Sahara desert in Eastern Senegal, eastward to southern Chad and south into Angola. It also extends into adjacent parts of Sudan and Zaire. Its distribution in eastern Africa is focal, and extends from Ethiopia in the north to Malawi in the south. The foci of onchocerciasis in eastern Africa are widely varied. Little or no information is available on the disease and its vector in some countries like Burundi, Mozambique, and Zambia, whereas data on countries like Kenya and Tanzania are extensive. Eradication of onchocerciasis had been achieved in foci in two countries, namely Kenya and Uganda.

## 2.6. VECTOR AND DISEASE DISTRIBUTION IN NIGERIA

Crosskey (1956; 1981b) pointed out that the geographic distribution of *S. damnosum* in northern Nigeria was governed by its geology. There is therefore a clear correlation between the occurrence of endemic onchocerciasis and basic geology. Vector and disease are largely circumscribed to areas where the Precambrian basement rock forms the land surface and is exposed in the river-beds in the shape of resistant rocky rapids that break the flow producing rapids that provide the classic type of fly breeding-habitat. Diverse landscape have been produced like the extensive plains in Kano, rocky mountainous areas in former Gongola State and undulating semi-forest country in south western Nigeria. All these provide many rocky-bedded rivers extensively inhabited by the vector (Crosskey, 1981b).

In the mid north and north-east, minor areas of volcanic intrusion occur that provide conditions of broken water similar to the Precambrian rapids. The largest is the Biu Plateau

which gives rise to the ancient lava composing the Hawal bed of the Hawal River on the border of the former Borno and Gongola States. Continuos natural vector breeding is found in the Precambrian rapid between Kainji dam and Jebba, but no natural breeding is observed in the sedimentary reaches upstream from Jebba in Kogi State.

Budden (1956) found the distribution of endemic onchocerciasis to be limited by the distribution of the *Simulium* vector. The disease was found to occur in each of the 12 provinces of northern Nigeria which include Adamawa, Bauchi, Benue, Borno, Ilorin, Kabba, Kano, Katsina, Niger, Plateau, Sokoto and Zaria. In these areas, no obvious correlation was found between the biting rate of *S. damnosum* and the incidence and intensity of infection in the human population. In Abuja, the infection rate was found to be moderate but with an abundance of flies.

Breeding sites of six species of the blackfly (*Simulium* sp.) have been mapped out along six streams and rivers in Anambra State (Iwuala and Ezike, 1980). In Cross River State, Braide *et al.*, (1980) reported breeding sites of the blackfly (*Simulium* sp.) along sixteen streams and rivers, and found infected individuals in Obudu, Ogoja, Akamkpa, Ikom and Calabar local government areas.

In eastern Nigeria, forest cytospecies of *S. yahense* and *S. squamosum* constitute a substantial proportion of the flies present there while the population of savannah cytospecies is relatively smaller and apparently concentrated in the northern localities (Akpa, 1988). It has been observed that there could be possible movement of savannah cytospecies into the forest zone in the dry season in Nigeria (Akpa, 1988).

A summary of information so far available on the distribution of *S. damnosum* complex species in Nigeria shows *S. damnosum* s.s., *S. squamosum*, *S. sirbanum* and *S. soubrense* to be present in the area north of the Benue-Niger confluence. South of the river Benue, with

the river Niger as the western border are found *S. damnosum*, *S. squamosum*, *S. sirbanum* and *S. yahense*. In the western part of the country (with River Niger as the upper limit) are *S. damnosum*, *S. squamosum*, *S. sirbanum* and *S. sanctipauli* (Vajime *et al.*, 1987).

More recent cytotaxonomic surveys of known onchocerciasis areas in Nigeria (Mafuyai *et al.*, 1996) revealed the presence of *S. damnosum* s.s. (including Volta form), *S. sirbanum* (including *S. sudanense*), *S. squamosum, S. yahense, S. soubrense* and the Beffa form of *S. soubrense* from four bioclimatic zones (Sudan savannah, Guinea savannah, montane forest and forest). *Simulium damnosum* s.s. and *S. sirbanum* were both found to be widespread in the savannah, with *S. sirbanum* the more northerly of the two. *Simulium damnosum* s.s. was observed in a rain-forest site, river Oshun at Ede, in south-western Nigeria. *Simulium yahense,* normally considered a forest dwelling cytospecies, was identified from a single forest location, Agbokim waterfalls in Cross River State. *Simulium squamosum* was widespread across Nigeria in montane forest, forest and Guinea savannah zones but not in the Sudan savannah zones. It was the dominant species in the montane area throughout the year. The Beffa form of *S. soubrense* was found in the major rivers of the south-west. Mafuyai *et al.* (1996) confirmed in their study that a relationship existed between vector distribution, geology and bio-climatic zonation.

Endemic onchocerciasis is known in the Galma valley (Crosskey, 1953). This valley lies between Zaria and Jos and its river is about 160 kilometres east of Kaduna. The Galma River pursues a north-westerly course to the outskirts of Zaria. A survey of 3,000 persons throughout the Galma valley (32 villages and hamlets) showed 1,116 individuals (37.1 %) to have microfilaria in their skin snip, 318 (10.6 %) to have nodules and 32 (1.1 %) individuals to be totally blind.

Furthermore in Kaduna State, onchocerciasis has been reported in seven villages in Kachia local government area and is said to be mesoendemic in three of these, Iri, Iburu and Doka

(Galadima, 1989). In the Hawal Valley of northern Nigeria (Borno State), onchocerciasis has been found to be prevalent and it has led to village desertion (Bradley, 1972). Not only were the victims of onchocerciasis found to desert the village, but the continued development of village life was deterred compared to areas free from the disease. Kainji dam in Niger State was completed in 1968 and the lake thus formed inundated the stretch of the River Niger which hitherto had notorious foci for *S. damnosum* breeding and onchocerciasis transmission (Adekolu-John, 1982). This change could probably explain the low prevalence of onchocerciasis in the lakeside villages above the dam as compared to villages close to the River Niger below the dam and the River Oli. The lakeside villagers were found to have started to lose their parasites due to the natural control provided by the newly created lake. Kanji lake is located within the northern Guinea savannah. Galadima (1989) working in Niger State observed that onchocerciasis was hyperendemic in four villages (Bari, Kwakuti, Kaliko and Zungu). Gemade and Dipeolu (1983) found *S. damnosum* at Kuhe, Manor and Gube areas of Kawada local government area of Benue State. The flies breed at various points along the rivers Amire-Kiriki and Katsina Ala. Out of 1,558 individuals examined in some villages in Benue State, 1,227 (78.8 %) were found to be infected with *O. volvulus* (Gemade and Utsalo, 1990). The mean microfilarial density was about 20 per skin snip. Prevalence of the disease showed a sharp rise between ages 6-12 years, more or less stable density between ages 20-50 years followed by a drop after the age of 50 years and above.

In Plateau State, the disease has been observed (11.1% infection rate) with low blindness rate in the Assob River area (Onwuliri *et al.*, 1987). Higher infection rates were observed in the Jarawa District area which is surrounded by fast flowing tributaries of the Jarawa river system (Nwoke *et al.*,1989). The usual clinical manifestation of onchocerciasis, skin depigmentation, hanging groin, and nodules were observed in different parts of Plateau State (Nwoke *et al.*, 1987a), and 22.3 % of the sampled individuals were positive for microfilaria of *O. volvulus* in their skin (Nwoke *et al.*, 1987b). The Plateau State studies

also showed a sex and age related difference in onchocercal infection. Males had a significantly higher infection rate and percentage infection which showed a gradual increase with age. Further studies of 41 villages in Plateau State showed differences in endemicity and intensity of infection between villages. It was observed also that the number of both microfilariae carriers and cases of clinical onchocerciasis increased with age (Ufomadu *et al.*, 1992). In southern, eastern and western Nigeria, the distribution of the disease and its vector have not been widely investigated as in the northern parts. Studies however have shown that onchocerciasis is more prevalent in the northern and eastern states than in the western States of Nigeria (Hunponu-wusu and Somorin, 1977). In Lagos State, they found onchocerciasis in only 95 patients, the majority of who came from endemic foci outside Lagos in the former states of Bendel, Imo, Ondo, Cross River, Anambra, Benue and Kwara. Ogunba (1981) reported 48.8 % onchocerciasis positive cases in Idi-Ata village in Oyo State. Wyatt (1971) observed onchocerciasis in the Ibarapa area of Oyo State. The area has savannah vegetation and numerous small rapidly flowing seasonal rivers with rocky beds. Infection here was found to be probably less in northern Nigeria due to the observation of lower count of microfilaria per mg of skin and the smaller number of subcutaneous nodules (Kershaw *et al.*, 1954; Budden, 1956).

## 2.7 Disease and Vector distribution in Cameroon and Bioko

In Cameroon, Anderson *et al.* (1974) observed that savannah onchocerciasis was characterised by a higher skin concentration of microfilariae, more skin atrophy, more microfilarial invasion of the cornea and 2·5 times as much blindness. By contrast, in the heavily infected forest villages, there were more nodules, more skin depigmentation and more cases of hanging groin. A four year follow up of an onchocericiasis study in Cameroon (Anderson *et al.*, 1976) showed that although the incidence of blindness was low in the rain forest, the overall incidence of impaired vision and ocular pathology was hardly

less than in the savannah. The lesions however were less severe and occurred at a later age. Regarding annual transmission of onchocerciasis in the rain forest, transmission potential of *S. damnosum* has been found to depend mainly on the absolute numbers of fly bites and to a lesser extent on the microfilarial reservoir available to these flies (Duke *et al.*, 1972).

The prevalence of infection and disease due to *Onchocerca volvulus* in regions at different altitudes and distances from the nearest *Simulium* breeding sites in a forested area of Cameroon showed altitude to be positively correlated and distance from breeding site to be negatively correlated with several clinical indicators of onchocerciasis (Menduza Aldana *et al.*, 1997). Specimens of *S. damnosum* from Cameroon were collected as early as 1916 in Tinto, 1932 in Victoria and 1950 in Kumba (Lewis, 1960). *Simulium mengense* was discovered as a new species (Vajime and Dunbar, 1977) in the Menge river in Cameroon after the initial report of Vajime and Dunbar (1975) describing the original eight cytospecies of the *Simulium damnosum* complex in West Africa.

A survey of *Simulium* vectors was conducted by Renz and Wenk (1984) along the main water courses in the Tcholliré (8°24'N, 14°10'E) and Touboro (7°46'N, 15°21'E) foci of northern Cameroon. These foci are situated between the Guinea savannah area to the south (Adamawa mountains) and the Sudan savannah plains to the north towards Garoua, where onchocerciacis is not endemic. Results have showed the vectors of onchocerciasis to be mainly *S. damnosum* s.s. and *S. sirbanum* together with small proportions of *S. squamosum.*

A survey of the northern part of Cameroon showed *S. damnosum* s.s. and *S. sirbanum* to be common in the savannah zones, while *S. squamosum* was found principally in the forest zones. *Simulium yahense* occurred locally in the west and *S. mengense* was found scattered widely throughout the area (Traore-Lamizana & Lamasson, 1989).

The presence of *Onchocerca volvulus* was first described on the island of Bioko in 1932 (Pittaluga and Goyanes, 1932). In 1936, the disease, onchocerciasis was described here (Najera Angula, 1936) and in 1954, a survey conducted in the northern and southern part of the island revealed 2·5% onchocerciasis carriers among 4 000 examined persons (Gonzalez Vincente, 1956). Onchocerciasis prevalence has been found to be greater in Bioko than on the mainland part of Equatorial Guinea (Calvo Pico, 1962). The most recent survey found onchocerciasis to be hyperendemic with a prevalence of 75% and almost the entire population of 62 000 inhabitants is estimated to be at risk from the disease (Mas *et al.*, 1990; 1995). Epidemiological studies in the area have revealed the usual symptoms of the disease such as the presence of palpable nodules (onchocercomata) dermatitis, pigmentation changes, cutaneous atrophy and lymphoedemas (Mas *et al.*, 1995). Blindness rate on the island is similar to that seen in the forest endemic areas of West Africa (WHO, 1984).

The vector of onchocerciasis in Bioko is known to be a member of the *S. damnosum* species complex (Cheke *et al.*, 1997). Cytotaxonomic identification of the larvae from this area revealed a "Bioko form" which is closely related to *S. yahense* (Wilson *et al.*, 1994). All the larval population from Bioko have been found to be similar to each other (Wilson *et al.*, 1997) and closely related to *S. squamosum* and *S. yahense*. Full karyotype analysis failed to reveal fixed inversion differences but the Bioko population were found to be cytotaxonomically distinct from both *S. squamosum* and *S. yahense* in terms of sex-linkage of inversion 2L-18. This inversion is normally considered to be absent from *S. squamosum* and X-linked in *S. yahense* (Boakye, 1993), but in Bioko, it is strongly Y-linked in all populations (Wilson *et al.*, 1997). Morphological, protein electrophoresis and DNA studies have all confirmed that the "Bioko form" of *S. damnosum* is taxonomically distinct and genetically isolated from *S. damnosum* s.l. populations on the mainland, but classified within the *S. squamosum* sub-complex and closely related to *S. yahense* (Wilson *et al.*, 1994).

All adult female flies from Bioko have been classified as *S. yahense* on the basis of their morphological characters such as dark fore coxae, dark antennae, dark wing tufts, black arculus and dark scutellar setae (Wilson *et al.,* 1994). Evidence of the relative isolation of the "Bioko form" cytospecies has been drawn from the prevailing wind direction, which blows across the island towards the mainland (Teran, 1962). It is thought to be highly unlikely that flies could migrate to Bioko from the west. Also, it is extremely unlikely that flies could migrate to the island from the east or north-east, because this is contrary to the usual prevailing winds. Easterly winds are known to be often associated with line squalls. In other parts of West Africa, available evidence suggests that line squalls do not carry migrating *S. damnosum* s.l. (Garms *et al.,* 1979; 1982; Cheke and Garms, 1983; Baker *et al.*, 1990).

However, while population analysis show the distinct nature of the Bioko population, individual specimens can not be identified with certainty due to the absence of fixed inversion differences. In summary, morphological evidence suggests that members of the *S. damnosum* complex in Bioko are a form of *S. yahense*, but, clearly are not *S. squamosum.* This evidence regarding affinity to *S. yahense* has important implications when considered against the background that *S. yahense* is one of the most sedentary members of the *S. damnosum* complex (Wilson *et al.,* 1997).

## 2.8 VECTOR CONTROL AND CHEMOTHERAPY

Chemo-therapeutic agents against *O. volvulus* have long been used as a means of treating onchocerciasis but the development of chlorinated hydrocarbon insecticides revolutionised the prospects for vector control (Walsh *et al.*, 1979). Several *Simulium* control schemes were executed in Africa in the late 1940's and 1950's. There was the dichloro-diphenyl-trichloroethane (DDT) larviciding waged in Kenya against *S. neavei* which resulted in its eradication from the country (McMahon *et al.*, 1958).

Larviciding with DDT was also attempted in Abuja, Nigeria, but although a great reduction in vector populations was achieved for several years, the epidemiological results were disappointing (Davies *et al.*, 1962; Davies, 1968). In Nigeria, a fairly large area was brought under control to protect the work force engaged on the Kainji hydro-electric project in Niger State (Hitchen and Giony, 1966). Though there was a regular influx of *S. damnosum*, the limited objectives of their campaign were achieved (Walsh, 1970).

A form of control without chemicals has been observed in Tanzania (Muro and Mziray, 1990; Muro and Raybould, 1990). Here increased exposure of breeding sites by deforestation seems to have produced a decline in the *S. neavei* population. For ecological reasons however, deforestation can not be recommended as a control means for onchocerciasis (Muro and Mziray, 1990) but information on the fate of vectors when natural forest is replaced by plantations and other farming activities could be useful (Muro and Raybould, 1990).

The Onchocerciasis Control Programme (OCP) under the auspices of the WHO was established in West Africa in 1974. This programme came as a result of the recognition of major health problems the disease onchocerciasis posed in the savannah belts. Its original area of operation included Burkina Faso, Ghana, Cote d'Ivoire, eastern Mali, Togo, Niger and Benin. In 1987/88, its activities were extended to include parts of Guinea –Bissau, western Mali, Senegal and Sierra Leone. The initial control strategy used by OCP was vector control involving weekly larviciding (WHO, 1985). Now in its final stage, treatment with ivermectin has been incorporated into its control activities. This is because despite the success of the OCP campaign based on *Simulium* larviciding, it realised that improved chemotherapy would be essential to maintain gains made against onchocerciasis after the larviciding might have stopped, as was scheduled at the time for 1997, or in the event of multiple insecticide resistance (WHO, 1981).

By 1994 (twenty years since its establishment) the OCP had recorded outstanding results (WHO, 1994) with onchocerciasis virtually removed from the original seven countries and no new infections occurring throughout the extension areas. In more specific terms, 9 million children born within the original OCP area since programme operations started had been spared the risk of onchocercal blindness, 30 million people protected from infection, 185, 000 had been prevented from going blind and 1.5 million had lost their onchocercal infection (Molyneux and Davies, 1997; WHO, 1994).

However, to sustain this success and ensure the continued elimination of onchocerciasis in these countries of West Africa, the public health services are being equipped to detect and deal with instances of transmission that may occur. This devolution process, where countries themselves assume responsibility for onchocerciasis control is OCPs plan for sustainability of its achievement.

Ivermectin, produced by Merck, Sharp and Dohme (MSD) and produced under the name Mectizan, is the first new drug to be registered for treatment of human onchocerciasis in the last 40 years (Awadzi *et al.*, 1985). Ivermectin is now supplied free of charge to individuals and organisations in the endemic areas. On the basis of pre-registration clinical trials and community trials (Awadzi *et al.*, 1985; Brown and Neu, 1990; Remme *et al.*, 1990; Taylor, 1990), ivermectin has been found to be an effective non-toxic, single dose (150 mcg/kg) microfilaricide. It has the added advantage of being able to act as a microfilarial suppressant by blocking the exodus of microfilaria from the uteri of the adult female worm. By drastically reducing the numbers of microfilariae in inhabitants of an area and thus reducing the chances of blackflies picking up the parasite, ivermectin can therefore lower the rate of the transmission of the diseases.

The OCP has supported extensive research (through the Onchocerciasis Chemotherapy Project (OCP) and Macrofil) to seek an effective chemotherapeutic agent which will kill

adult *O. volvulus*. The prospects of finding a compound with the characteristics of Mectizan *i.e.* a safe drug with limited side effects which can be used for mass treatment without medical supervision, is remote within the time frame of the programme (Molyneux, 1995). One of the candidate compounds, Amocarzine, has been assessed as a potential microfilaricide in Africa following studies in Ecuador that indicated its efficacy against adult worms (Molyneux & Davies, 1997). The earlier used drugs (Diethylcarbamazine (DEC) which kills the microfilariae and Suramin which kills the adult nematodes) cannot be used safely on a large scale for the treatment of onchocerciasis. DEC is usually administered daily over a 2-week period every three months for up to 10 years. Suramin must be administered intravenously under hospital supervision (Duke, 1987).

Recent years have seen considerable progress in onchocerciasis control and a change of emphasis. The number of treatments administered globally has increased rapidly over the period 1988-1996 (Mectizan Programme Notes, 1997). The commitment by the manufacturers of Ivermectin, through the Mectizan Donation Programme was the incentive for the development of a partnership of non-governmental development organisations (NGDOs) involved in blindness prevention, international organisations (WHO and the World Bank), governments of endemic countries and a donor consortium to establish the African Programme for Onchocerciasis Control (APOC). This programme would cover all endemic African countries outside the OCP and its main objective is to establish an effective and self sustainable community-based ivermectin treatment throughout the remaining endemic areas in Africa and to eliminate the disease by vector control in selected foci.

The establishment of APOC is expected to bring about better financing of onchocerciasis control activities to the endemic African countries under its operational areas. This is because APOC is based on the concept of partnership between NGDOs and governments in the organisation of the National Onchocerciasis Task Force and at the local level, the development through NGDOs and regional or district government of the appropriate

community directed delivery systems. APOC has indicated that treatment would be confined to hyper-endemic and meso-endemic villages identified by Rapid Epidemiological Assessment (REA) and mapped through Rapid Epidemiological Mapping of Onchocerciasis (REMO).

Country maps are therefore required before funding is provided by APOC (Molyneux and Davies, 1997). It is expected that NGDOs and government contribution should be at least 25% of the funding requested from APOC, whether in cash or kind. Projects would be funded against a background of a comprehensive national plan and each project would be expected to demonstrate a trend to sustainability. The concept of sustainability here refers to the ability of communities following initial external involvement to maintain the viability and continuity of the ivermectin treatment process in the absence of external support.

The on going onchocerciasis programmes in Nigeria, Cameroon and Equatorial Guinea may now benefit from APOC activities and as earlier indicated the island of Bioko is already enjoying this regarding the proposed vector eradication programme of APOC there.

## 2.9 SOCIO-ECONOMIC IMPACT OF THE BLACKFLY AND ONCHOCERCIASIS

In a community where onchocerciasis is endemic, up to 60% and above of persons are usually infected. *Onchocerca volvulus* infection of the skin and lymph nodes causes much stress and disfigurement but is very rarely disabling (Duke, 1990). The ocular lesions of onchocerciasis is what causes the true disablement such that in an area with high infection rate, up to 20% of individuals may be totally blind (Vajime, 1982). The above evidence on the socio-economic impact of onchocerciasis in a community support the following points earlier made by Hamon and Kartman (1973):

- Farming capacity is lowered below survival levels in a hyper-endemic area with 20% blindness.

- River valleys are deserted due to lack of farming activities and intolerable nuisance of *Simulium* bites with less fertile uplands becoming overcrowded.
- Loss of valuable source of protein (fish) results due to desertion of rivers.
- Great cost experienced in the protection of labour force engaged in activities such as dam constructions.
- The cost of caring for the disabled will be shouldered by the society.

This accounts for the socio-economic disaster of desertion and depopulation as seen in some parts of Plateau and Benue states in Nigeria (Nwoke, *et al.*, 1987a; Gemade and Utsalo, 1990), Hawal valley in the southern part of Bornu State of Nigeria (Bradley, 1972) and in the Volta River basin area (Cueller, 1972; Hunter, 1976; Philippon, 1977; Le Berre *et al.*, 1978). The economic costs of onchocerciasis in Kwara State was found to be largely in terms of loss in productivity and cost of treatment (NISER, 1988). Very bad situations have been observed in the Guinea Savannah belt stretching across Africa from Senegal in the West to Sudan in the East. Here up to 15% of the whole population and 40% of the male population may be blind and the mortality rate of blind persons over 30 years of age is 3-4 times as high as among sighted persons in the same group (Prost and Vangelade, 1981). Such a community would cease to be economically viable after a few years and inhabitants would move away to a less dangerous area deserting the fertile lands near the rivers in exchange for less productive land away from *Simulium* breeding sites (Duke, 1990).

In recent years the public health importance of onchocercal skin disease has been studied among endemic communities. A large multi-country study in Ghana, Nigeria, Cameroon, Uganda and Tanzania showed convincingly that onchocercal skin disease imposes a grave burden on the sufferer and its symptoms have important personal and psycho-social effects not only on the affected individuals, but also on their families and communities (WHO, 1995b). This study emphasised that onchocercal skin lesions are an important cause of stigma and affect people's self-esteem.

Onchocercal skin disease has been known to affect adolescent girls in a rural Nigerian community (Amazigo, 1993). Here the skin lesions are believed to be inherited through a mother's poisoned blood. An infected girl is usually stigmatised and this extends to other members of the family. Such infected girls are found to marry at a later age than uninfected girls. Though infected males also suffer stigmatisation, the humiliation on the females appears to be more pronounced. With this array of negative effects, it is clear that the control of onchocerciasis will bring about great socio-economic benefits to affected communities.

## 2.10 COMMUNITY PARTICIPATION IN ONCHOCERCIASIS CONTROL

Ignorance of the cause of onchocerciasis has been found to be typical in most endemic areas (Nwoke *et al.*, 1987a; Braide *et al.*, 1990). In such situations, knowledge of the association between the blackfly and onchocerciasis is lacking, making it difficult to persuade such communities to change cultural/occupational habits that promote the spread of the disease. It has been advocated that for successful control of the disease, community participation should commence with an understanding of the disease and persist through all control stages. Communities must be persuaded to change existing habits, beliefs and practices that encourage onchocerciasis infection (Braide *et al.*, 1990). The ultimate goal of any control programme should aim at creating awareness and involvement through information sharing to sustain the control programme, even in the absence of official supervision.

A WHO multi-country study in Africa (Mali, Ghana, Nigeria, Cameroon and Uganda) was carried out to identify and develop single acceptable and sustainable methods for community directed treatment with ivermectin. The study was designed to ascertain whether programme directed approaches developed and proposed to the community or community designed methods, identified by the communities themselves could be more effective. This study concluded that community directed treatment is feasible and effective

and successful in a range of diverse settings, thereby suggesting its replicability (WHO, 1996; Molyneux and Davies, 1997). It is expected that effective control within the APOC countries will depend on the sustainable delivery of ivermectin through community-directed treatment. This will be through ensuring adequate community involvement which will sustain delivery after the support to projects has ceased.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 STUDY AREAS

Sampling sites for the present study areas were chosen within a broad transect from the northern extremes of distribution in Nigeria through its southern bioclimatic zones into south west Cameroon near Mount Cameroon, and further south to parts of Bioko in Equatorial Guinea (Fig 3.1).




**Fig 3.1** – The three countries in West Africa studied

### 3.1.1 Nigeria

Nigeria lies between latitudes 4°N and 14°N and between longitudes 13°E and 15°E. It has a great variety of physical landscapes as compared with many other West African countries such as Mali and Niger, and as many as twelve physical regions are recognised in the country (Udo 1978). The Nigerian study transect begins in the northern Sudan savannah extremes of Katsina and Kano states down to the forested areas in Imo and Cross River states in the south (Fig 3.2). The Sudan savannah climate consists of a strong harmattan and a rainy and dry season. The harmattan is a dry dusty wind blowing down from the Sahara desert, which begins in December and ends in February.

Moving down south through the Guinea savannah zone and into Plateau state, there is an undulating highland of an average height of 1260m above sea level. The height endows this area with a fine semi-temperate climate (Udo 1981). Within the geographical "Niger-Benue Trough", which separates the north Central Highlands from the south-eastern landscape and the Cross River plains, is Benue state which spans through two vegetational belts. This state has rain forest vegetation covering the western part of the state and Guinea savannah-type vegetation in the southern and northern-most parts respectively. The typical rain forest sites in Nigeria include areas in the Cross River, Imo and Enugu states. The major rivers are the Cross river, which runs through the length of Cross River state, emptying its water into the Atlantic Ocean, the Oji river which is situated between Enugu and Onitsha, a locality only a few kilometres away from the Udi Hills area.

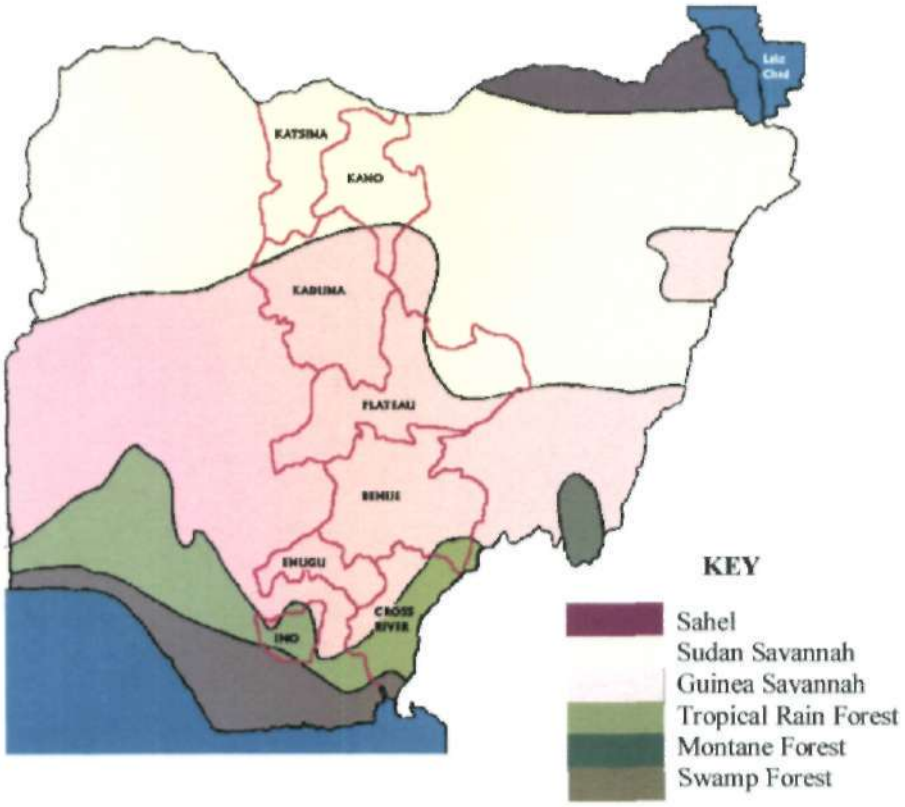



**Fig 3.2** – Study areas in Nigeria

The Isuikwuato-Okigwe area of Imo state is a hilly area with a lot of streams and rivers. The courses of the major rivers run mainly over Precambrian geological formations of various kinds with few exceptions of sedimentary or alluvial formations at certain narrow banks (Fig 3.3).

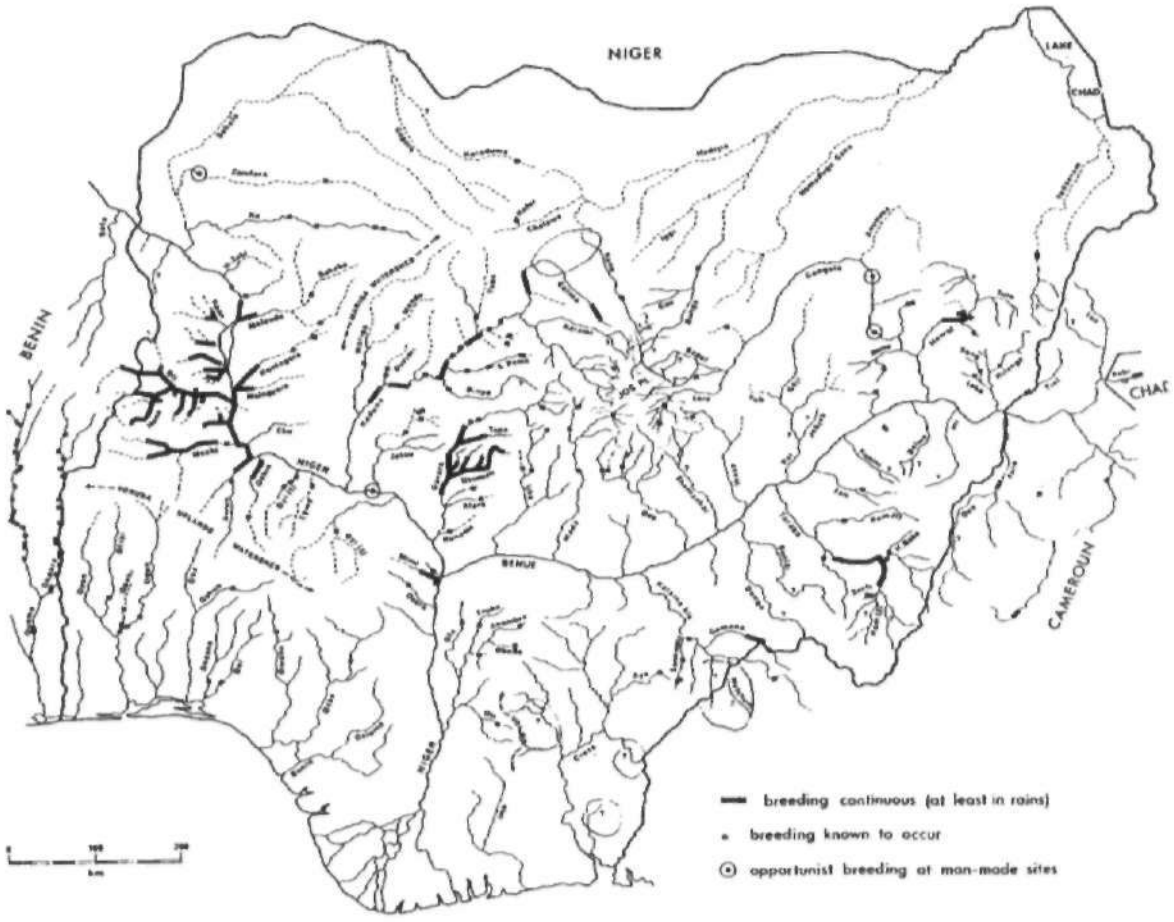



**Fig 3.3** – *Simulium damnosum* breeding sites in Nigeria (Crosskey, 1979).

### 3.1.2 Equatorial Guinea

Equatorial Guinea is situated south of Cameroon. Its capital city is Malabo which can be found on the island of Bioko (Fig 3.4). Bioko is a volcanic island with cloud-covered volcanoes providing the backdrop to almost every view on the island. It is 72km long and 35km wide, covering an area of 200km². It is situated off the coast of Cameroon between latitudes 3°12'N and 3°48'N and longitudes 8°25'E and 8°56'E. To the west of the island is the sea and to the south lie the islands of Principe, São Tome and Annobon. The study transect passes from Mount Cameroon across the sea to the island of Bioko.

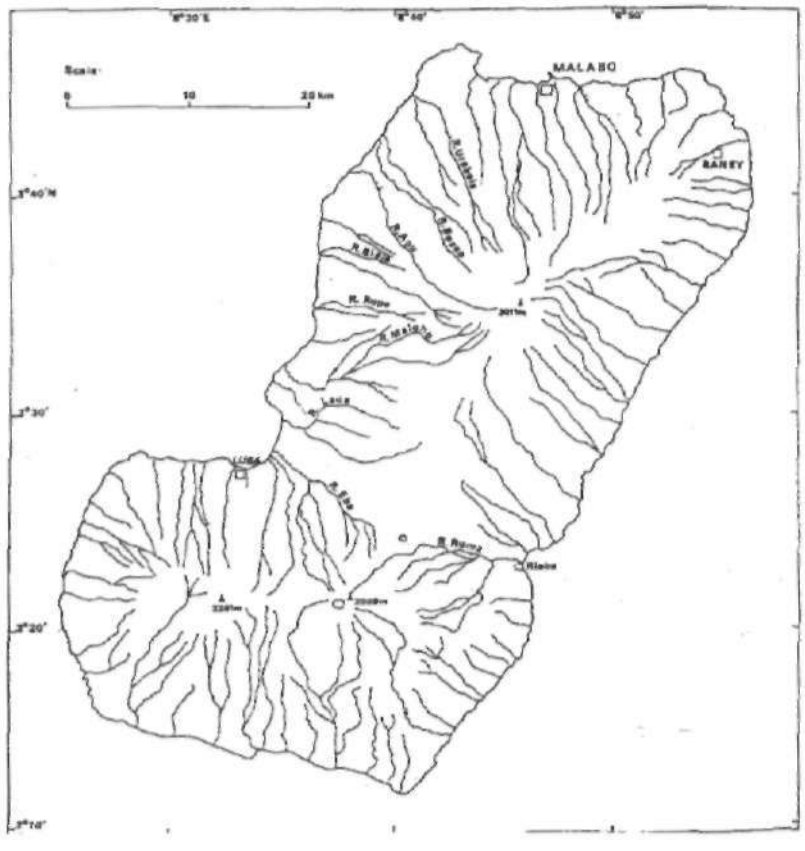



**Fig 3.4** – The island of Bioko showing some of its rivers and tributaries

The climate/vegetation is tropical rain forest. Towards the north of the island is a distinct rainy and dry season, the rainy season lasts from July to October, and the dry season from November to March. The dry season is caused by the north-east Harmattan winds blowing from the Sahara desert, bringing in dry air. In the south, the influence of the Harmattan is less and there is rainfall throughout the year. The heaviest rains however, coincide with the rainy season in the north. Bioko is said to be one of the wettest areas in Africa, and in fact, in the World (Teran 1962). The island, being volcanic, provides steep slopes drained by numerous small fast flowing streams that originate on the mountain slopes and serve as breeding sites for *Simulium*.

### 3.1.3 Cameroon

The Republic of Cameroon is in western Africa and is bounded on the north by Lake Chad, on the east by Chad and the Central African Republic, on the south lies the Congo, Gabon and Equatorial Guinea. On the west lie the Atlantic Ocean and Nigeria. The country forms a bridge between western and central Africa and has a total area of 475 442 square kilometres. Douala is the country's largest city but its capital is Yaoundé.

Cameroon has four distinct topographical regions. In the south lies a coastal plain, a region of dense equatorial rain forest. In the centre of the country is the Adamawa Plateau, a region with elevations reaching about 1370m above sea level. This is a transitional area where forest gives way to the northern savannah country. In the far north, savannah gradually slopes into marshland, which surrounds Lake Chad, and in the west there is an area of high mountain forest of volcanic origin. Located in the west is Mount Cameroon (4095m), the highest peak in western Africa and an active volcano. The country's most fertile soils are found in this region.The study transect passes south west across the border

from Cross River State in Nigeria to the Mount Cameroon area of western Cameroon (Fig 3.5). Cross River State itself arises in western Cameroon, but the study transect passes south of this to include the streams draining from Mount Cameroon.




**Fig 3.5** The Republic of Cameroon

## 3.2 FIELD PROCEDURES

### 3.2.1 Collection and storage of *Simulium damnosum* larvae

*Simulium damnosum* s.l. larvae were found attached to rocks or vegetation in fast flowing points of rivers, streams or waterfalls. For cytological analysis, larvae of the penultimate instar with white pupal respiratory filament histoblast (Dunbar, 1972) were preferable, although larvae of other stages showing discernible polytene chromosomes were also used.

In warm weather, larvae have been known to be adversely affected after removal from the water and the giant chromosomes begin to deteriorate. This led to the "ice collecting" method of Dunbar (1972). This method was employed during the course of this study but an alternative method was used when ice was not readily available.

Ice blocks were made in plastic containers and carried to the field in an icebox, together with the chemicals for larval fixation (absolute alcohol and glacial acetic acid) and other accessories. Larvae were collected from different substrates found in faster flowing points of the water body with the help of forceps. Each larva was placed immediately on the ice block for about 5 minutes. They were then blotted dry on filter paper and dropped immediately into freshly prepared cold fixative (2 parts absolute alcohol: 1 part glacial acetic acid) in McCartney (Bijou) bottles. When possible, the abdomen of the larvae was split open before dropping it in the fixative. The concentration of the fixative used in this study had been shown to give better results than the usual Carnoys fixative - 3 parts absolute alcohol: 1 part glacial acetic acid (Vajime and Prigadarshini, 1987; Vajime, pers. comm.).

To avoid the larval fluid from diluting the fixative and changing it from a colourless to a greenish liquid (and consequently reducing its effectiveness), not more than about 20-30 larvae were put in each Bijou bottle. Before final storage, the fixative in which the larval material was stored was changed twice and in the laboratory, the samples were stored in the deep freezer at around –20°C.

In some of the areas of Nigeria where samples were collected, there was no electricity supply and one had to rely on the use of refrigerators in the Government Health Centres where there were generators. These usually could not sustain power for periods long enough to make ice and so only ice packs were stored and used for fieldwork. Larval samples from these areas were fixed immediately without dissection in the field in cool, freshly prepared fixative. The chemicals were kept cool in an icebox filled with ice packs. Sample collection in Cameroon and Equatorial Guinea was carried out using the above described methods and stored in deep-freezers until ready for transportation to the Netherlands and London.

In some instances where collection was to be made from more than one site in a day and time was limited for fixing in the field, sticks/leaves on which larvae are attached were collected in polyethylene bags. The mouth of each bag was tied, allowing in some air and these bags were placed in the icebox. This was then transported to the laboratory where the larvae were fixed and stored in the freezer. A survey of various prospective breeding sites was carried out in the Nigerian study area (Table 3.1) and later sampling was restricted to areas that showed either presence of the *Simulium damnosum* complex or where cytological preparations were successful. Field trips involved visiting the sites in Nigeria. Collection data are shown for Nigeria (Table 4.1) and for Bioko and Cameroon (Tables 4.6 and 4.7).

**Table 3.1** - Rivers Prospected in Nigeria and *Simulium damnosum* s.l. status

| Date | River | Site | State | Vegetation | *Simulium damnosum* status |
|---|---|---|---|---|---|
| 12/4/89 | Amire-u-Tamen | Ikyogen | BNS | G.Savannah | +'ve |
| 12/4/89 | Amire-u-Tamen | Manyan | BNS | G.Savannah | +'ve |
| 19/4/89 | Mateh River | Bottom Hill | CRS | Forest | -'ve |
| 20/4/89 | Matu | Bessenge | CRS | Forest | +'ve |
| 12/5/89 | Ibe River | Aking | CRS | Forest | +'ve |
| 26/5/89 | Ehom River | Aking | CRS | Forest | -'ve |
| 26/5/89 | Udip River | Akamkpa | CRS | Forest | -'ve |
| 11/10/89 | Agbokim Waterfalls | Agbokim | CRS | Forest | +'ve |
| 11/10/89 | Mfum boarder River | Mfum, Ikom | CRS | Forest | -'ve |
| 9/11/89 | Gora River | Kudaru | KDS | G.Savannah | +'ve |
| 9/11/89 | Mekahu River | Kudaru | KDS | G.Savannah | +'ve |
| 28/11/89 | Selina River | Kudaru | KDS | G.Savannah | +'ve |
| 30/11/89 | Kogum River | Kogum | KDS | G.Savannah | +'ve |
| 12/1/90 | Korum River | Itu Agoi | CRS | Forest | +'ve |
| 26/1/90 | Kwa Waterfalls | Aningeje | CRS | Forest | +'ve |
| 7/8/90 | Galma River | Kudaru | KDS | G.Savannah | +'ve |
| 20/8/90 | Tiga Dam, Tiga | point A & B | KNS | S.Savannah | +'ve |
| 22/8/90 | Maska Dam | Maska | KTS | S.Savannah | +'ve |
| 5/9/90 | Jarawan Kogi | Federe | PLS | Montane | +'ve |
| 4/10/90 | Ngu Attai River | Bebi | CRS | Montane | +'ve |
| 13/10/90 | Mkomon | Waya | BNS | G.Savannah | +'ve |
| 11/12/90 | Oji River | Oji Power Station | ENS | Forest | +'ve |
| 27/2/95 | Afi River | Boki | CRS | Forest | -'ve |
| 27/2/95 | Okpon | Obubra | CRS | Forest | -'ve |
| 13/4/95 | Ibi River | Umulolo, Okigwe | IMO | Forest | +'ve |

***Key:***
CRS - Cross River State; IMO - Imo State; ENS - Enugu State; KTS - Katsina State; BNS - Benue State; KDS - Kaduna State; KNS - Kano State; PLS - Plateau State; G. savannah - Guinea savannah; S. savannah - Sudan savannah; +ve - positive; -ve - negative.

### 3.2.2. Identification of *S. damnosum* complex larvae

During the first field trips, carried out in late 1989 and up to the early part of 1990, *S. damnosum* s.l. larvae were identified using a low power dissecting field microscope. Their paired conical processes (tubercles) on the dorsal surface of the abdomen and the hairy proleg (Crosskey, 1960) identified them. In some cases, the tubercules were prominent but in others there was need for a very close examination. Later on, it became possible to

identify the larvae (*S. damnosum*) with the naked eye relying mainly on their usually darker coloration. Confirmation of the material collected was done before dissection in the laboratory and this applied also to the Bioko and Cameroon larvae

### 3.2.3. Physical and chemical parameters of breeding sites of Nigerian *S. damnosum* complex

Since the availability of larval material and the quality of chromosomes obtained from squash preparations are all known to be affected by varied water/nutritional conditions, some parameters were measured in the different collection sites. It was also necessary to determine whether aquatic habitat range is similar for breeding sites in Nigeria as elsewhere in Africa (Grunewald, 1976a; 1976b; Quillévéré *et al.*, 1976; 1977). Temperature readings were made using a mercury thermometer whereas pH and conductivity (dissolved solids) were measured using portable Hanna pH and conductivity meters. These were usually submerged in water for 30 - 60 seconds before readings were made. Current was measured with a locally produced pitot tube. This was an L-shaped piece of glass tubing with a vertical length of about 60.9 cm and a horizontal limb of about 10.2 cm. It was immersed in water with the horizontal limb pointing upstream. The pressure of water in the stream/river forces water into the tube and the level above the river on the vertical limb is noted by means of a graduated scale on this limb. The velocity of the current at a particular depth was calculated as reported by Dowdeswell, (1959) using the formula;

$$V = \sqrt{2hg}$$

Where $V$ = the speed of the current

$g$ = acceleration due to gravity (981 cm/sec$^2$)

$h$ = the height of the column above the river surface

## 3.3. LABORATORY PROCEDURES

### 3.3.1. Dissection of Larvae

Larvae were dissected to expose the salivary glands before staining. This was done by placing the larvae on a clean slide in a drop of the fixative and inserting two sharp needles near the top of the bulge on the abdomen and tearing the body wall through about two thirds of the entire length of the larvae from the posterior end. The glands were thus exposed and could be recognised.

The salivary glands are large transparent paired structures that extend posteriorly to about two thirds of the body length and then curve back. The best chromosomes are usually obtained from the first one third of the posterior end.

### 3.3.2. Staining of Larvae

Two staining methods were employed, the Feulgen and the lacto-acetic orcein methods (Dunbar, 1972) with slight modifications (Boakye, pers. comm.).

#### 3.3.2.1 Feulgen

In this method, dissected larvae were first washed in distilled water for five minutes to remove the fixative. Hydrolysis was then carried out - hot hydrolysis with 1.0 N HCl at 65°C for 7 minutes (Dunbar, 1972) or cold hydrolysis with 5.0 N HCl at room temperature for about 30 minutes (Dunbar, 1972; Post, pers. comm.). Time and temperature are very critical for the hot hydrolysis, so it was usually more convenient to use the cold hydrolysis as this worked equally well.

At the end of hydrolysis, the larvae were removed from the HCl onto filter paper to blot out the excess acid. These were then placed in a tightly stoppered bottle containing a small amount of Feulgen stain and kept in a dark cupboard. The staining period varied from 15 minutes to 3 hours, depending on the Feulgen batch. Through experimenting for different times, for each newly prepared Feulgen stain, an average time was usually obtained whereby relatively well stained chromosomes were obtained. Larvae were then removed from the stain and placed in tap water (which had an effect on the stability of the stain) for 5 - 20 minutes.

#### 3.3.2.2 Lacto-Acetic Orcein

The dissected larvae were washed for about 5 minutes in distilled water, blotted dry and put for 1 minute into 10% HCl which was pre-warmed to 45 °C. The larvae were then removed and blotted dry and placed on a slide with a drop or two of lacto-acetic orcein stain. These were left in the stain for about 1 hour. The larvae were transferred into distilled water to remove excess stain. This step was later eliminated after a few trials, because the intensity of the stain reduced significantly creating problems with identification of the chromosome banding patterns.

### 3.3.3. Differentiation and Mounting

Individual larvae from each of the different staining methods were then mounted on clean slides in a drop of 50% acetic acid for about 5 minutes, to soften and swell them and to remove excess stain from the tissues (differentiation). The glands and paired gonads (located at the posterior end of the abdomen) at this point became visible because the DNA

in the nuclei of their numerous and densely packed cells had picked up the stains. The salivary glands were then isolated and anything which would impede squashing was removed. Larvae were then separated into sexes at this point by observing gonadal shape. The testes are usually pear-shaped and in older larvae they may be seen to be divided into "sacs".

The ovaries are long, banana-shaped structures. In younger larvae, they appear long and thin without a central lumen, while in older larvae they are much broader and a lumen is present (Dunbar, 1972). After sexing, the salivary glands were transferred to a clean slide on a drop of 50% acetic acid. They were squashed under a cover slip with a gentle tap between filter paper. Sometimes when Feulgen staining of the cells of the glands was too light, re-staining with lacto-acetic orcein was done by adding a drop of this stain to the 50% acetic acid on the slide before squashing.

After the squashing process, the cover slip was temporarily ringed with rubber solution or nail varnish to prevent the cells drying out. The slide was then viewed under a compound microscope. When the slide showed satisfactory spread, this temporary mount was read and the species complex identified. For further detailed study, the slide was made permanent so that it could be read with oil immersion lens to obtain full details of the banding patterns.

#### 3.3.3.1. Permanent mount

The dry ice method (with modifications) for making slides was used in this study (Dunbar, 1972). This involved placing the slides with cover slips on a flat surface in a deep freezer

for about 30 minutes or longer. The slides were then removed from the freezer and the cover slip prised off with a razor blade. Both cover slip and slide were then immersed in 100% absolute alcohol for 2-3 seconds after which excess alcohol was drained off and the slide and cover slip reunited with a drop of mounting medium - euparal.

#### 3.3.3.2 Photomicrography

For further detailed identification/interpretation of chromosome banding patterns and for recording inversions or other features of interest, photographs were taken with a 35mm camera fitted to the microscope.

### 3.3.4. Cytotaxonomic Analysis

On the basis of the division of the three chromosomes of *Simulium damnosum* s.l. into 100 units by Vajime and Dunbar (1975), the different cytospecies encountered in the study were identified by the various inversion differences that exist between them. The published keys of Vajime and Dunbar (1975) with further modifications of Post (1986), Vajime (1989) and Boakye (1993) were employed in the identification process. The banding pattern of the polytene chromosomes was compared with the published standards (Vajime and Dunbar, 1975) and any fixed or heterozygous inversions noted. Identifications of sibling species were then made according to the inversions found.

*Simulium squamosum* was identified as identical to Vajime and Dunbar's standard with no fixed inversions. *Simulium yahense* was identified by the inversion 2L-18 as described by Vajime and Dunbar (1975) which is almost completely X-linked and absent from other cytospecies. *Simulium damnosum* and *S. sirbanum* were identified by the criteria of Vajime

and Dunbar (1975). The breakpoint modifications of inversions as described by Post (1986) do not affect the basic criteria for identification (Post, pers. comm.)

# CHAPTER FOUR

# RESULTS

## 4.1 NIGERIA

Identifications were made from fourteen sites spanning seven states of the country. This was through the extreme savannah vegetation of Katsina state in the north, into the montane forest of Plateau state with its undulating highlands, to the typical forest zones in the south and east of the country. Four cytospecies of the *Simulium damnosum* complex were observed in these areas; *S. squamosum, S. yahense, S. damnosum* s.s. and *S. sirbanum* (Table 4.1).

*Simulium squamosum* was identified as identical to Vajime and Dunbar's (1975) standard with no fixed inversions. *Simulium yahense* was identified by the inversion 2L-18 as described by Vajime and Dunbar (1975). All females were homozygous 2L-18/18 and all males were heterozygous 2L-18/St. *Simulium damnosum* and *S. sirbanum* were identified by the modified criteria of Vajime and Dunbar (1975) (Boakye, 1993). *Simulium damnosum* was identified by the independent fixed interspecific inversion 2L-C and the heterozygous inversion 2L-C/C.8 was observed more in males. *Simulium sirbanum* was identified by the interspecific inversion 2L-3.8 and both males and females were homozygous for this inversion.

### 4.1.1. Distribution of *Simulium damnosum* s.l.

The identification of the *S. damnosum* s.l. cytospecies in Nigeria showed *S. squamosum* to have the highest frequency (58%). This was followed by *S. sirbanum* (21.3%), *S.*

*damnosum* s.s (17.3%) and *S. yahense* which was the least abundant (3.3%) in Nigeria (Tables 4.1 & 4.2). Virtually all the *S. squamosum* observed were found in the forest area (Table 4.2). *Simulium sirbanum* though observed in all the vegetational zones, predominated in the Sudan savannah zone. *Simulium damnosum* occurred throughout the four climatic zones but it predominated in the Guinea savannah. The *S. yahense* number was very small, with a total of only five and all of these were in the forest zones (Table 4.2).

**Table 4.1** - Cytospecies identification and distribution in Nigeria

| Site | River/ Location | Co-ordinates | Bioclimatic Zone | Cytospecies | | | | Total number of identifications |
|---|---|---|---|---|---|---|---|---|
| | | | | *sq* | *yah* | *dam* | *sir* | |
| Aningeje | Kwa Falls | 05°08'N 08°31'E | Forest | 21 | - | 1 | 2 | 24 |
| Agbokim | Agbokim Waterfalls | 05°45'N 08°54'E | Forest | 2 | 4 | 1 | 1 | 8 |
| Obudu (Bebi) | Ngu Attai | 06°28'N 09°13'E | Montane Forest | 1 | 1 | 1 | 1 | 4 |
| Ugep (Itu Agoi) | Korum River | 05°45'N 08°07'E | Forest | 8 | - | 1 | - | 9 |
| Dalle | Kogum River | 09°25'N 08°13'E | Guinea Savannah | - | - | 4 | - | 4 |
| Assob | Assob Bridge | 09°31'N 08°37'E | Montane Forest | 2 | - | - | - | 2 |
| Tiga (A&B) | Tiga Dam | 11°32'N 08°24'E | Sudan Savannah | 1 | - | 2 | 22 | 25 |
| Maska Dam | Maska Dam | 11°25'N 07°18'E | Sudan Savannah | - | - | - | 3 | 3 |
| Kudaru | Galma River | 10°39'N 08°30'E | Guinea Savannah | - | - | 2 | 2 | 4 |
| Obudu (Bessenge) | Matu River | 06°37'N 09°18'E | Forest | - | - | 2 | - | 2 |
| Obubra (Aking) | Ibe River | 05°20'N 08°34'E | Forest | 2 | - | 9 | 1 | 12 |
| Umulolo | Ibu River | 06°30'N 07°30'E | Forest | 34 | - | - | - | 34 |
| Katsina Ala | Katsina Ala River | 07°10'N 09°25'E | Guinea Savannah | - | - | 3 | - | 3 |
| Enugu | Oji River | 06°17'N 07°17'E | Forest | 16 | - | - | - | 16 |
| **TOTAL** | | | | **87** | **5** | **26** | **32** | **150** |

*Key: Sq - S. squamosum, yah - S. yahense, dam -. S. damnosum, Sir - S. sirbanum*

**Table 4.2** - Distribution of Cytospecies in the different bioclimatic zones in Nigeria

| Bioclimatic Zone | Cytospecies | | | |
|---|---|---|---|---|
| | *squamosum* | *yahense* | *damnosum* | *sirbanum* |
| Forest | 83 | 4 | 14 | 4 |
| Montane Forest | 3 | 1 | 1 | 1 |
| Guinea Savannah | - | - | 9 | 2 |
| Sudan Savannah | 1 | - | 2 | 25 |
| **TOTAL** | 87<br>58·0% | 5<br>3·3% | 26<br>17·3% | 32<br>21·3% |

## 4.1.2. Cytotaxonomy of *Simulium damnosum* s.l.

*Simulium yahense*, *S. damnosum* and *S. sirbanum* were identified as mentioned previously using the modified criteria of Vajime and Dunbar (1975). For these cytospecies, there was no reason to suspect any new inversion sequences, however, for *S. squamosum*, full karyotype analysis was carried out to ascertain the possible existence of inversions or other polymorphic characteristics that were not used in the initial identification of the cytotype and that may have been unique to Nigerian material (Table 4.3).

Vajime and Dunbar (1975) had described sex differences in *S. squamosum* material from Cameroon. They observed a non-pairing altered centric segment on chromosome 1 (1Ca) (Plates 1). This phenomenon was found mainly in males. In Nigeria, *S. squamosum* was found to exhibit this sex-determining mechanism in some of the sites with varying frequencies (Table 4.4). A female *S. squamosum* from Ibe river in Cross River State was found to possess 1Ca (Table 4.4) and it also had a split centromere on chromosome 3 (Table 4.3).

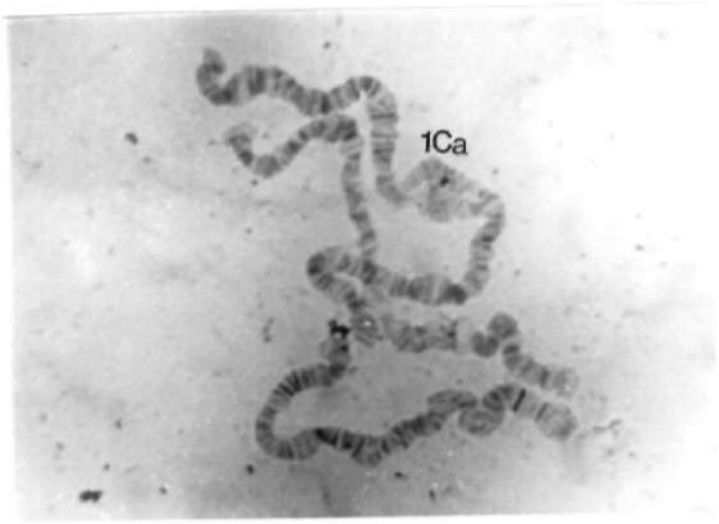



Plate 1: *Simulium squamosum* showing split centromere of chromosome 1

**Table 4.3** - Full Karyotype Analysis of Nigerian *Simulium damnosum* complex

| Country/Site/ID Number | Number Examined | Cytospecies | Sex | Full Karyotype Observations* |
|---|---|---|---|---|
| Ibu River (95/1) | 4 | *squamosum* | M | 1C split |
| | 1 | *squamosum* | ? | 1C split |
| Ibu River (96/1) | 8 | *squamosum* | M | 1C split |
| | 1 | *squamosum* | ? | 1C split |
| | 2 | *squamosum* | M | 1C not split |
| Ibe River (95/8) | 1 | *squamosum* | F | 1C split, 3C split |
| **TOTAL** | **17** | | | |

* All three chromosomes were observed and specific features recorded as shown; ?, sex unknown

**Table 4.4** *S. squamosum* - relationship between sex and the centromere region of chromosome 1 (1Cs/1Ca)

| Site (State) | Males identified | | Females identified | | Unidentified | | Total 1Ca observed |
|---|---|---|---|---|---|---|---|
| | W 1Ca | WO 1Ca | W 1Ca | WO 1Ca | W 1Ca | WO 1Ca | |
| Korum River (Cross River) | 2 | 1 | 0 | 0 | 3 | 3 | 5 |
| Kwa Falls (Cross River) | 3 | 0 | 0 | 0 | 7 | 9 | 10 |
| Ibe River (Cross River) | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| Ibu River (Imo State) | 12 | 5 | 0 | 12 | 3 | 1 | 15 |
| Oji River (Enugu State) | 0 | 9 | 0 | 6 | 0 | 1 | 0 |
| TOTAL | 17 | 15 | 1 | 19 | 13 | 14 | 31 |

*Key*: W = with, WO = without

### 4.1.3 Physical and Chemical parameters of *S. damnosum* s.l. breeding sites in Nigeria

Measurements of water temperatures, current, pH and conductivity were made at the breeding sites through the months of August to December in 1990, and during January of 1992. the temperatures of the sites ranged from 22°c to 33°c (table 4.5). The measurement

of pH showed most sites to be slightly alkaline, ranging from 7·1 to 7·9. Two sites gave neutral pH readings of 7·0, these were the river Amire-u-Tamen at Ikyogen and Manyam. Agbokim water falls had a weak acid pH of 6·5 and both Kwa falls and Mateh river in the southern (Cross River State) area had near neutral pH values of 6·9. Conductivity ranged from 0.1-0.6 µS and water current readings were between 1.17 and 2.12 m/s.

**Table 4.5** - Temperature, pH, conductivity and current speed of water at sampling sites.

| Period | Site | Temp (°C) | pH | Cond (µS) | Current (m/s) | Cytospecies | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *sq* | *yah* | *dam* | *sir* |
| Aug'90 | Galma river | 24 | 7.2 | 0.4 | 1.657 | - | - | P | P |
| | Tiga Dam A | 25 | 7.9 | 0.5 | 1.716 | P | - | P | P |
| | Tiga Dam B | 26 | 7.4 | 0.6 | 1.930 | P | - | P | P |
| | Maska Dam | 24 | 7.2 | 0.6 | 1.930 | - | - | - | P |
| | Assob Bridge | 22 | 7.9 | 0.4 | 1.930 | P | - | - | - |
| Sept'90 | Galma river | 26 | 7.6 | 0.5 | 2.124 | - | - | P | P |
| | Kogun river | 31 | 7.2 | 0.4 | 1.930 | - | - | P | - |
| | Jarawa Kogi | 29 | 7.3 | 0.3 | 1.400 | ? | ? | ? | ? |
| Oct'90 | Kwa falls | 23 | 6.9 | 0.1 | 1.657 | P | - | P | P |
| | Agbokim falls | 30 | 6.5 | 0.2 | 1.172 | P | P | P | P |
| | Ngu Attai river | 30 | 7.1 | 0.2 | 0.990 | P | P | P | P |
| | Mateh river | 23 | 7.4 | 0.1 | 1.400 | ? | ? | ? | ? |
| | Mkomon river | 31 | 6.9 | 0.2 | 1.716 | ? | ? | ? | ? |
| | Amire-u-Tamen (Ikyogen) | 31 | 7.0 | 0.2 | 1.772 | ? | ? | ? | ? |
| Dec'90 | Ngu Atta river | N | 7.0 | N | 1.534 | P | P | P | P |
| | Mateh river | 30 | 6.9 | N | 1.172 | ? | ? | ? | ? |
| | Amire-u-Tamen (Ikyogen) | 33 | 7.1 | N | 1.400 | ? | ? | ? | ? |
| | Amire-u-Tamen (Manyam) | 30 | 7.0 | N | 1.400 | ? | ? | ? | ? |
| Jan'92 | Kwa falls | N | 7.8 | 01 | N | P | - | P | P |

*Key*: N =. Not determined; P = Cytospecies found; - = Cytospecies not found; ? = *S. damnosum* s.l. but cytotype could not be determined due to poor chromosome quality.

## 4.2 CYTOTAXONOMY AND DISTRIBUTION OF *S. DAMNOSUM* S.L. IN EQUATORIAL GUINEA (BIOKO) AND CAMEROON.

In the island of Bioko, Equatorial Guinea, larval specimens were identified from 22 sites (Table 4.6). These larval populations were found to be similar to each other when cytotaxonomically identified and were closely related to *S. squamosum* and *S. yahense*. Full karyotype analysis did not reveal any inversion differences but 2L-18 was found to be strongly Y-linked in this population, as opposed to being X-linked in *S. yahense* and absent from *S. squamosum* (Boakye 1993). A polymorphic inversion, 3L-I was observed in two females, one male and one larva of unknown sex (Plate 1a). All the Bioko specimens were therefore found to conform to a single cytotype which basically was standard on all chromosome arms except for 3L-I (which was polymorphic) and 2L-18 which was Y-linked.

The Cameroon larval specimens produced rather poor quality preparations. Cytospecies identified here were *S. squamosum* and *S. mengense* (Table 4.7). *Simulium squamosum* was identified as identical to that described by Vajime and Dunbar (1975) with no fixed inversions and *S. mengense* by the triple inversion sequence 2L-34.41.42 and also by the inversion 2L-43.44 (other inversion differences exist in this cytospecies, Vajime and Dunbar (1977)). Practically however *S. mengense* was identified by a very conspicuous expanded centromere region on chromosome 1 (Vajime and Dunbar, 1977) before confirming it with the above inversion. *Simulium squamosum* had the highest frequency of occurrence (84%) and was observed in five of the seven study sites. *Simulium mengense* frequency distribution was 16% and was found in the River Sanje and River Mossingili (Table 4.7). Full karyotype analysis on one female *S. squamosum* revealed a heterozygous 1S arm). The inversion 3L-I was found on one *S. squamosum* male specimen from Bille and this same specimen had a split centromere on chromosome1 (Plates 2).

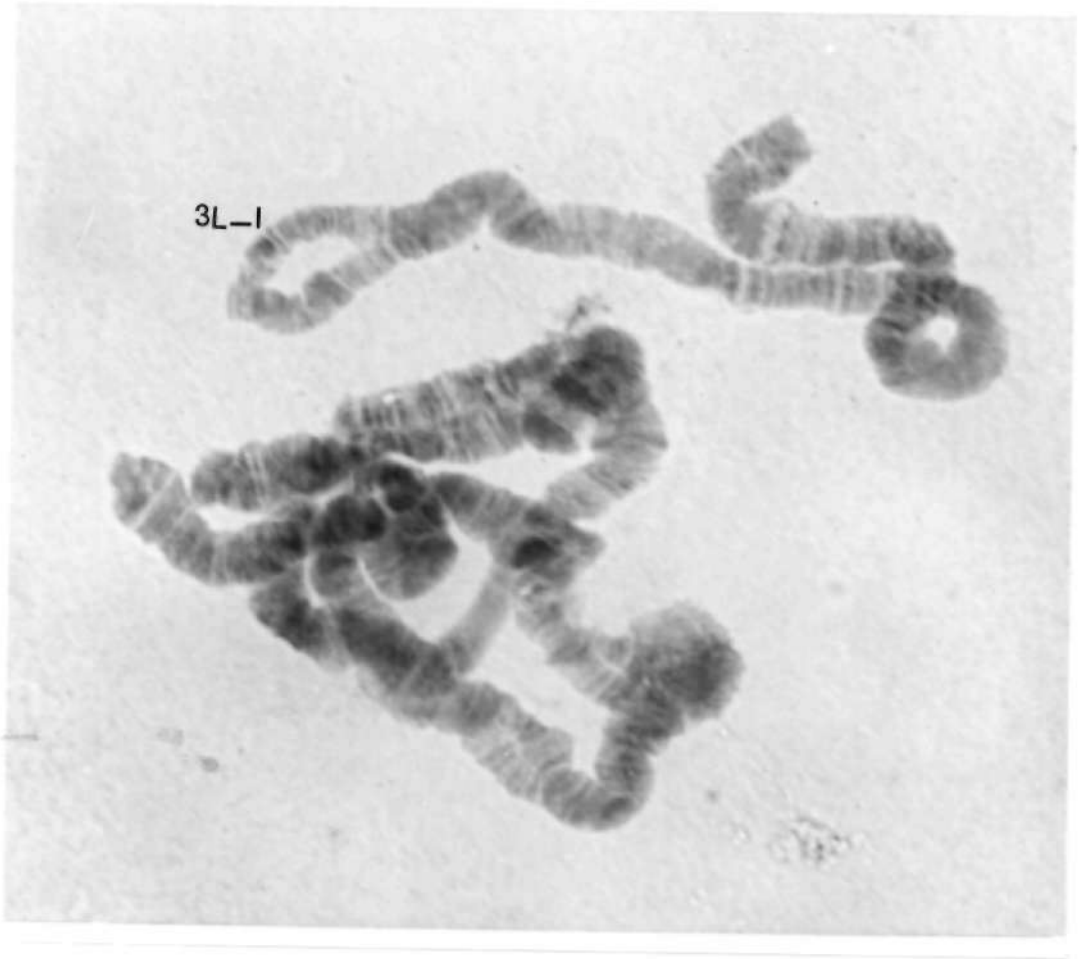



**Plate 2**: *Simulium squamosum* showing 3L-I

**Table 4.6** - Cytotaxonomy and distribution of *S. damnosum* in Equatorial Guinea (Bioko)

| Site | River/ Location | Co-ordinates | Cytospecies | | | | Collection Dates | Total number of identifications |
|---|---|---|---|---|---|---|---|---|
| | | | *sq* | *yah* (BF*) | *dam* | *sir* | | |
| *Bioko* (Site 81) (Site 82) | *River Matogi* River Lada | 03°31'N 08°37'E 03°30'N 08°37'E | - | 12 | - | - | 13/04/96 | 12 |
| *Bioko* | *River Rupe* | 03°35'N 08°37'E | - | 10 | - | - | 20/04/96 | 10 |
| Bioko | River Grande | 03°23'N 08°44'E | - | 20 | - | - | 23/04/96 | 20 |
| Bioko | River Ruma | 03°23'N 08°44'E | - | 18 | - | - | 23/04/96 | 18 |
| Bioko | Irrigation Channel 1 | 03°27'N 08°35'E | - | 14 | - | - | 24/4/96 | 14 |
| Bioko | Irrigation Channel 2 | 03°27'N 08°35'E | - | 16 | - | - | 24/04/96 | 16 |
| Bioko | River Boala (Upstream) | 03°27'N 08°35'E | - | 7 | - | - | 24/04/96 | 7 |
| Bioko | River Musula Dam | 03°27'N 08°36'E | - | 4 | - | - | 24/04/96 | 4 |
| Bioko | River Muedebe | 03°18'N 08°44'E | - | 13 | - | - | 29/04/96 | 13 |
| Bioko | Ilagyi River | 03°15'N 08°43'E | - | 18 | - | - | 30/04/96 | 18 |
| Bioko | River Leke | 03°14'N 08°37'E | - | 1 | - | - | 02/05/96 | 1 |
| Bioko | River Ole | 03°16'N 08°28'E | - | 1 | - | - | 03/05/96 | 1 |
| Bioko | River 61 (River Eneca) | 03°34'N 08°37'E | - | 8 | - | - | 08/05/96 | 8 |
| Bioko | River Ope | 03°37'N 08°37'E | - | 3 | - | - | 08/05/96 | 3 |
| Bioko | River Maloho | 03°32'N 08°36'E | - | 7 | - | - | 08/05/96 | 7 |
| Bioko | River Rupe | 03°35'N 08°37'E | - | 5 | - | - | 08/05/96 | 5 |
| Bioko | River Bala | 03°38'N 08°38'E | - | 1 | - | - | 08/05/96 | 1 |
| Bioko | River Apu | 03°41'N 08°39'E | - | 2 | - | - | 08/05/96 | 2 |
| Bioko | River Cibita | 03°16'N 08°26'E | - | 4 | - | - | 09/05/96 | 4 |
| Bioko | River 82 (between Lada & Matogi Rivers) | 03°30'N 08°37'E | - | 2 | - | - | 10/05/96 | 2 |
| Bioko | River Matogi | 03°31'N 08°37'E | - | 19 | - | - | 10/05/96 | 19 |
| Bioko | River Timbabe | 03°45'10"N 08°45'15"E | - | 20 | - | - | 22/07/96 | 20 |
| **TOTAL** | | | - | **205** | - | - | | **205** |

*Key: Sq - S. squamosum, yah* (BF) - *S. yahense* Bioko form, *dam -. S. damnosum, Sir - S. sirbanum.*

**Table 4.7** - Cytotaxonomy and distribution of *S. damnosum* in Cameroon

| Site | River/ Location | Co-ordinates | Cytospecies | | | | | Collection Dates | Total number of identifications |
|---|---|---|---|---|---|---|---|---|---|
| | | | men | sq | yah | dam | sir | | |
| Bussumbu | River Limbe | 04°02'N 09°12'E | - | 18 | - | - | - | 07/07/96 | 18 |
| Mutengene (opposite plantation) | River Benoe | 04°05'N 09°19'E | - | 18 | - | - | - | 07/07/96 | 18 |
| Likomba | River Likomba | 04°05'N 09°20'E | - | 4 | - | - | - | 07/07/96 | 4 |
| Ombe | River Ombe | 04°05'N 09°17'E | - | 2 | - | - | - | 07/07/96 | 2 |
| Refros Camp 3 | River Sanje | 04°15'N 08°57'E | 4 | - | - | - | - | 08/07/96 | 4 |
| Sodem Camp | River Mossingili | 04°15'N 08°57'E | 7 | - | - | - | - | 08/07/96 | 7 |
| Muyuka | River Yoke | 04°18'N 09°26'E | - | 16 | - | - | - | 07/07/96 | 16 |
| TOTAL | | | 11 | 58 | - | - | - | | 69 |

*Key: Men-S. mengense, Sq - S. squamosum, yah - S. yahense, dam -. S. damnosum, Sir - S. sirbanum.*

# CHAPTER FIVE

# DISCUSSION

The geographic distribution of the cytospecies of onchocerciasis vectors in Nigeria has been reviewed by Crosskey (1981; 1987) and additional information exists from other researchers (Vajime and Gregory, 1990; Akoh *et al.*, 1987; Akpa, 1988). The most recent information is provided by Mafuyai *et al.*, (1996) and the present study area included a number of sites from these earlier studies along with new locations. While former studies seemed to concentrate more on the northern part of Nigeria, the present one has more information in southern and eastern areas.

Out of the six dominant species of *S. damnosum* complex described by Vajime and Dunbar (1975) and Vajime and Quillévéré (1978), only four were observed, *S. squamosum, S. yahense, S. damnosum* s.s. and *S. sirbanum* (Tables 4.1 & 4.2). *Simulium squamosum* was found to have the highest frequency distribution (58%) which covered mainly forest zones. Only one specimen was observed in the Guinea savannah zone at Tiga dam. Mafuyai *et al.* (1996) found *S. squamosum* to be widespread in Nigeria, occurring in the forest, montane and Guinea savannah zones, but not in Sudan savannah. The situation was different in the present study since *S. squamosum* was found at a single site in Sudan savannah thus extending the known bioclimatic distribution of *S. squamosum* in Nigeria to cover all zones. The opportunistic nature of *S. squamosum* described by Garms and Vajime (1975) and Vajime (1976) therefore is concordant with this very widespread distribution.

However, an alternative explanation could be that in Nigeria, *S. squamosum* is a generalist rather than a specialist species. Further observations would be necessary to distinguish these possibilities.

The frequency of *S. sirbanum* was the second highest (21.3%) followed by *S. damnosum* s.s. (17.3%) and *S. yahense* (3.3%). *Simulium sirbanum* was found in both the forest (15.6%) and savannah (84.4%) regions with more of it occurring in the savannah regions. This cytospecies has been known to show a strong preference for Sudan savannah conditions (Garms and Vajime, 1975; Vajime, 1976), for example in the arid regions of Mali and Burkina Faso; and its existence however in the forest zone shows that in Nigeria, *S. sirbanum* does move into forest type vegetation (Akpa, 1988).

*Simulium damnosum* was observed in all the vegetational zones (57.7% in forest, 42.3% in savannah). It is known to be one of the typical savannah species and in the OCP it has been found to predominate in the Guinea and transitional zones (Garms and Vajime, 1975; Vajime, 1976). Its mode of occurrence in the present study tends to reflect a species that is more or less opportunistic. The fact that it is one of the only two members of the *S. damnosum* complex (the other being *S. mengense*) found in both East and West Africa (Dunbar and Vajime, 1981) shows that it has an exceptionally wide overall distributional range.

*Simulium yahense* was observed only in the forest zone. In the montane forest of the Ngu Attai river in Cross River State, its frequency was 20% and in the typical forest climate of Agbokim waterfalls, it was 80%. This species has been known to be more restricted to the forest zone than *S. squamosum* and Vajime and Dunbar (1975) found that it required conditions for breeding that were quite different from other species within the complex. Only a single specimen was recorded sympatric with other species (Vajime and Dunbar, 1975). *Simulium yahense* is also known for its preference for small water courses (Garms and Vajime, 1975).

Three out of six forest sites where it was not recorded were big rivers (Oji, Ibu and Korum rivers). *Simulium yahense* was not observed in the Guinea savannah vegetational zone as reported by Akoh *et al.* (1987).

Sample areas in this study were chosen based on sites that had earlier been identified as positive breeding sites, areas where information on cytospecies distribution was available, or from known onchocerciasis endemic areas. However, cytological data for Korum and Ibe rivers in Cross River State and Ibu river in Imo State were unknown. The general distributions of *S. damnosum* complex in this study were as expected, designated forest-type cytospecies were found to predominate in forest vegetational zones and savannah cytospecies were found in savannah areas.

*Simulium squamosum* identified in Nigeria was found to have no fixed inversion and were all identical to that described by Vajime and Dunbar (1975). However, there was an observed geographic variation with regards to the sex mechanism of chromosome 1 in three forest sites (Table 4.4). This was characterized by a long non-pairing region of the centromere and had earlier been described by Vajime and Dunbar (1975) as a slightly larger expanded region lacking certain dark staining bands (Plates 1a & 1b). The frequency of occurrence of this sex-mechanism in males in the three sites were as follows; 63·5% in Korum river; 43% in Kwa falls; and 42% in Ibu river. Oji river with a pure *S. squamosum* population did not show the phenomenon.

Mafuyai *et al.* (1996) observed the same sex chromosome linkage in Kwa falls. Other chromosomal variations have been observed in *S. squamosum*. In Guinea, Sierra Leone and western Côte d'Ivoire, sex determination was found to be related to a band dimorphism of the centromere of chromosome 3 (SCIII). About 80% of males here were found to show this band polymorphism (Boakye, 1993). Also, a new subspecies has been found within the *S. squamosum* complex from East Zaire, *S. squamosum kitetense* (Post and Elsen, 1989). It

differs from *S. squamosum* by four fixed inversions; 1L-101; 2L-18; 2S-101 and 3L-101 of which 1L-101, 2S-101 and 3L-101 are unique to the subspecies.

In view of the taxonomic weight accorded to sex chromosome variations in Simuliidae, including the *S. damnosum* complex and the observations of sex determination in Cameroon (Vajime and Dunbar, 1975; Traore-Lamizana and Lemasson, 1987) and in Nigeria (Mafuyai *et al.,* 1996 and the present study) it has been proposed that the current observed three different populations in Nigeria and Cameroon be recognized as informal geographic races (Traore-Lamizana *et al.,* in press – see Appendix B).

These three *S. squamosum* populations have been described as *squamosum* "A" with the sex linked long non-pairing arm of chromosome 1 (1Ca), originally described by Vajime and Dunbar (1975) and is distributed throughout Cameroon except in the Sanaga river and around Mount Cameroon. In Nigeria, its distribution spreads across the Cameroon border into the south east (present study; Mafuyai *et al.* 1996). *Simulium squamosum* "B" which appears to be restricted to the Sanaga river in Cameroon and is characterised by the shorter and distinctive non-pairing region of 1Cb. *Simulium squamosum* "C" which has no sex-linked chromosome variation and is found distributed throughout most of Nigeria and around Mount Cameroon (Mafuyai *et al.,* 1996; present study). A mixture of *S. squamosum* "A" and "C" were observed north of Mount Cameroon and this also is the case in the present study some forest sites (Table 4.4).

A single identified female larval sample from Ibe river was found to have the non pairing centromere region of chromosome 1 and a split centromere on chromosome 3. More of these observations need to be made before commenting on the issue. Within the *S. damnosum* complex, water temperature, current speed, turbidity, pH and conductivity factors are said to play some part in restricting the geographical or seasonal distribution of the larval habitats of different cytospecies (Davies and Crosskey, 1991).

*Simulium damnosum* s.l. in Nigeria is found to breed in an average temperature of 27°C. In the savannah zone, temperature range was between 24°C and 33°C and the forest range was 22°C to 30°C. This fell within the observations of Grunewald (1981) in the savannah and tropical rainforest zones of West Africa for members of the *S. damnosum* s.l. cytospecies. High temperature readings of 30°C and above for all breeding sites were obtained during the hot and drier periods of the year (October – January). The Kogum river (with *S. damnosum* s.s. population) was the only site that had 30°C temperature in the month of September. Also, Kwa waterfalls, with a high *S. squamosum* population showed some exception in the temperature status during the month of October. The reading here was 23°C compared with 30°C - 31°C range for other sites during the same period. This lower temperature in a *S. squamosum* breeding site contradicts the general observations of lower breeding site temperatures for *S. yahense* (Grunewald, 1976a; 1976b; Quillévéré *et al.*, 1976; 1977). The low temperature at Kwa falls was again surprising since it was measured from the collection point, in the middle of the river, away from tree canopies or any form of shading, and it was expected that the heat of the sun's rays on the rocks would affect the temperature of the water. The observation therefore does not fall in line with that of Ocran *et al.* (1982) who concluded that temperature is closely related to the size of the river, or distance from the source, and that small shaded tributaries carry cooler water than the main rivers. On the basis of Ocran's assumption, Agbokim waterfalls should have given a lower temperature reading at the time (October) since the area in which larvae were collected and the measurement taken was under the shade of tree canopies. Another reason for such expectation (i.e. lower temperatures) in Agbokim would be based upon its typical *S. yahense* population (Grunewald, 1976a; 1976b; Quillévéré, 1976; 1977).

The overall observation here showed savannah sites to tend towards a more alkaline nature with a range of 7·0 to 7·9 while the forest rivers ranged from being slightly acidic as in Agbokim waterfalls (pH 6·5) to neutral to slightly alkaline pH (6·9 – 7·1). One montane

forest site (Assob bridge) recorded a pH of 7·9 (Table 4.5). These observations are not too far from the weak acid/weak alkaline recordings said to be peculiar to *S. damnosum, S. sirbanum* and *S. squamosum* (Grunewald, 1976a; Quillévéré *et al.*, 1976; 1977). The pH recordings of the breeding sites of *S. squamosum* and *S. yahense* were found to show differences. Two different recordings were made in Kwa waterfalls, a mainly *S. squamosum* breeding site. These were 6·9 (in October) and 7·8 (in January). This showed a tilt from near neutral to alkaline conditions. Grunewald (1976a) found *S. squamosum* to breed in a more or less neutral range of 6·9 –7·0. In the typical *S. yahense* breeding site at Agbokim waterfalls, an acidic pH of 6·5 was recorded which coincides with the acid habitat observed by Grunewald (1976a). Another site that gave two clearly different readings at different times of the year was Mateh river, a Guinea savannah site, where cytospecies found were non-*damnosum* (Table 4.5).

The conductivity of the water in the breeding sites of the *S. damnosum* species complex were found to show differences between the forest and savannah zone sites. In the savannah zone the conductivity range over all the sites was 0.3 – 0.6μS, while only readings of either 0.1 or 0.2μS were obtained in the forest zone with one montane forest site, Jarawa Kogi having a conductivity value of 03μS. In this site, cytospecies identification was unsuccessful (Table 4.5).

Water current is a physical parameter known to affect organisms in lotic habitat. Although examples of Simuliid larvae completing development in totally stagnant water are known (Crosskey, 1973), the majority of species require flowing water for larval development (Colbo and Watson, 1981). Blackfly larvae are known to depend upon a particular current velocity not only for their feeding activities but also for preventing the accumulation of oxygen depleted water around them (Grunewald, 1981). Members of this complex have been recorded in sites with current velocity ranging from 0·4m/s to 2·4m/s (Elsen and

Hebrard, 1977). The general observation with regard to current velocity measurements in the present study showed a range of 0·99m/s – 2·12m/s. The highest value of 2·12m/s was recorded in the Guinea savannah site at Galma river where *S. damnosum* and *S. sirbanum* cytospecies were found. The lowest recording of 0·99m/s was in the montane forest site of Ngu Attai river where all four cytospecies were recorded but only a single specimen of each cytospecies was identified. More non-*damnosum* species were found at this site.

Sites in the savannah zones appeared to have higher velocity (1·4 – 2·12m/s) compared to the forest zone breeding sites (1·17 – 1·77m/s). The water current reading in the typical *S. yahense* breeding site at Agbokim waterfalls was 1·17m/s, the second lowest overall reading. Whether the following data on water current in the different breeding sites have any relevance to the distribution of different *S. damnosum* species complex can not be determined here. It was however established by Wu (1931) that there existed an association between current velocity and larval Simuliid distribution.

This study confirmed the presence of a "Bioko form" of *S. yahense* in Bioko as earlier described by Wilson *et al.* (1997). It also confirmed the presence of the low polymorphic inversion 3L-I (Wilson *et al.*, 1997) in this population (Plate 1a). Cytotaxonomic, morphological, protein electrophoresis and DNA studies of this "Bioko form" confirm that it is an endemic form, taxonomically distinct and genetically isolated from the *S. damnosum* s.l. population on the mainland. It is however confined within the *S. squamosum* sub-complex and closely related to *S. yahense* (Wilson *et al,* 1994). Only few studies on the island's vectors have been made (Wilson *et al.*, 1997; Cheke *et al.*, 1997; McCall *et al.*, in press) and the extent of its distribution are unknown. However, Bioko is the only island known to have endemic human onchocerciasis and *S. damnosum* s.l. has not been found on the other islands of Annobon (J.Mas unpublished data) or São Tome (Santos Gracio, 1990). The island of Principe remains unsurveyed for blackflies.

In Cameroon, *S. squamosum* and *S. mengense* were the two cytospecies observed in the study areas. *Simulium squamosum* has been identified in south western Cameroon (Flook and Post, 1996) and also in the north (Traore-Lamizana & Lemasson, 1987). The first observations of *S. mengense* in Cameroon was by Vajime and Dunbar (1977) in the Menge river and this cytotype was later found scattered throughout the northern part of Cameroon (Traore-Lamizana & Lemasson, 1987). Very few records exist for *S. damnosum* cytospecies distribution in south and west Cameroon (Post, per. comm.). Cameroon so far is the only West African country where *S. mengense* has been recorded, the other country being Tanzania in East Africa (Dunbar & Vajime, 1981).

# CHAPTER SIX

# SUMMARY AND CONCLUSION

Cytological studies of *S damnosum* s.l. from different bioclimatic zones in Nigeria revealed the presence of four cytospecies; *S. squamosum*, *S. yahense*, *S. damnosum* s.s. and *S. sirbanum*. *Simulium damnosum* s.s. exhibited a wide distributional range through the main forest and savannah vegetational zones and *S. yahense* was very restricted in its distribution, occurring as expected, only in the forest zone. *Simulium squamosum* was found, in this study, mainly in the forest zone, contrary to expectation (Garms and Vajime, 1975; Vajime, 1976). New cytospecies information for Nigeria from this study came from two sites in the south; Korum and Ibe rivers in Cross River State and one from the east; Ibi river in Imo State. Until now, cytological studies had not been carried out in these sites. Korum and Ibi rivers displayed typical forest type cytospecies distribution. Korum river had 89% *S. squamosum* and 11% *S. damnosum*, whilst Ibi river had 100% *S. squamosum*. Ibe river had the following cytospecies distribution; 17% *S. squamosum*, 75% *S damnosum* and 8% *S. sirbanum*. This indicates that this site had cytospecies living sympatrically, with a higher proportion of *S. damnosum*, the so called "opportunistic" cytospecies (Dunbar and Vajime, 1981). *Simulium soubrense* and the Beffa form of *S. soubrense* were not observed in this study as earlier recorded in Nigeria by Mafuyai *et al.*, (1996).

In the forest zone *Simulium squamosum* exhibited sex chromosome differences of the non-pairing altered centric segment of chromosome 1 (1Ca). This chromosomal difference was earlier observed in Cameroon (Vajime and Dunbar, 1975) and in Kwa falls in Nigeria (Mafuyai *et al*, 1996). This sex chromosome determining mechanism of *S. squamosum* was found in sympatry with individuals of the same cytospecies in which it was absent. Oji river, a pure *S. squamosum* breeding site in the forest, had only the *S squamosum* type that lacks the sex linked mechanism *Simulium squamosum* in Nigeria therefore is shown to have two distinct populations.

The observed temperature range in the Nigerian breeding sites fell within that recorded by Grunewald (1981) for forest and savannah *S. damnosum* s.l. species. Hydrogen ion concentration (pH) in the *S damnosum* s.l. breeding sites were found in general to be slightly alkaline. Forest and savannah conductivity values had a range of 0.3-0.6μS in savannah sites and 0.1-0.2μS for forest sites. Current velocity in the savannah zone was 1·4 – 2·14m/s compared to 1·17 – 1·77m/s in the forest sites.

The effect of these physical/chemical parameters on the distribution of the different cytospecies can not be determined from the data collected. However, it can be said that these parameters do seem to differ to some extent in the different vegetational zones, and may consequently be related to cytospecies distribution. More investigation in this area is required to make any conclusive statement.

On the island of Bioko (Equatorial Guinea) a single cytospecies was observed, *S. yahense* "Bioko form". This "Bioko form" is confined within the *S. squamosum* sub-complex and is closely related to *S. yahense* (Wilson *et al.* 1994). Morphologically, it is classified as *S. yahense* (Wilson *et al.*, 1994) but cytotaxonomically it has no fixed inversion differences from either *S. squamosum* or *S. yahense* but it differs from both in the pattern of sex linkage of inversion 2L-18 which is Y-linked.

*Simulium squamosum* and *S. mengense* were the two cytospecies observed in the Cameroon sites. *Simulium squamosum* had earlier been observed in this south western part of Cameroon (Flook and Post, 1996). The presence of *S. mengense* in Cameroon is also confirmed in the present study. This cytospecies distribution has so far remained unique to Cameroon in West Africa (Vajime and Dunbar, 1977; Traore-Lamizana and Lemasson, 1987) and the other observation has been in Tanzania in East Africa (Dunbar and Vajime, 1981).

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[M. Traore-Lamizana, S. Somiari*, H. B. Mafuyai, C. G. Vajime and R. J. Post. *Medical and Veterinary Entomology* (1998), In press].

* formerly S. A. E. Bassey

# APPENDIX A

**A practical guide to the identification of sibling species of the *Simulium damnosum* complex in Nigeria.**

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# 1 INTRODUCTION

## 1.1 Need for Cytotaxonomic identification

The initial use of cytological parameters in larval identification in Uganda revealed four and later nine cytological segregates (Dunbar, 1966; 1969). This confirmed *S. damnosum* to be a species complex. In 1975, Vajime and Dunbar described eight cytospecies from West Africa, *S. damnosum s.s., S. sirbanum* and *S. sudanense, S. squamosum, S. soubrense, S. sanctipauli, S. yahense* and *S. dieguerense.* Ever since this pioneering period, there have been various revisions and interpretations of these initial observations.

Larval chromosome pictures of the *S. sanctipauli* sub-complex have proved difficult to interpret. *Simulium sanctipauli* and *S. soubrense* share a host of floating inversions and were distinguished from each other by the inversion 2L-7 (Vajime and Dunbar, 1975). However, this inversion has been found to be polymorphic across West Africa (Quillévéré, 1975; Quillévéré *et al.*, 1982; Meredith *et al.*, 1983) and so it is not suitable as a species diagnostic inversion. Post (1986) used two new fixed inversions (1L-A and 2L-A) to distinguish three species *S. sanctipauli, S. soubrense* and *S. soubrense* 'B'. 'Beffa' form (Meredith *et al.*, 1983) was considered an intraspecific variant within *S. soubrense* and two further species (*S. leonense* and *S. konkourense*) have been described from Sierra Leone and Guinea (Boakye *et al.*, 1993). *Simulium sirbanum* and *S. sudanense* are now considered to constitute a single cytospecies, *S. sirbanum*, with strong evidence of incipient speciation (Vajime, 1989) but the species status of *S. damnosum* s.s. and *S. sirbanum* has been supported. *Simulium dieguerense* is almost restricted to Guinea and Mali (Boakye & Mosha, 1988).

Vajime and Dunbar (1975) reported sex determination associated with chromosome 1 in Cameroon. In Guinea, Sierra Leone and Western Cote d'Ivoire, sex determination is related to a band dimorphism of the centromere of chromosome 3 (SCIII) (Boakye, 1993). *Simulium squamosum* has 2L-18 as a floating inversion. This inversion is that which distinguishes *S. yahense* from *S. squamosum* and here it is found to be sex-linked. More

females are homozygous (2L-18/18) whilst more males are heterozygous (2L-St/18) (Vajime and Dunbar, 1975).

Knowledge of cytotaxonomic separation of the different cytospecies is important in all onchocerciasis endemic areas. In Nigeria where the Nigerian Onchocerciasis Control Programme (NOCP) has been set in motion to carry out control of the disease through ivermectin distribution, very little is known about the role of the different sibling species in the role of onchocerciasis transmission. Major gaps exist in the knowledge of the vectors in Nigeria especially in the east, west and southern parts of the country. Attention was drawn to this cytotaxonomic gap in Nigeria in 1977 by the World Health Organisation (WHO) and it urged that priority be given to Nigeria to complete the taxonomy of the complex in West Africa. In Crosskey's appraisal of current knowledge of *S. damnosum* s.l. in Nigeria in 1979, he mentioned the need to taxonomically identify members of the complex as one of the major requirements in the provision of new data for control planning. The other requirement was the acquisition of data on geographical locations of breeding sites of *S. damnosum* s.l. Crosskey (1981) indicated that the incidence of the disease is not distributed uniformly across the country, but a clear correlation exists with basic geology, that is, vector breeding sites are most commonly found where the Precambrian basement rock is exposed to break the flow of water and create rapids. Crosskey (1981), recognised five endemic zones of the Precambrian rocks and four small foci on sedimentary rocks along the Niger valley (Fig 1).

Cytotaxonomic information on *S. damnosum* s.l. species complex in Nigeria has however been sparse (Vajime & Dunbar, 1975; Dunbar & Vajime, 1981; Gregory, 1982; Post, 1986; Crosskey, 1987; Vajime & Gregory, 1990). There has been the lack of a complete identification key for Nigeria or even the provision of idiograms to indicate inversion differences among members of the complex. Also there is the absence of an overview or regional picture of Nigeria's vector population status. Studies on the constituent members of the *S. damnosum* complex in West Africa have almost been entirely confined to the countries of the Onchocerciasis Control Programme (OCP) and have been recently summarised by Boakye (1993).

In planning any control programme, it is necessary to first acquire precise knowledge of the different species (Le Berre & Fiasorgbor, 1985). This could lead to the correlation of the different forms with epidemiologically significant factors such as anthropophily and the tracing of migratory patterns or insecticide resistance distribution (Post, 1986). Species identification would normally be carried out by a trained cytotaxonomist, but simple identification instructions that can be followed and understood by any non-specialist would be a useful tool for the routine identification of Nigerian cytospecies. Such instruction should provide a step by step basis of identification of the diagnostic features of the three chromosomes of the *S. damnosum* complex, which will then lead to the recognition of the basic inversions used in separating the different cytospecies.

This paper is aimed at providing the means through which specific identification of Nigerian materials can be made. It therefore includes, complete cytotaxonomic instructions for the non-cytotaxonomist. It is intended that with practice any entomologist can use this paper to identify all Nigerian *S. damnosum* sibling species.

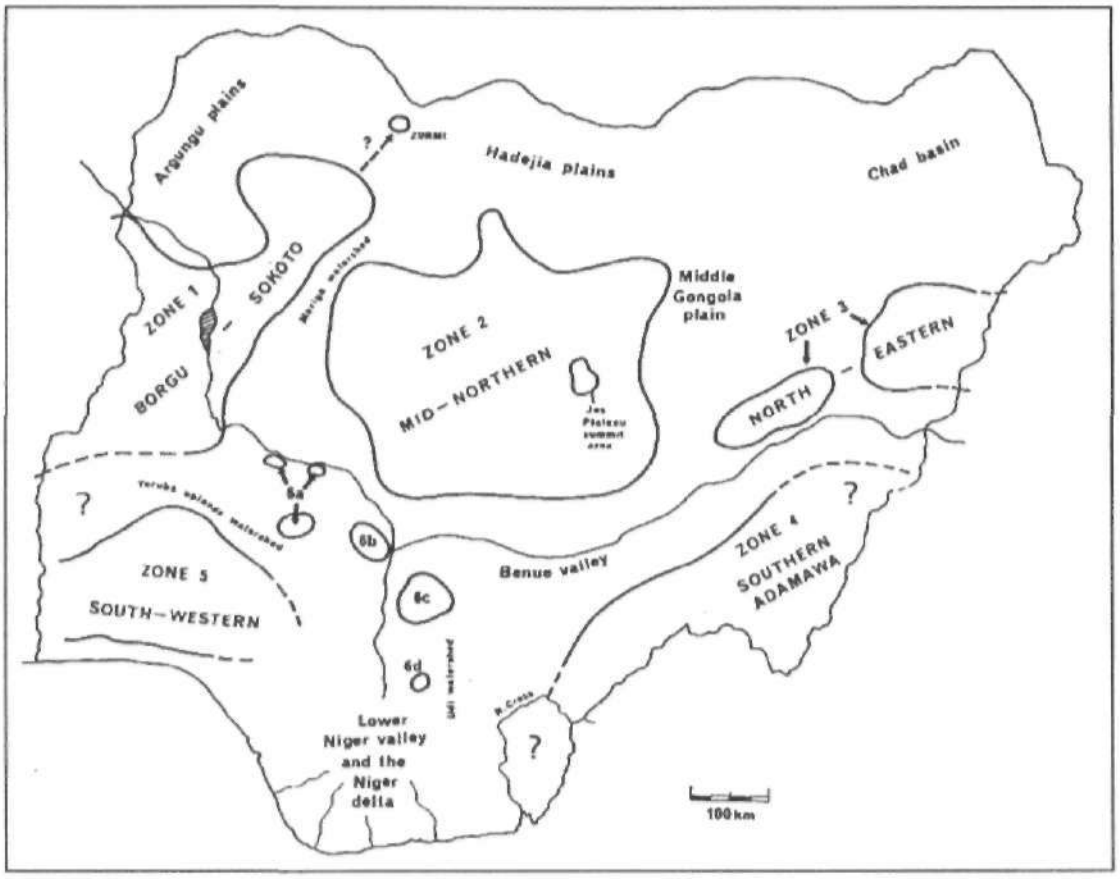



**Fig 1** – The five recognised onchocerciasis endemic zones in Nigeria (Crosskey, 1981).

## 1.2. Giant/Polytene chromosomes

The cytotaxonomic method of Simuliid sibling species identification is considered the most reliable (Le Berre and Fiasorgbor, 1985) and it has been used as a reference point in various fields of research. It is the "gold-standard" in the development of new identification tools.

Cytogenetic analysis of *S. damnosum* complex is feasible due to the fact that their salivary gland polytene chromosomes are easily identifiable and they have a low chromosome number 2n=6 (*i.e.* three pairs).

Giant/polytene chromosomes are found primarily in well-differentiated organs that are engaged in vigorous, often secretory, metabolic activities for example, in salivary glands, intestines and malphigian tubules. These tissues grow by an increase in cell size rather than number. Along these individual chromosome fibres are numerous dense spots called

chromomeres, which contain much-folded deoxyribonucleic acid (DNA). Homologous or matching chromomeres of neighbouring filaments are fastened together to form bands. Chromosomes resulting from this growth process are said to be polytene and have multi-strand structure resembling a rope (White, 1973).

The bands vary in thickness and contain high concentrations of DNA and histones (proteins associated with DNA). Each giant chromosome in a set within a cell has its own characteristic sequence or pattern of banding which appears in the homologous giant chromosome of every individual of the species. Spectacular changes in appearance can occur at some of the genetic loci of polytene chromosomes to bring about swellings or puffs. Specialised puffs like the balbiani rings exist. Formation of a puff involves the accumulation of acidic protein, despirilisation of DNA within the region where the puff is to be formed, synthesis of ribonucleic acid (RNA) and the storage of newly synthesised RNA (Beerman, 1965).

## 1.3 Inversions

An inversion is a 180° reversal of the banding pattern of a section of a particular polytene chromosome. A single chromosome can be either inverted (I) or un-inverted (S). However an individual diploid fly can be either homozygous inverted (II),or heterozygous (IS), or homozygous un-inverted (SS), that is, exhibiting the standard sequence. Inversions can be fixed or floating. Fixed inversions are entirely homozygous (II or SS) and the existence of fixed inversions allows absolute recognition of species. In a population sub-divided as 'A' and 'B', if all individuals in population A have the un-inverted sequence (SS) and those of population B have an inverted sequence (II), the absence of a heterozygous (IS) between the two kinds of homozygotes would show no interbreeding and attest to two distinct reproductive units (*i.e.* two species). Population 'B' would differ from population 'A' by one fixed inversion. Floating inversions can be in the homozygous (SS, II) or heterozygous (IS) state. According to simple Mendelian inheritance, a floating inversion in a population will occur in Hardy-Weinberg equilibrium (Dobzansky, 1951; Mayr, 1970). A floating inversion at 50% frequency on the chromosome produces one quarter homozygous each for the standard and inverted sequence and half heterozygous ($^1/_4$ SS, $^1/_2$ IS, $^1/_4$ II). However, for a floating inversion to occur naturally in a Hardy-Weinberg equilibrium, the traits must be

under normal Mendelian inheritance and the population freely breeding. If a species begins to split into two (incipient speciation) and a particular inversion is involved, the Hardy-Weinberg equation will quickly indicate this. Usually the first sign being a rather low number of heterozygous (Vajime and Dunbar, 1975). Different species of Simuliidae sometimes have distinctly different sets of floating inversions. However, some floating inversions have been found to be shared by two or more closely related species as floating inversions in both, or sometimes as inversions floating in one species but fixed in the other. Such floating inversions are thought to have been in the parent population and as speciation occurred both new species retained those floating inversions. Usually, the degree of similarity in the floating inversions shared and their proportion can be taken as a rough indication of the relative time since divergence.

In most species as in Man, chromosomes in sperm and egg determine the sex of offspring. In the Simuliidae, males are known to have an X and Y chromosome, while females have two X-chromosomes. Most species are found to show some form of sex chromosome differentiation of the banding pattern of their sex chromosomes. Sex chromosome differentiation may involve inversions and/or band polymorphism. Band polymorphisms are distinguishing features found on one or both arms of a chromosome. There are differences in appearance of one or more bands, which are stable and inherited. They could appear homozygously or heterozygously and in some cases they have been found to be linked to sex chromosomes and as such used as a sex-determining factor. They are sometimes associated with a local area of non-pairing (split) of the polytene chromosome arm, especially when they are sex-linked. These bands are distinguished from puffs which, even though they are found occurring throughout the generation of a particular species, are not inherited but are determined by the expression of genes in particular environments or particular stages of the larval life cycle.

## 1.4 Cytoforms of *S. damnosum* complex in Nigeria

Cytotaxonomic studies of the *S. damnosum* complex in Nigeria revealed nine cytoforms from different parts of the country (Vajime & Dunbar, 1975; Dunbar & Vajime, 1981; Gregory, 1982; Roberts, 1985; Crosskey, 1987; Akoh *et al.*, 1987; Vajime & Gregory, 1990; Wilson *et al.*, 1994; Mafuyai *et al.*, 1996). These are;

1. *S. damnosum* Theobald s.s.
2. *S. sirbanum* Vajime & Dunbar
3. *S. sudanense* Vajime & Dunbar
4. Volta form of *S. damnosum* complex (sensu Vajime & Gregory, 1990)
5. *S. squamosum* Enderlein
6. *S. yahense* Vajime & Dunbar
7. *S. sanctipauli* Vajime & Dunbar
8. *S. soubrense* Vajime & Dunbar
9. Beffa form of *S. soubrense* (sensu Meredith et al., 1983).

The cytotaxonomic status of *S. sudanense* and the Volta form is not universally accepted (Crosskey, 1987; Vajime, 1989; Boakye, 1993) and in this paper are considered synonymous with *S. sirbanum* and *S.* damnosum s.s.. *Simulium sanctipauli* is unknown east of Togo except for one unconfirmed record in Nigeria. Not all published identifications have distinguished the Beffa form and typical form of *S. soubrense*, but those which do were all Beffa form and probably all *S. soubrense* in Nigeria are the Beffa form. A summary of the distribution pattern of members of the complex in Nigeria is shown in Table 1.

**Table 1** - Distribution of *S. damnosum* complex in Nigeria (Mafuyai *et al.*, 1996 and Somiari, 1998)

| Climatic zones | Cytospecies | | | | |
|---|---|---|---|---|---|
| | *damnosum* | *sirbanum* | *squamosum* | *yahense* | *soubrense* |
| Sudan savannah | 30 | 141 | 1 | - | - |
| Guinea savannah | 162 | 31 | 59 | - | 1 |
| Montane Forest | 20 | 2 | 182 | 1 | - |
| Forest | 16 | 4 | 120 | 53 | 20 |

## 2. LABORATORY PROCEDURES

To observe these chromosomes and the specific features (landmarks, inversions) good chromosome preparations have to be made.

## 2.1. Collection and storage of *S. damnosum* larvae

Large larvae of *S. damnosum*, without the dark histoblast, were collected from fast flowing points of rivers, streams or falls. They are not usually found in the smallest and slowest streams. They are attached to substrates such as dead leaves, plants and stones.

Larvae are collected with the help of forceps and placed in sample bottles (bijou bottles) containing freshly prepared fixative (2 parts absolute alcohol : 1 part glacial acetic acid). A 15ml bottle should contain not more than about 20 larvae. The fixative with larval specimens is changed about twice before final storage. First after 2 to 5 minutes, then after 1 hour. Bottles are labelled using pencil on plain paper which is stored inside the bottle. Labelling must include the following information;

i. Date of collection
ii. Name of rivers and site
iii. Latitude and longitude

For best results, chemicals should be cold, that is, carried in an ice box to the field and fixed specimens should be preserved in the refrigerator. Such specimens can be kept up to one year or more in the refrigerator. However, in the absence of a refrigerator, specimens should always be kept in a cool place, away from direct sunlight.

## 2.2. Morphological Identification

Using a dissecting microscope, the *S. damnosum* s.l. larvae can be identified from other *Simulium* larvae on the basis of the following characteristics (Crosskey, 1960)

i. Scales on the thoracic proleg
ii. Dorsal tubercules

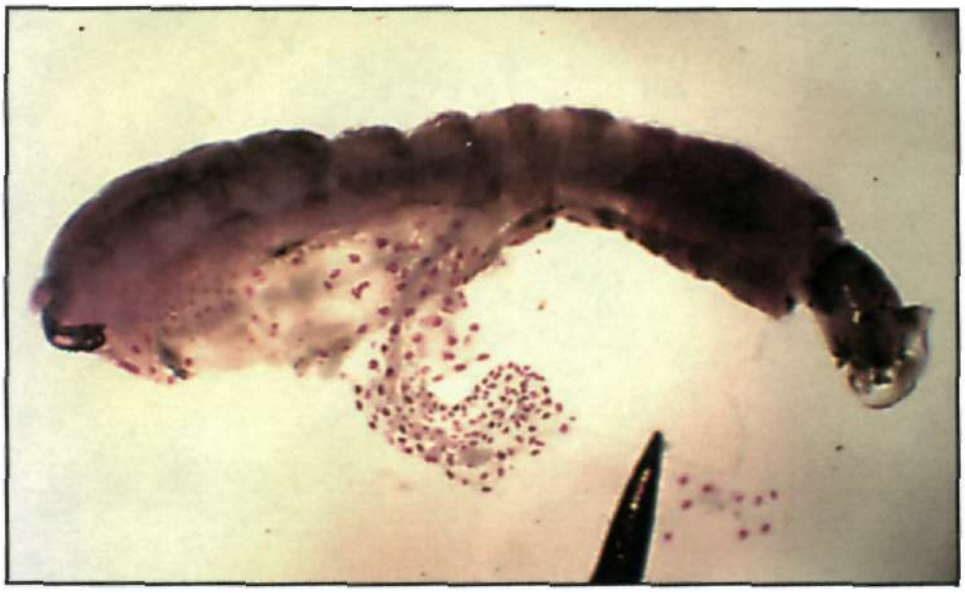


**Fig.2** – Dissected larva of *S. damnosum* s.l. showing stained (pink) salivary glands (*picture courtesy Magda Charalambous).*

## 2.3. Chromosomal preparations

**Dissection**

i. Place larvae on a clean slide and insert two sharp needles ventrally towards the posterior bulge of the abdomen.

ii. Using needles open the abdomen, approximately two-thirds of its entire length from the posterior end. The salivary glands are now exposed and can be seen.

*N.B.* The salivary glands are large transparent paired structures extending posteriorily to about three-quarters of the length and then doubling back.

**Staining Methods**

Either the Feulgen or lacto-acetic orcein staining protocol maybe used

Feulgen

i. Wash dissected larvae in distilled water for 5 minutes.
ii. Blot dry.
iii. Hydrolyse in 5NHCL at room temperature for 30 minutes.
iv. Blot dry.

v. Place larvae in stoppered bottle of Feulgen and keep in dark cupboard for 15 minutes to 1 hour.

Lacto-acetic orcein

i. Wash dissected larvae in distilled water for 5 minutes.
ii. Blot dry.
iii. Place larvae in pre-warmed (45 C) 10% HCL for 1 hour.
iv. Blot dry.
v. Place individual larvae on a slide with a drop or two of stain for 30 minutes to 1hour.

**Mounting**

i. Place individual larva (after staining) on a clean slide in a drop of 50% acetic acid for 5 min. (Glands and paired gonads become visible at this point, Fig 2).
ii. Determine sex by observing gonodal shape (Fig 3)

*N.B.* The gonads are located towards the posterior end of the abdomen. They lie close to the dorsal wall in the 6th segment, about the end of the mid gut. The testes are pear-shaped while the ovaries are banana-shaped.

iii. Transfer salivary glands to another clean slide in a drop of 50% acetic acid.
iv. Cover with a cover slip.
v. Squash gently, taping specimen between filter paper.
vi. View under compound microscope.

If a good (clearly stained and well spread) chromosome preparation is obtained, a temporary or permanent mount can be made for subsequent detailed analysis *i.e.* reading of bands using a microscope at around 400x magnification.

**Temporary Mount**

Ring the cover slip with rubber solution or nail varnish. This will slow down the drying out of the slide, which is normally stored in a freezer.

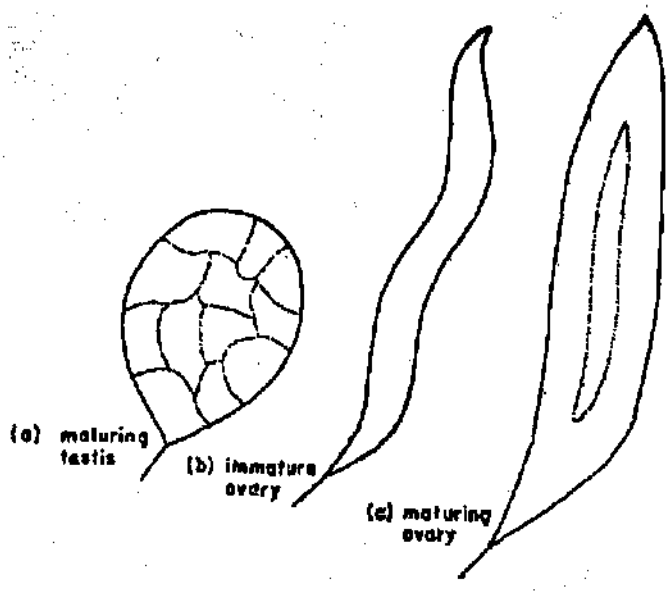



**Fig. 3** – Diagram of larval gonads (Dunbar, 1972)

**Permanent Mount**

The dry ice method of Dunbar (1972) can be employed with modifications.

i. Place slides (with cover slips) in a deep freezer for an hour or more, or even overnight.
ii. Remove from freezer and quickly prise off cover slip with the aid of a razor blade.
iii. Immerse cover slip and slide in 100% alcohol for 2 seconds.
iv. Drain off excess alcohol on filter paper and reunite slid and cover slip with a drop of mounting medium e.g. euparol.

# 3. CHROMOSOMAL DESCRIPTION

Vajime and Dunbar (1975) divided the three haploid chromosomes (*i.e.* the karyotype) from the tip of the short arm of chromosome 1 to the tip of the long arm of chromosome 3 into 100 units. These are referred to as the total complement length (TCL). Each chromosome is divided based on its relative proportion to the TCL. Chromosome 1 is 42% TCL, chromosome 2 is 30% TCL and chromosome 3 is 28% TCL. Letters 'S' and 'L' designate short and long arms of each homologous chromosome pair. The centromere is the marker constant in all three chromosomes and it divides each into its long and short arm. The designation 1S, 1L, 2S, 2L, 3S, 3L simply indicate the short and long arms of the three chromosomes (Plate 1).

## 3.1 Landmarks/Markers

Recognition of the three chromosomes is based on the observation of specific landmarks on them and these are as described below (see also Plates 1 – 9).

Chromosome 1

i. Expanded Region (EX) - a darkly stained region housing the centromere (C).
ii. Nucleolar Organiser (NO) - found on the short arm and appears as a region of broken bands giving the chromosome a broken image.

Chromosome 2

i. Double bubble (db)- a feature characterised by a ' puff ' or ' circle '.
ii. Balbiani ring (B) - large puff separated from db by 2-3 dark bands.
iii. Centromere - separated from ' B ' by 2-3 dark bands which sometimes appears as a single extended dark band.
iv. Parabalbiani Ring (PB) - found on the long arm with a concave/convex appearance.

Chromosome 3

i. Blister (b) - a puffy structure close to the arm, its identification is usually aided by a thick dark band just before it.




**Plate 1**: Full karyotype of standard sequence showing chromosome 1, 2 and 3 and some landmarks

# 4 DESCRIPTION OF CYTOSPECIES

## 4.1 *Simulium squamosum.*

The polytene banding pattern of *S. squamosum* is the arbitrarily chosen standard for the West African members of the *S. damnosum* complex (Dunbar & Vajime, 1972) and hence it is designated "St" in all chromosomes (Plate 2). It is the closest of this group to the East African forms from which it is differentiated by the inversion 1S-1 & 1L-3. These two fixed inversions differentiate all members of the West African *S. damnosum* complex from those in East Africa (Boakye, 1993).

There are three observed sex-determining mechanisms here, two of them on chromosome 1 and the other on chromosome 3.

i. A long centromeric non-pairing region on chromosome 1 (Ca). This is found mainly in males and this condition was first observed in Cameroon (Vajime & Dunbar, 1975) and in southeastern Nigeria (Mafuyai *et al.*, 1996; Traore-Lamizana *et al.*, in press).

ii. A short distinct non-pairing region on the centromere of chromosome 1 (Cb) and also found mainly in males (Traore-Lamizana *et al.*, in press). This feature is characteristic of *S. squamosum* found in the Sanaga river of Cameroon.

iii. A band dimorphism of the centromere of chromosome 3 (Sc III) found mostly in males and reported from Guinea, Sierra Leone and Côte d'Ivoire (Boakye, 1993). In some *S. squamosum* species there is no sex-linked chromosome variations ( i.e. no non-pairing of 1C) and this form is distributed through out most of Nigeria (Mafuyai *et al.*, 1996; Somiari, 1998-unpublished data).

A few heterozygotes of 2L-18 have been found for both sexes of *S. Squamosum* in parts of Nigeria - Jos Plateau (30%), SouthWest Nigeria (36%) and Nothern Cameroon (5%) (Traore-Lamizana *et al.*, 1998 - In press). *Simulium squamosum* is wide spread in Nigeria and has been identified from montane forest, forest and Sudan savannah zones.

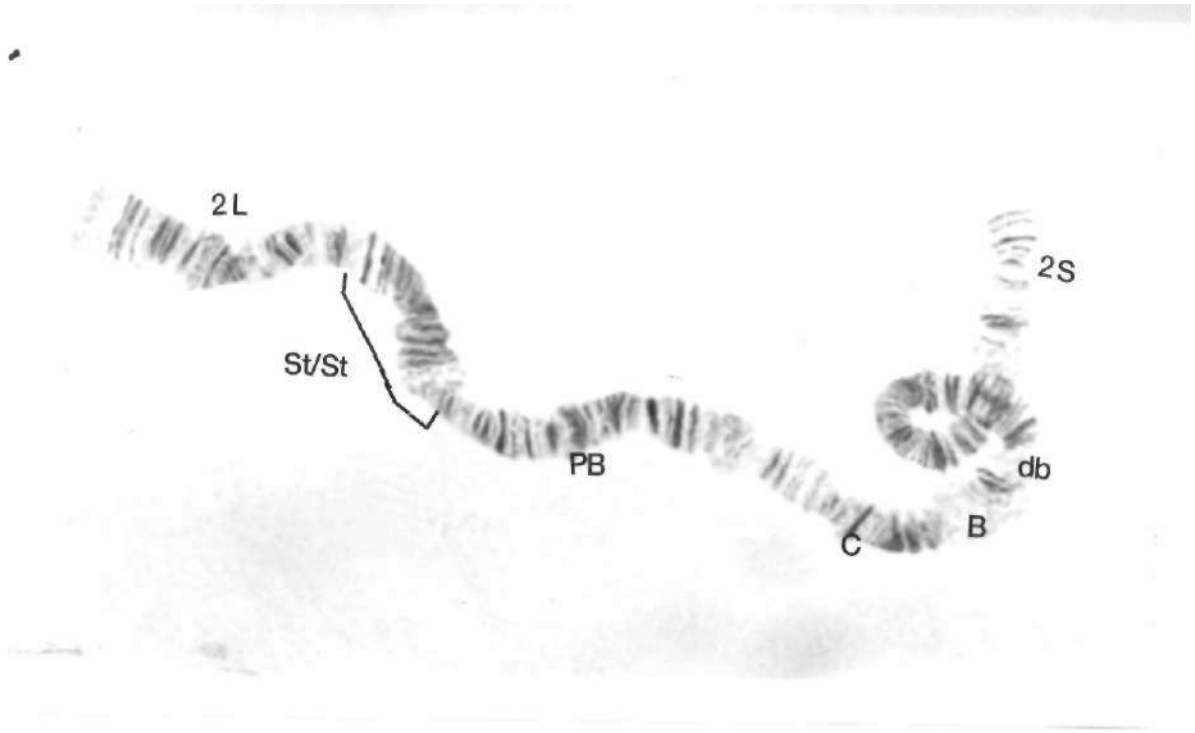



**Plate 2**: Chromosome 2: Standard sequence of *Simulium squamosum* (2L-St/St)

## 4.2. *Simulium yahense*

This is the closest of the West African *S. damnosum* sl to the chosen standard *S. squamosum*. Both species show no fixed inversion differences (Quillevere, 1975), their specific status having been confirmed by iso-enzyme electrophoresis (Meredith and Townson, 1981; Garms and Zillmann, 1984; Thompson *et al.*, 1990). Chromosomally, this species is distinguished from *S. squamosum* by the inversion 2L-18 (2L-18-18) (Plate 3).

Sex determination is on chromosome 2 associated with inversion 2L-18 (plate 4). Many more females than males are found to be homozygous for this inversion and males are heterozygous, hence 2L-18 is associated with sex determination with high frequency on the X-chromosome and low frequency on the Y-chromosome (Vajime and Dunbar, 1975). In Nigeria the situation conforms to this cytotaxonomic description. Identification problems could arise when *S. yahense* is found sympatric with *S. squamosum* since *S. squamosum* has 2L-18 as a floating inversion (Boakye, 1993). In Nigeria five specimens heterozygous for 2L-18 were identified as *S. squamosum* (not *yahense)* because they showed no evidence of sex-linkage (Mafuyai *et al.*, 1996). This species is known to have a focal distribution pattern (Vajime and Dunbar, 1975). In Nigeria, it has been observed only in the forest zone in the southeastern part of the country (Mafuyai *et al.*, 1996).

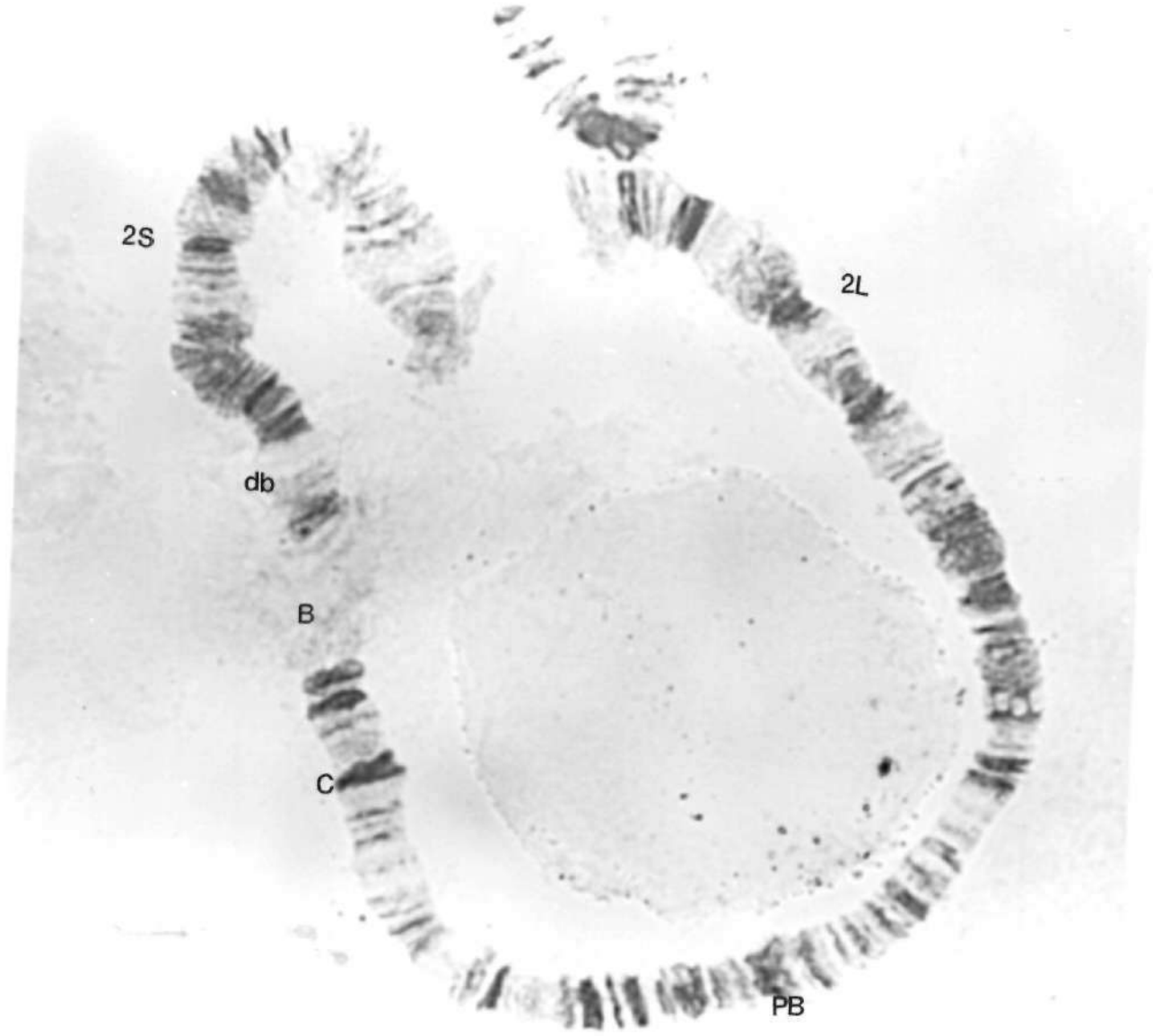



**Plate 3**: Chromosome 2: Homozygous sequence of inversion 18 of *Simulium yahense* (2L-18/18)

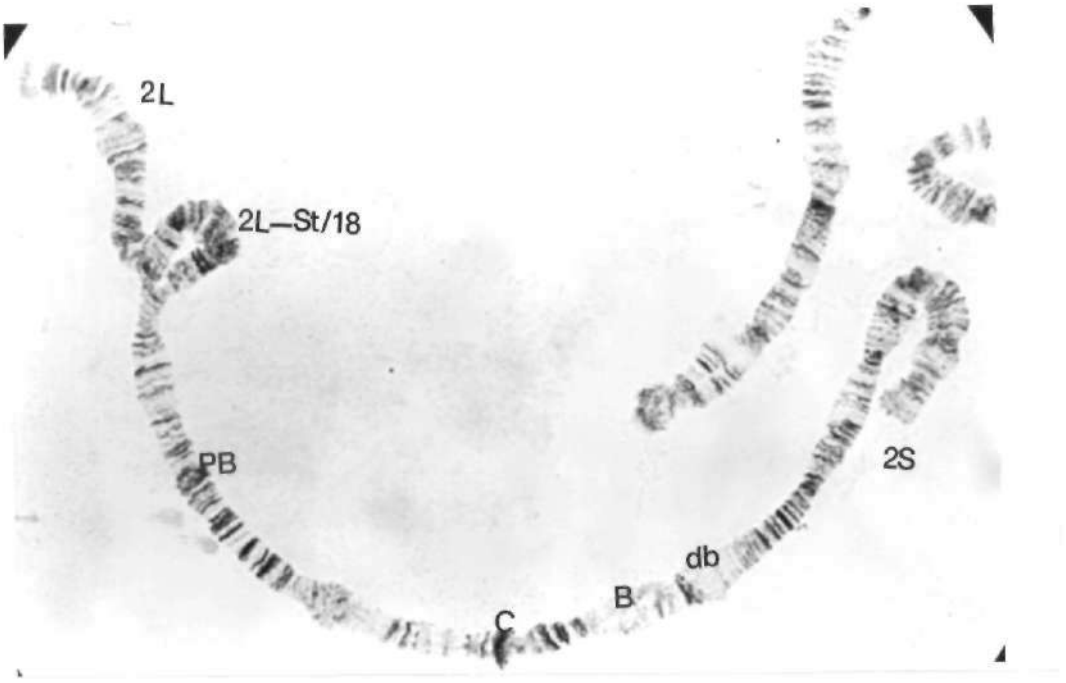



**Plate 4**: Chromosome 2: Heterozygous sequence of inversion 18 of *Simulium yahense* (2L-18/St)

## 4.3. *Simulium damnosum* s.s.

On the basis of chromosome 2, *S. damnosum* s.s. differs from *S. squamosum* by fixed inter-specific complex inversion 2L-C (plate 5). Dunbar and Vajime (1981) reported a difference in *S. damnosum* s. s. found in the OCP area from the type species of Uganda and called it the Volta form. However, the separate taxonomic status of these cytoforms is universally accepted (Boakye, 1993). Sex determination is on chromosome 2 and associated with the inversion 2L-8. Most females are found to be homozygous 2L-C/C and most males are heterozygous 2L-C/C.8 (plate 6).

Its distribution in Nigeria is wide spread in the savannah region and it has been observed breeding in a rain forest site in south-western Nigeria and along River Okpara, where it forms an international border between Nigeria and Benin Republic.

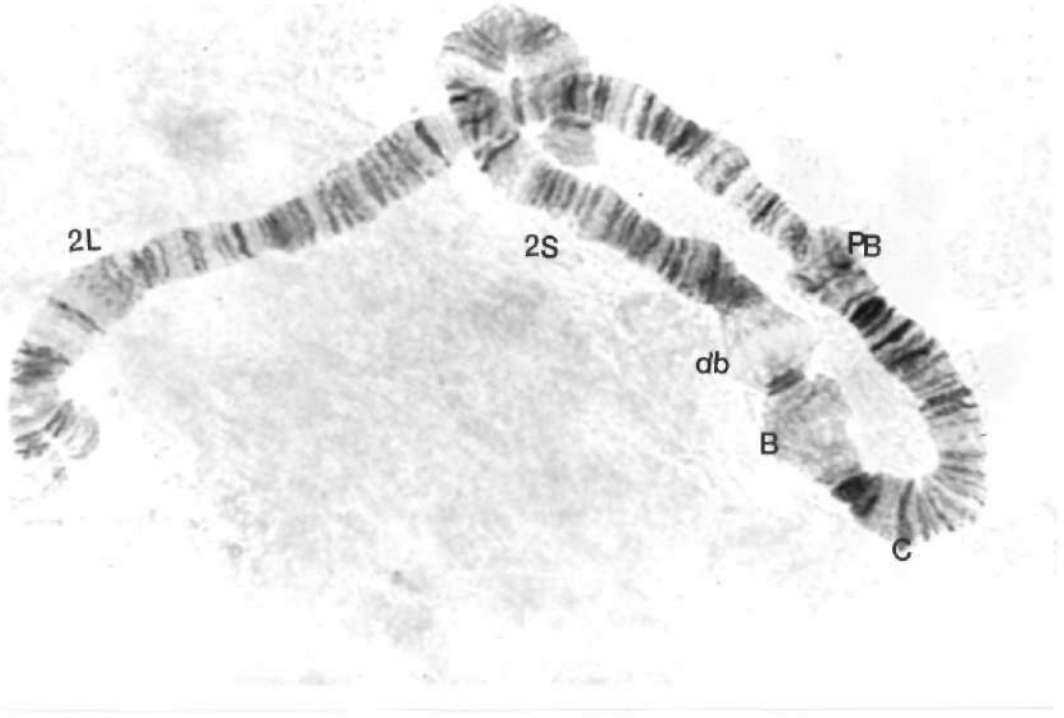



**Plate 5**: Chromosome 2: Homozygous sequence of inversion C of *Simulium damnosum* (2L-C/C)

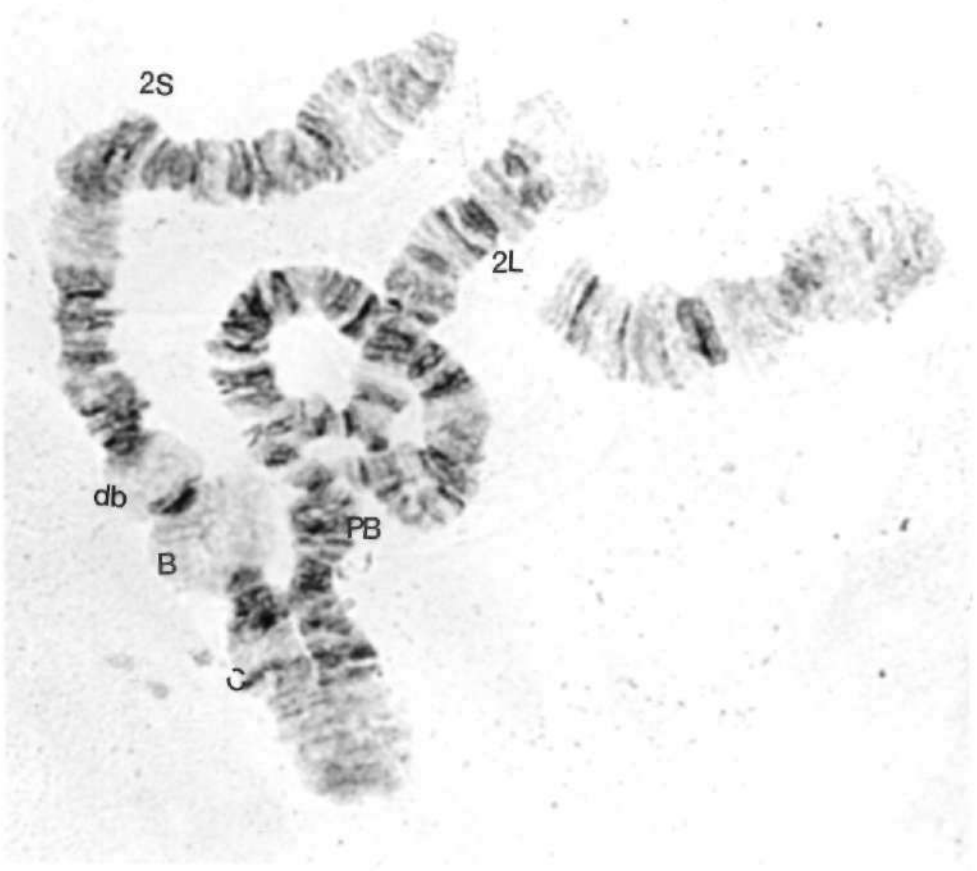



**Plate 6**: Chromosome 2: Heterozygous sequence of inversion C and 8 of *Simulium damnosum* s.s. (2L-C/C.8).

### 4.4 *Simulium sirbanum*

This is made up of two cytoforms, *S. sirbanum* and *S. sudanense* that were initially thought to be different species, but now found to constitute a single cytospecies (Vajime, 1989). Individuals of this species are separated from *S. squamosum* by the fixed inversions 1L-1, 2L-C.8 and 3L-2 (Plate 7). They differ from *S. damnosum* s.s. by the inversion 2L-8 which is fixed and diagnostic in both males and females.

Sex determination is on chromosome 1, associated with the inversion 1S-3 in a complicated way (Vajime & Dunbar, 1975). X chromosomes can be either 1S-St or 1S-3, such that in any particular population females are usually either 1S-St/St or 1S-3/3. Y chromosomes can be either 1S-St or 1S-3, but in such a way that in any particular population males are usually heterozygous. Hence Vajime and Dunbar (1975) described two sorts of populations, one in which females are 1S-St/St and males 1S-St/3 (*S. sirbanum*) and the other in which females are 1S-3/3 and males are 1S-St/3 (*S. sudanense*), but the situation is more complicated and Vajime (1989) prefers to treat them all as belonging to a single species *S. sirbanum*.

In Nigeria it is found distributed mostly in savannah bioclimate where it is associated with the more severe blinding foci of onchocerciasis.

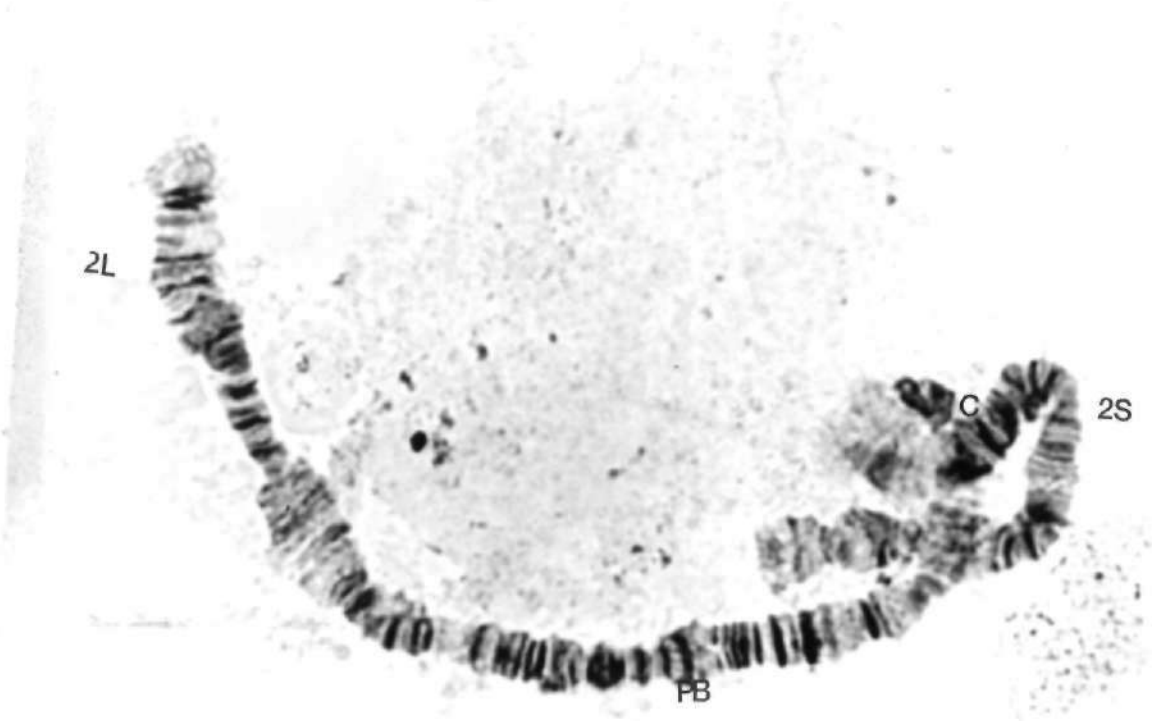



**Plate 7**: Chromosome 2: Homozygous sequence of inversion C and 8 of *Simulium sirbanum* (2L-C.8/C.8)

## 4.5 *Simulium sanctipauli* s.l.

This is a sub-complex of four species, namely, *S. sanctipauli, S. soubrense, S. leonense* and *S. konkourense* (Boakye *et al*, 1993) but *S. leonense* and *S. konkourense* have not been found east of Guinea. The diagnostic inversion for members of this sub-complex is 2L-4 (Boakye, 1993) but in addition, each member has some fixed inversion which differentiate it from the other species of the sub-complex. *Simulium sanctipauli s.s.* (sensu Boakye *et al.*, 1993) is differentiated from the other members by the presence of the fixed interspecific inversion 2L-A (Post, 1986) (Plate 8).

All members of the *S. sanctipauli* sub-complex lacking the inversions, 1L-A and 2L-A are *S. soubrense* (Post, 1986) (Plate 9). The Beffa form of *S. soubrense* often shows a distinctive sex-linked association with an inversion designated 2S-6b (Meredith *et al.*, 1983). Males are heterozygous for the inversion and it is absent in females.

*Simulium sanctipauli* has been reported from the middle of the northern part of Nigeria (Akoh, *et al.*, 1987; Crosskey, 1987), but these identifications were based on the cytotaxonomic criteria of Vajime and Dunbar (1975) without the recent modifications of Post (1986) and hence need to be re-confirmed. *Simulium soubrense* has been found in the major rivers of the south west and from River Gurara and river Okpara on the boarder with Benin (Meredith *et al.*, 1983; Cheke *et al.*, 1987). The records refer to the Beffa form of *Simulium soubrense*, but a few older records do not distinguish the Beffa form from the typical form. In summary, *S. sanctipauli, S. soubrense* and the Beffa form of *S. soubrense* have been identified from Nigeria, but it is possible that in fact only the Beffa form occurs. These cytotaxonomic descriptions are presented as a key in section 5.

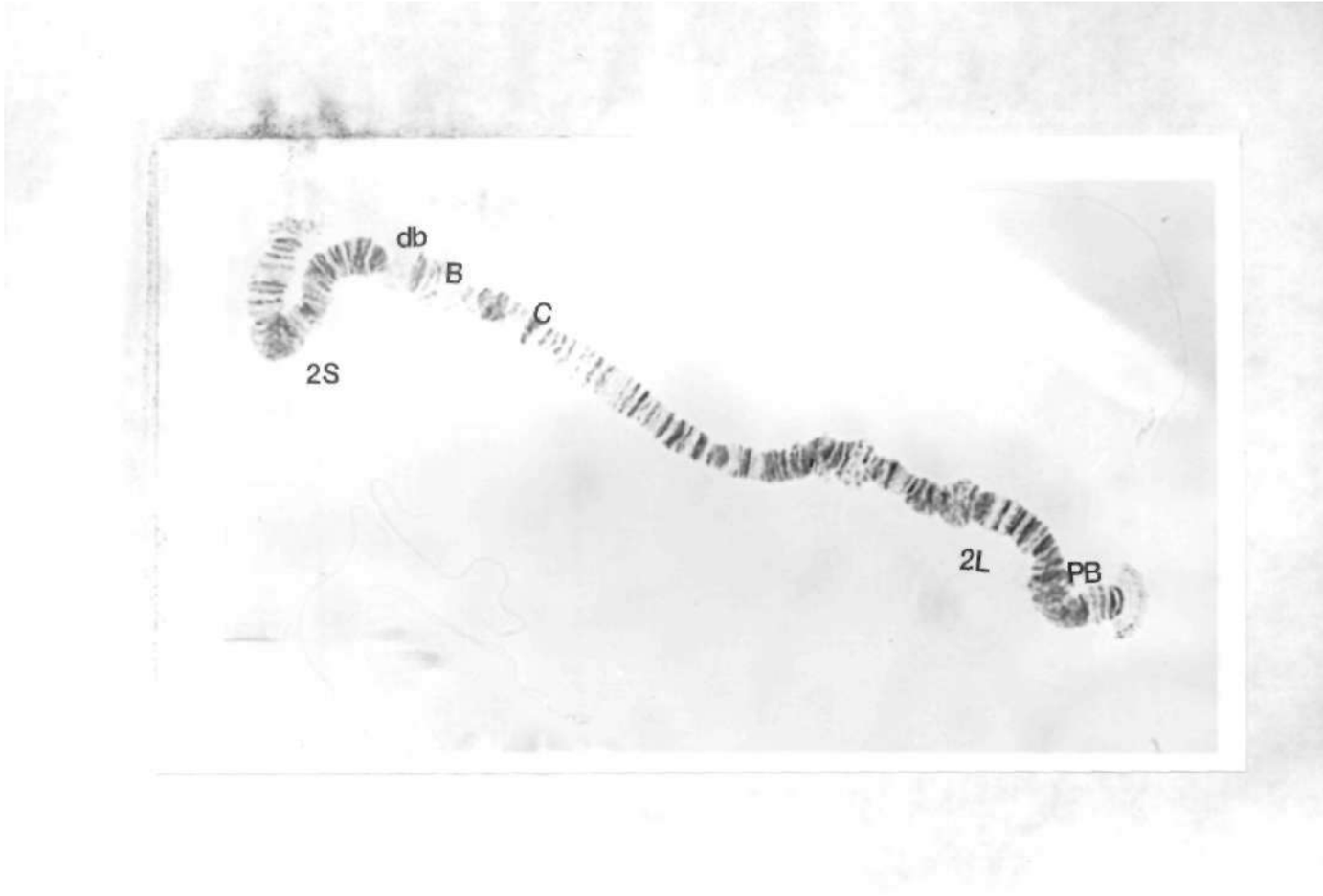



**Plate 8**: Chromosome 2: Homozygous sequence of inversion 4, 6, 7 and A of *Simulium sanctipauli* (2L-4.6.7.A/4.6.7.A).

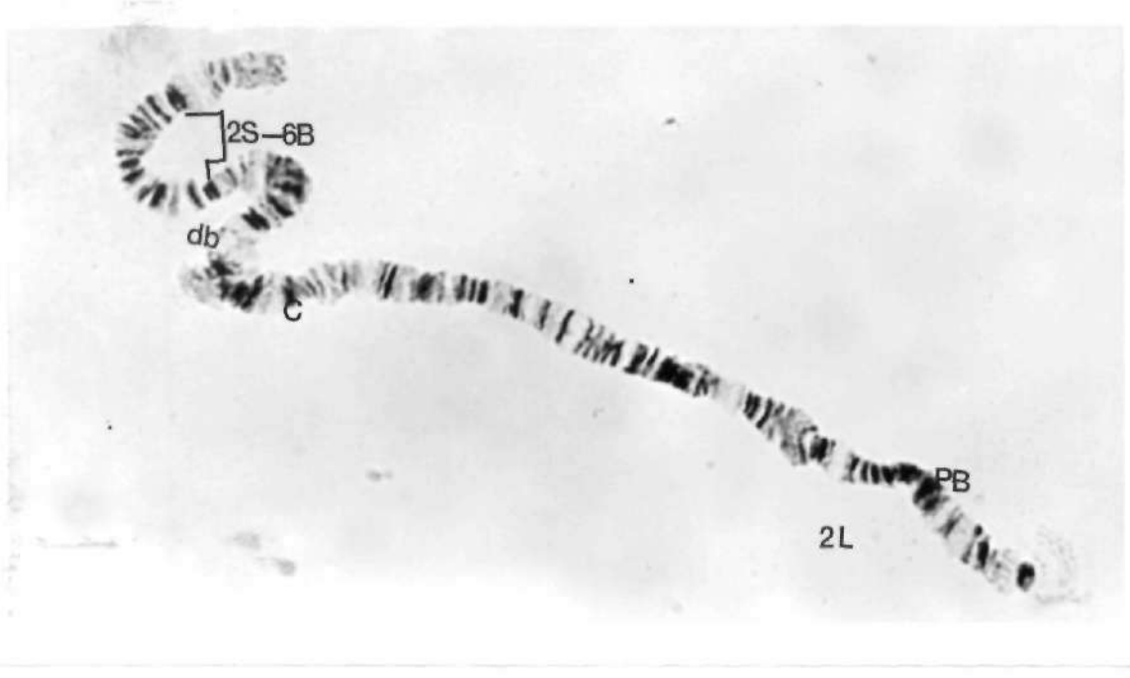



**Plate 9**: Chromosome 2: Homozygous sequence of inversion 4, 6 and 7 of *Simulium soubrense* (2L-4.6.7/4.6.7) with positions of the inversion 2S-6B shown to indicate *S. soubrense* Beffa form.

## 5 KEY TO *Simulium damnosum* COMPLEX POLYTENE CHROMOSOME IDENTIFICATION USING CHROMOSOME 2

| | |
|---|---|
| **1.** 2L – St/St | *S. squamosum* |
| 2L – St/18 or 18/18 | 2 |
| 2L – Other | 3 |
| **2.** 2L - 18/18 (F), 2L - St/18 (M) sex linked | *S. yahense* |
| 2L – 18 not sex linked | *S. squamosum* |
| **3.** 2L - C/C | 4 |
| 2L - 4.6/4.6 | 5 |
| **4.** 2L - C.8 sex linked, 2L - C/C (F) 2L - C/C.8 (M) | *S. damnosum* |
| 2L - C.8/C.8 | S. sirbanum |
| **5.** 2L - 4.6/4.6 + 2S - St/St | *S. soubrense* typical form |
| 2L - 4.6/4.6 + 2S - 6b (sex linked) | *S. soubrense* Beffa form |
| 2L - 4.6.A/4.6.A | *S. sanctipauli* |

# APPENDIX B

# Cytotaxonomy and sex determination variations within *Simulium squamosum* (Diptera: Simuliidae) from Cameroon and Nigeria.

*Medical and Veterinary Entomology*

SHORT COMUNICATION

**Cytotaxonomy and sex chromosome variation within *Simulium squamosum* (Diptera: Simuliidae) from Cameroon and Nigeria**

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**Keywords.** *Simulium damnosum* complex, *S. squamosum*, onchocerciasis, cytogenetics, sex-chromosomes, taxonomy, sibling species, Cameroon, Nigeria.

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Short title: *Simulium squamosum from Cameroon and Nigeria*

The vectors of human onchocerciasis in Nigeria and Cameroon are members of the *Simulium (Edwardsellum) damnosum* Theobald complex. Nine sibling species were originally described by Vajime and Dunbar (1975, 1977) from West Africa on the basis of chromosome differences. Of these *S. damnosum s.str.*, *S. sirbanum* Vajime and Dunbar, were recorded from both countries, with *S. squamosum* Enderlein and *S. mengense* Vajime and Dunbar additional for Cameroon. Traore-Lamizana and Lemasson (1987) surveyed the northern half of Cameroon and published distribution maps for the different cytospecies. Within this area *S. damnosum s.str.* and *S. sirbanum* were common in the savanna zones, while *S. squamosum* was found principally in the forested zones, extending its range north into the savanna rivers during the rainy season. *Simulium yahense* Vajime and Dunbar occurred locally in the west, and *S. mengense* was found to be scattered widely throughout the area. The situation for Nigeria has been reviewed by Mafuyai *et al.* (1996). *Simulium damnosum s.str.* and *S. sirbanum* are commonly distributed throughout the savanna areas, with *S. sirbanum* tending towards a more northerly distribution, and *S. damnosum s.str.* also extending into the more forested areas of the southwest and southeast. The *S. sanctipauli* subcomplex (*S. sanctipauli* Vajime and Dunbar and *S. soubrense* Vajime and Dunbar) has been recorded from the southwest and surprisingly also around the Jos Plateau. *Simulium yahense* is reliably known from only two sites in the southeast, whereas *S. squamosum* has been widely recorded from all the major bioclimatic zones except in the sudan savanna, and becomes the dominant species in montane areas.

*Simulium squamosum* is generally considered to be a rather variable species. It has a wide but patchy distribution in forest, guinea savanna and montane areas of both west and central Africa, from Sierra Leone and Guinea in the West to Zaire and Central African Republic in the East (Crosskey, 1987). Focal distribution of this sort might be expected to lead to intraspecific taxonomic variation and even speciation, and geographical variation in genetical traits is known for DNA probes (Post and Flook, 1992), isoenzymes (Thomson *et al.*, 1990) and chromosomes (Boakye, 1993). The frequencies of a number of floating inversions is not uniform across the entire range of the species, but fixed inversion differences (inversions which have only been found homozygously in any part of the range) are not known except for *S. squamosum kitetense* which is found in the extreme southeast of the distribution (Elsen and Post, 1989). However, geographic variation in the sex-chromosomes has been reported by a number of authors (Vajime and Dunbar, 1975; WHO, 1988; Elsen and Post, 1989; Boakye, 1993). Sex-chromosomes are generally considered to be particularly sensitive indicators of taxonomic divergence in the Simuliidae (White, 1978; Rothfels, 1979) and as such have been used to distiguish a number of important sibling species and geographic races within the *S. damnosum* complex, for example *S. yahense* (Vajime and Dunbar, 1975), the Beffa form of *S. soubrense* (Meredith *et al.*, 1983;

Post, 1986) and the Djodji form of *S. sanctipauli* (Surtees *et al.*, 1988). The objective of this study was to describe the patterns of sex-chromosome variation within *S. squamosum*, which was discovered during routine surveys of *S. damnosum* complex cytospecies distribution in Cameroon and Nigeria, and to consider the taxonomic significance.

Collection sites where *S. squamosum* has been found are listed and mapped by Traore-Lamizana and Lemasson (1987) for northern Cameroon, and for the whole of Nigeria by Mafuyai *et al.* (1996). These sites along with additional localities in southern Cameroon are mapped on Fig. 1. Final and penultimate stage larvae were collected from their riverine breeding sites and fixed in Carnoy's solution (3:1:1 ethanol:acetic acid:chloroform, or 3:1 ethanol:acetic acid, or 1:1 ethanol:acetic acid). These samples were then stored at 4°C for chromosome preparation. Polytene chromosomes were prepared from the larval silk glands by dissection and staining in feulgen or orcein according to standard procedures (Dunbar, 1972; Quillévéré, 1979). The resultant chromosome preparations were then compared with the standard chromosome maps published by Vajime and Dunbar (1975), Quillévéré (1975), Elsen and Post (1989) and Boakye (1993).

Most populations of the *S. damnosum* complex examined from Cameroon and Nigeria were found to conform to the cytotaxonomic descriptions and standards of Vajime and Dunbar (1975, 1977). This included *S. yahense* which was found to show typical sex linkage of inversion IIL-18 (homozygous in 97% of females and heterozygous in 65% of males). However, some geographic cytotaxonomic variation was observed within *S. damnosum s.str.* and *S. sirbanum* from Nigeria, *S. mengense* from Cameroon and *S. squamosum* from both countries.

No fixed inversions were found to differentiate any populations of *S. squamosum* from the standard karyotype (Vajime and Dunbar, 1975). However, inversion IIL-18 is normally considered to be diagnostic between *S. squamosum* and *S. yahense*, but a few heterozygotes were found in both sexes of *S. squamosum* in parts of northern Cameroon, around Mount Cameroon (5%), the Jos plateau (3%) and southwest Nigeria (36%).

Vajime and Dunbar (1975) described differences between the sexes in material from Cameroon. Females (n = 81) were all homozygous standard (ICs), and 43% of males (n = 69) showed a long centromeric non-pairing region on chromosome I (ICa: Fig 2), indicating a sex-differential region. *Simulium squamosum* from Cameroon, outside the Sanaga river and Mount Cameroon area, (6587 larvae examined) was found to conform to this description. However the centromeric non-pairing region of chromosome I was clearly different in males from the Sanaga river (ICb: Fig 3). All 593 females and 286 males were homozygous standard, but 449 (61%) males had the modified sex-differential region. Sites around Mount Cameroon presented a mixture of situations. *Simulium squamosum*

sampled from rivers draining southeast from the mountain (n = 91 larvae) showed neither non-pairing of IC in either sex, nor any other sex-linked chromosome variation. *Simulium squamosum* from two sites north of the mountain (river Fiango at Bikili dam, and river Menge near Ikilivindi) showed a non-pairing ICa in some members of both sexes (St = 4 males and 6 females; non-pairing = 3 males and 5 females).

A total of 315 larvae of *S. squamosum* were identified from 14 sites from Nigeria. From 13 sites no sex-linked chromosome variation of any sort was observed, although two female larvae from the southwest had a non-pairing IC. However, 25 larvae examined from Kwa Falls were found to possess the same sex-chromosome system that occurs throughout most of Cameroon (ICa), and was originally described by Vajime and Dunbar (1975).

The observations suggest that there are three sex-chromosome systems co-existing within *S. squamosum* from Cameroon and Nigeria. These are mostly geographically seperated and by definition indicate genetic differences between the populations. In view of the taxonomic weight normally accorded to sex-chromosome variation in the Simuliidae, including *S. damnosum* complex, it is proposed to recognise these different populations as informal geographic races labelled *S. squamosum* 'A'-'C'. The distribution of the three forms is clearly seperate (Fig 1), except north of Mount Cameroon in southwest Cameroon where a number of anomalies were found. The results of Vajime and Dunbar (1975) and the older samples collected and analysed for this study (1979-1983) indicated the presence of *S. squamosum* 'A', but more recent samples (1989-1994) indicate a mixture of S. squamosum 'A' and 'C' in this area. Further sampling is required to determine the exact situation.

*Simulium squamosum* 'A' is the typical form with the sex-linked long non-pairing ICa (Fig 2) originally described by Vajime and Dunbar (1975). It is distributed throughout Cameroon except in the Sanaga river and around Mount Cameroon where it appears to interbreed with *S. squamosum* 'C'. Its distribution spreads across the border into southeast Nigeria where it has been found at a single locality.

*Simulium squamosum* 'B' appears to be restricted to the Sanaga river where it has not been found sympatrically with any other member of the *S. damnosum* complex. It is characterised by the shorter and distinctive non-pairing region of ICb (Fig 3) found in 61% of males. It is presumed to be responsible for the heavy man-biting in the area which resulted in the larviciding control operations to protect the population of the Song Loulou dam area. As a result *S. squamosum* 'B' has become resistant to temephos (Traore-Lamizana *et al.*, 1985) and chlorphoxim (Traore-Lamizana and Klein, unpublished data).

*Simulium squamosum* 'C' has no sex-linked chromosome variation

and only very rarely shows any non-pairing of IC. It is the form distributed throughout most of Nigeria, but also occurs around Mount Cameroon. To the north of Mount Cameroon populations occur which appear to be a mixture of *S. squamosum* 'A' and 'C'. The long non-pairing ICa occurs in some larvae, but it is not sex-linked. This suggests that the two forms are meeting in this area and interbreeding. *Simulium squamosum* 'C' is a proven vector of onchocerciasis in Nigeria (Mafuyai *et al.*, in press).

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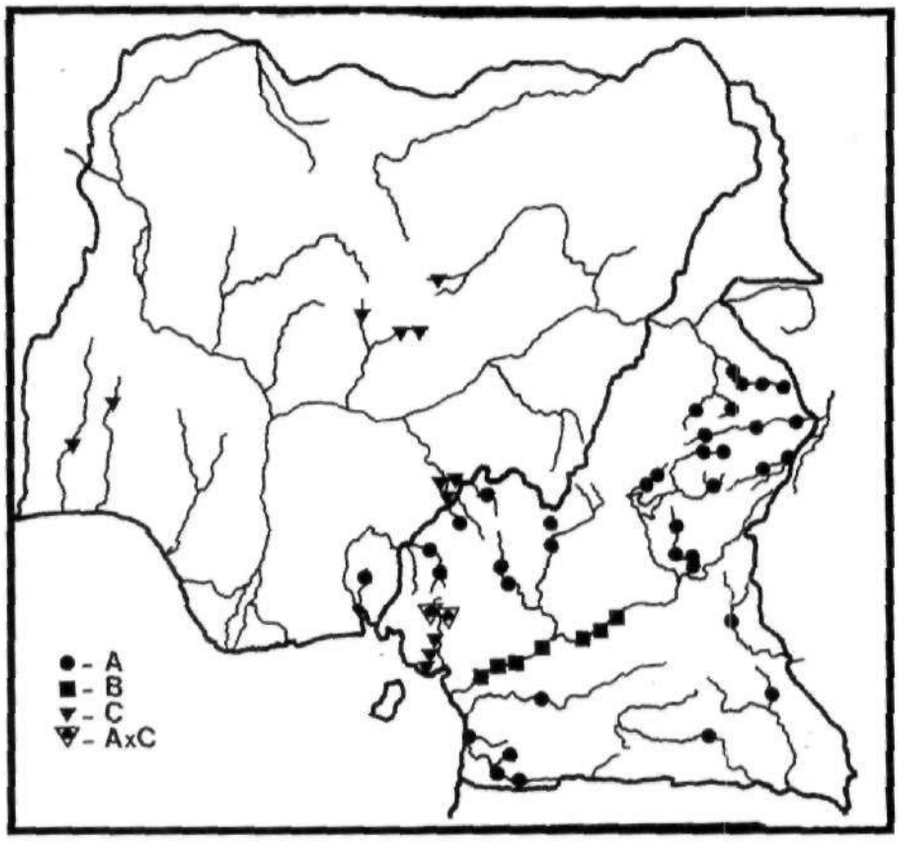



Fig. 1: Map showing the distribution of *Simulium squamosum* in Cameroon and Nigeria, classified according to sex-chromosome systems as *S. Squamosum* "A", "B" and "C".




Fig 2: Centromeric non-pairing sex-differential region from the male of a typical *Simulium squamosum* (1Ca). N = nucloelus organiser




Fig 3: Centromic non-pairing sex-differential region from a male of the sanaga population of *Simulium squamosum* (1Cb). N = nucleolus.