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Full Length Research Paper

Cytospecies identifications of vectors of human onchocerciasis in south eastern Nigeria

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Cytogenetic studies were carried out on the larvae of the members of the *Simulium damnosum* complex from 10 sites across the different bioecological zones in Southeast Nigeria. A total of 334 identifications belonging to 3 cytospecies (*S. squamosum*, *S. yahense* and *S. damnosum)* were made. The ICb variant of *S. squamosum* was observed for the first time in Nigeria. The seasonal abundance and geographical distribution of the sibling species generally conformed to that which had been observed further west in the OCP area, with the exception of *S. damnosum*, which was generally more plastic in its distribution.

Key words: *Simulium damnosum* complex, *S. squamosum*, *S. yahense*, cytogenetics, sibling species, cytospecies.

INTRODUCTION

Blackflies (Diptera: Simuliidae) or buffalo gnats of the *Simulium damnosum* Theobald complex are the only vectors of human onchocerciasis in West Africa. According to a WHO report (1995), Nigeria now has the largest number of people blinded by onchocerciasis, accounting for almost 40% of world cases of onchocercal blindness (Mafuyai et al., 1997). Presently, there are about 120,000 cases of blindness due to the disease and in communities where the intensity of infection is high, up to 10% of the population may be blind (Abiose, 1990; Anosike et al., 2001). The distribution of *Simulium* breeding and hence onchocerciasis is, however, not even across Nigeria but there is a clear correlation with basic geology (Crosskey, 1981), as the riverine conditions which create suitable breeding sites for the vector are common mostly where the African Precambrian basement rock is exposed to break the flow of water and create rapids. Confinement of breeding sites to Precambrian areas is, however, not absolute as harder strata in the sandstones of sedimentary areas and volcanic intrusions especially in parts of the mid-north and the northeast can also cause lodging of the river beds that breaks the flow, thereby creating breeding sites. As part of a feasibility study for onchocerciasis control in Nigeria, Crosskey (1981) identified 5 major endemic zones on the Precambrian rocks, with a further series of 4 small foci on sedimentary rocks along the Niger valley making a sixth zone.

Over twenty years after the identification of vector breeding areas in relation to onchocerciasis foci, the cytotaxonomic identity of *Simulium damnosum* complex population in Nigeria has remained insufficiently studied. Most published records were either concentrated in the middle of the country from the Guinea Savanna belt (i.e. Kaduna, Plateau and Nassarawa States) or rather generalized (e.g. Osun, Oyo and Niger States) (Mafuyai et al., 1996), thus leaving much of the country inadequately surveyed. From these studies, nine cytoforms of the *Simulium damnosum* complex were reported from different parts of the country including *S. damnosum*, Theobald s. str., *S. sirbanum*_Vajime and Dunbar, *S. sudanense* Vajime and Dunbar, the Volta form of the *S. squamosum* complex (Vajime and Gregory, 1990), *S. squamosum* Enderlein, *S. yahense* Vajime and Dunbar, *S. sanctipauli* Vajime and Dunbar, *S. soubrense*

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Table 1. Cytospecies identification and geographical distribution of *Simulium damnosum* complex in the study area.

Locality					Cytospecies				Number
Site No	Name of River	Geographical Coordinates (Lat. / Long.)	Bioclimatic Zone	Season	*squa*		*yah*	*dam*	(n)
					ICs	ICb			
1	ATAN	7°50', 5°08'	Forest	D	27	-	-	-	27
2	AKPA-IKPONG	8°05', 5°04'	Forest	D	19	-	-	4	23
3	IMO	7°23', 5°42'	Mosaic Forest	D	39	-	-	3	42
4	IBII	7^0 22', 5°55'	Mosaic Forest	D	5	1	18	-	24
				W	9	2	14	-	25
5	IBII	7°23', 5°57'	Forest	D	9	17	-	-	26
				W	14	4	-	-	18
6	IBU	7°22', 5°57'	Forest	D	7	-	13	-	20
7	UGBI	7°18', 5°56'	Forest	D	14	4	2	-	20
				W	4	-	6	2	12
8	IYI-AKA	7°20', 6°00'	Forest	D	10	-	14	-	24
9	IDODO	7°42', 6°35'	Mosaic / Guinea Savanna	D	10	5	-	17	32
10	OKPOKWU	7°52', 6°50'	Forest Interphase / Transitional Zone	D	5	-	-	7	12
				W	6	-	-	23	29

D = Dry season, W = wet season, squa = *squamosum*, yah = *yahense*, dam = *damnosum*, ICb = short asynaptic centromeric region and ICs = synaptic centromeric region.

Vajime and Dunbar and the Beffa form of *S. soubrense* (Meredith et al., 1983), but of these the distinct cytotaxonomic status of *S. Sudanese* and Volta form is not universally accepted (Boakye, 1993; Crosskey, 1987; Vajime, 1989;).

Any control of onchocerciasis involving larvicidal treatments of breeding sites requires reliable species identification. This is, because, there are marked differences between the individual species in the *S. damnosum* complex with respect to macro and microgeographical distribution (Crosskey, 1981). There are also variations in larval habitat (Garms and Vajime, 1975), host preference and vectorial capacity (Garms et al., 1979; Quillévéré, 1979;), susceptibility to larvicides (Guillet et al., 1980), biting cycle and population age structure (Garms and Walsh, 1987). However, the conventional entomological identification techniques of adult blackflies are known to have technical and logistic limitations and this has greatly affected the assessment of control programmes (Dang and Peterson, 1980; Garms et al*.,* 1982) such that larval cytotaxnomy has remained the most reliable means of identification.

Again, very little is known about the role of the different sibling species of the *S. damnosum* complex in onchocerciasis transmission in Nigeria, and it is presumed that their relative importance will be rather similar to that found further West in the World Health Organization Control Programme (OCP) area (Mafuyai et al., 1996). Although the Rapid Epidemiological Mapping of Onchocerciasis (REMO/Atlas Geographical Information System (GIS), developed by WHO in collaboration with the Nigerian National Onchocerciasis Programme (NOCP) is good at assessing the current status of the disease in the human population through the analysis of easily detected symptoms (Ngoumou and Walsh, 1993), they are less efficient at rapid detection of changes in the epidemiology of the disease e.g. those which might be brought about by changes in the vector composition. Early detection of such changes requires information on the vector cytospecies composition, seasonal abundance and geographical distribution, which will be provided by cytotaxonomic identification. This study set out to employ cytological techniques in the establishment of the definite *Simulium* vector sibling species in the major biological onchocerciasis zones in the Southeast of Nigeria with attention to the variation in their seasonal and geographical distribution, in order to allow the filling in of the obvious gap in the understanding of the onchocerciasis vector ecology and dynamics in Nigeria.

MATERIALS AND METHODS

Collection of larvae

Larval collections of *S. damnosum* s.l for cytogenetic determination of sibling species were carried out for a period 12 months i.e. December – March for the dry season and May – October for the wet season. *S. damnosum* complex larvae were discriminated from other larval simuliids by recognition of the characteristic dorsal tubercles and the scales of the proleg (Crosskey, 1960). The best larvae for giant chromosomes are those in penultimate instar, with white pupal respiratory filament histoblasts or up to three-quarters of the way through the ultimate instar (i.e. with darkening pupal respiratory filament histoblasts) (Dunbar, 1995).

Cytological studies

The larvae were fixed in Carnoy's solution (3 parts absolute ethanol: 1 part glacial acetic acid) and polytene chromosome preparations were made as previously described (Boakye et al., 1993). Full karyotyping and cytospecies identifications were based on the criteria of Boakye (1993), Dunbar (1995), Meredith et al., (1983), Surtees et al., (1988), and Vajime and Dunbar (1975).

RESULTS

A total of 334 cytological identifications were made from 10 sites in the study area. The identifications, together with details of the geographical location of sampling sites, the bioclimatic zones and season are presented in Table 1 while Figure 1 shows the map of species composition, breeding sites, rivers and river basins. No new fixed inversion differences were observed. The new cytological identifications consisted of *S. squamosum* and *S.yahense* belonging to the *S. squamosum* sub-complex and *S. damnosum* belonging to the *S. damnosum* sub-complex.

S. squamosum was recorded from all the bioclimatic zones in the study area although it was more frequently observed in the dry season samples than in the wet and it was observed to be breeding in sympatry with *S. yahense* or *S. damnosum* or both in most areas. An allopatric breeding situation was observed to be exhibited by the *S. squamosum* species at one sample site, Atan river in Cross River State. *S, yahense* was found breeding in the mosaic forest in sympatry with *S. squamosum* in all the bioclimatic zones in the Southeast of Nigeria and it was found breeding in sympatry with *S. squamosum* in all cases.

Intraspecific chromosomal variation was observed in *S. squamosum* samples but this is indicated only in Table 1. Some of the *S. squamosum* sampled showed no sex-linked chromosome I with complete synapsis while in others, there was a short asynaptic centromeric region of chromosome I, conditions that have been designated ICs and ICb, respectively (Vajime and Dunbar, 1975). The ICb variants were observed at 4 sites (i.e. Ibii-Umulolo, Ibii-Aku, Ugbi and Idodo rivers), and this is the first time it is being observed anywhere in Nigeria (Ibeh, 2004).

DISCUSSION

The correct identification of individual member species of the *S. damnosum* complex in any locality is needed not

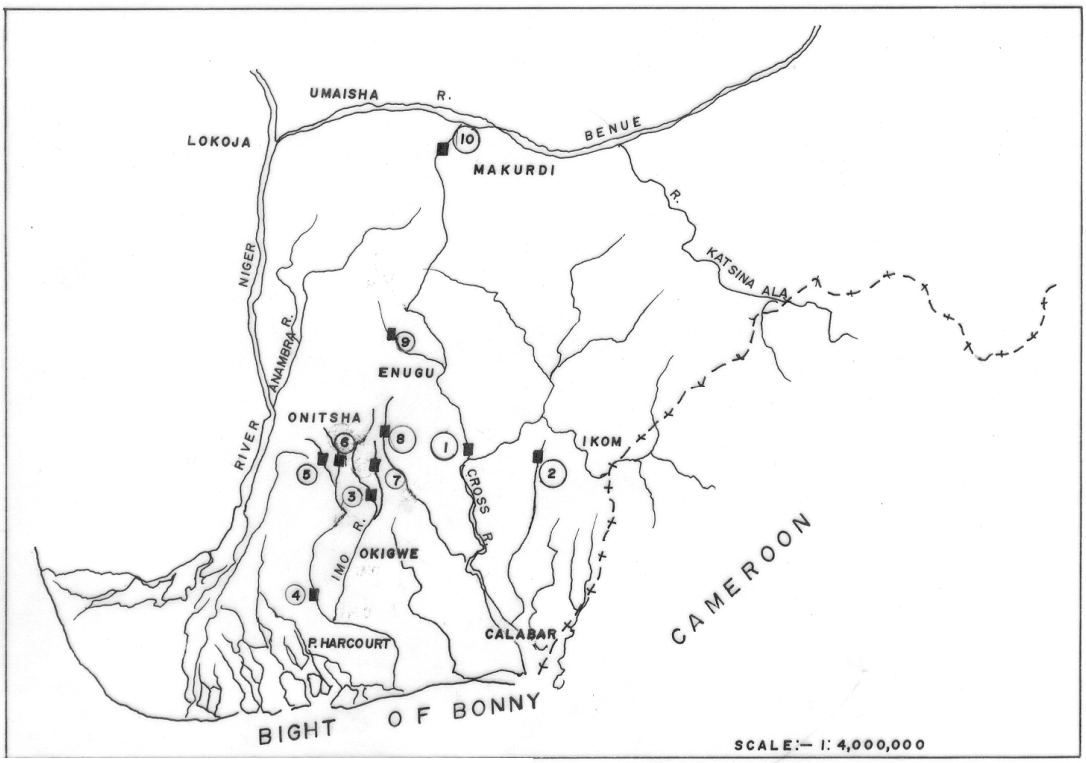

Figure 1. The geographical distribution of the cytospecies of *Simulium damnosum* complex in the study area. 1 to 10 are the sampling sites.

only for a better understanding of epidemiology but also in the practice of control operations. In the latter case, precise identification can be important for inferring the possible provenance of flies and for delimiting control zones.

The members of the complex sampled in the study area conformed to the standard cytotaxonomic descriptions by Vajime and Dunbar (1975) and also the species distribution in Nigeria by Mafuyai et al., (1996, 1997). *S. squamosum* has been known to be very widespread across Nigeria and has been identified from montain, forest and Guinea savanna zones, but not yet the Sudan Savanna, being the dominant species throughout the year in the mountain areas but more abundant in the dry season samples in the Guinea Savanna. *S. squamosum* is considered to be an efficient and important vector of *O. volvulus* in West Africa and has been known to have a patchy distribution in both west and central Africa. This focal distribution might be expected to promote taxonomic variation and potential speciation. A third variant of *S. squamosum*, ICa (with a long asynaptic centromere region on chromosome I) is known to be widely distributed throughout Cameroon (except in the Sanaga River) and had been observed from 3 sites in Nigeria in Kwa falls and Koram River in Cross River State and Ibii River in Imo State (Traore-Lamizana et al., 2001). The ICs variant has been observed to be widespread in Nigeria and the ICb variant had been known to be restricted to only the Sanaga River in Cameroon. The chromosomal variants of *S. squamosum* and the non-observance of hybrids between them in this study, coupled with previous observations within this species (Traore-Lamizana, 2001) indicates an expansion in the distributional range of ICb and also seem to suggest the beginning of the emergence of new species within this sibling species.

The allopatric pattern of breeding observed in Atan river would be very significant especially with respect to providing sources of “pure” larvae for experimental procedures i.e. locations with which larvicidal and transmission experiments could be conceived, tested and successfully applied in the campaign against onchocerciasis as was the case in the OCP area (Vajime et al., 1990).

Contrary to the report that *S. yahense* has not been identified from anywhere in Nigeria (Crosskey, 1987), the breeding of the species has been reported from different ecological zones in the nation. It has been observed in the Southern Guinea Savanna zones and also from Agbokim falls and Oji River in the Southeastern part of the country. In Oji River, it was found in sympatry with *S. damnosum* and *S. sirbanum* (Akoh et al., 1987). Its distribution is however patchy across the OCP area and it seems to require conditions for breeding quite different

from others in the complex as only a single specimen was recorded in sympatry with other species in the OCP (Fiasorgbor, et al., 1992; Vajime and Dunbar, 1975). The vectoral implication of *S. yahense* in the transmission of onchocerciasis was initially doubtful (Vajime and Dunbar, 1975) but it has been found to be associated with a forest-type epidemiology (Braide et al., 1980).

The *S. damnosum* cytospecies is the most widespread member of the complex, being the only one found across the continent. However, unlike the OCP area where the species was found mainly in the transitional zone between the forest and the savanna (Vajime, 1984), it is widespread throughout Nigeria. Again, while the predominant populations in the OCP area are the 'Volta' form, it is the 'Nile populations that are very common in Nigeria (Boakye et al., 1998; Vajime, 1984). *S. damnosum* is definitely a vector of onchocerciasis because it has been found closely associated with the disease where no other species is present (Vajime and Dunbar, 1979). *S. damnosum* has also been known to be a savanna species but its seasonal species distribution in this study seems to indicate its gradual invasion of the forest areas of the southeast of Nigeria. This will definitely pose a great risk because it is possible that a savanna onchocerciasis could get into this bioclimatic zone through the savanna vectors bringing the savanna strains of *O. volvulus* along with them.

In summary, cytological studies have had a tremendous impact on the investigation of simuliids, revealing a previously unimagined profusion of sibling species. Evidence is now available that in the *S. damnosum* complex, these cytologically identified species differ in biological characters of crucial importance to the epidemiology and control of human onchocerciasis.

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