MORPHOLOGICAL IDENTIFICATION OF SIBLING SPECIES OF THE *SIMULIUM DAMNOSUM* (Diptera:Simuliidae) COMPLEX FROM NIGERIA, CAMEROUN AND BIOKO

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Summary

Reliable identification of biting adult females of sibling species of the *Simulium damnosum* complex is essential for an understanding of the epidemiology and transmission of human onchocerciasis (=river blindness). A novel technique for morphological identification has already been developed for the Onchocerciasis Control Programme area of West Africa, and this method was tested on specimens obtained from further east. The method was found to be generally very reliable.

INTRODUCTION

Human onchocerciasis is a severely debilitating, blinding disease caused by infection with *Onchocerca volvulus* (Nematoda: Filarioidea). In West Africa the parasite is transmitted by blood-sucking adult females of sibling species of the *Simulium damnosum* complex (Diptera: Simuliidae). Blindness rates can reach as high as 15% in the savanna, with 100% infectivity (Duke, 1990). The major patterns of epidemiological variation are related to the taxonomy of the parasite and the vector (Post & Boakye, 1992), and so not all sibling species are equally important. Vector taxonomy is based upon the analysis of the polytene chromosomes from the larval silk glands, but there is clearly a requirement for the identification of the adult female, because it is this stage that actually transmits the parasite.

This requirement has long been a priority of the World Health Oganisation (WHO, 1978) and a subject of intensive study. An electrophoretic survey of 44 enzymes revealed species-specific variants for only two sibling species, *S. squamosum* and *S. yahense* (Meredith & Townson, 1981; Thomson et al, 1990). The G-C analysis of cuticular hydrocarbons revealed similar levels of variation both within and between species (Phillips et al, 1985). Specific DNA probes have been developed by Post and Flook (1992) for the identification of the three West African subcomplexes. However, morphological techniques have been found to be the most successful in terms of specificity, convenience and cost. Wilson et al (1993) have reviewed the use of morphological methods for the identification of the *S. damnosum* complex, and developed a new scheme which uses discriminant function analysis. They examined 1691 adult females from 27 sites throughout the area of the World

Health Organisation Onchocerciasis Control Programme, and considered 14 characters, including five that had already proved useful from previously published work. The identity of the specimens was known, mostly from correlated larval cytotaxonomy. The number of characters was reduced to the eight most useful by stepwise linear discriminant function (LDF) analysis, and the LDFs used for species discrimination. The LDFs can identify the *S. damnosum* subcomplex, the *S. sanctipauli* subcomplex, *S. squamosum* and *S. yahense* with an overall correct classification of over 98%. This identification scheme has been adopted for routine use by the Onchocerciasis Control Programme (C. Back, personal communication), and Wilson and Post (1994) are currently trying to integrate it with DNA methods for further species-discrimination within the subcomplexes.

National onchocerciasis control programmes are already operational or proposed in Nigeria, Cameroun and Guinea Ecuatorial and the success of these programmes will undoubtedly be improved by a better understanding of the local epidemiology, including vector species. The object of this paper is to assess the possibility that vector identification can be achieved in these countries using the scheme devised by Wilson et al (1993).

MATERIALS AND METHODS

Adult female flies were either reared from wild caught pupae or caught at human bait according to the methods described by Wilson et al (1993) and preserved in absolute ethanol. Larvae collected simultaneously were preserved in Carnoy's fixative and identified according to Boakye (1993). Collection sites are listed in Table 1.

Adult females were identified using the derived classification functions for the direct discriminant analysis method in Table 2 of Wilson et al (1993). This involved scoring the colour of the fore coxa, ninth abdominal tergite setae, wing tuft, arculus and scutellar setae, and the lengths of the forebasitarsus, antenna and foretibia. The classification functions aim to identify *S. squamosum*, *S. yahense*, *S. sanctipauli*, *S. soubrense* s.l. (including *S. leonense* and *S. konkourense*), *S. soubrense* Beffa form, and the *S. damnosum* subcomplex (*S. damnosum* s.s. and *S. sirbanum* together).

Table 1. Sample sites of adult female Simulium damnosum *complex.*

Country	Locality (Co-ordinates = N/E)	Date + Method[*]	
Nigeria	R. Ndip-Ekem at Agbokim Falls (6°55'/8°52')	02.x.90	RP
	R. Kwa at Kwa Falls (5°08'/8°31')	07.i.91	HB
	R. Galma at Kudaru (10°39'/8°31')	07.viii.90	RP
Cameroun	R. Fiango at Bikili Dam (4°37'/9°21')	05.v.89	RP
Bioko (GE)[#]	R. Apu at site 1 (3°41'/8°39')	18.v.89	RP

[*] RP = reared from pupae, HB = collected at human bait.
[#] GE = Guinea Ecuatorial

RESULTS AND DISCUSSION

The cytotaxonomic identification of correlated larval samples is presented in Table 2, and the morphological identification of adults in Table 3.

It is clear that the morphological identifications show a remarkably good correlation with the cytotaxonomic identifications of simultaneously collected larvae, and it can be deduced that the sibling species examined show a consistent morphology between the Onchocerciasis Control Programme area and the countries further east. This is particularly clear for the Nigerian samples, but the other samples show some interesting features.

Table 2. Cytospecies identifications of Simulium damnosum *complex.*

Sample	n	*sirb	dam	squa	yah	Bio
Agbokim Falls	25				25	
Kwa Falls	25			25		
Kudaru	22	3	19			
Bikili Dam	20		18	2		
Site 1	20					20

* *sirb* = *S. sirbanum*; *dam* = *S. damnosum* s.s.; *squa* = *S. squamosum*; *yah* = *S. yahense*; Bio = Bioko form of *S. squamosum* subcomplex (Post, unpublished data).

Table 3. Morphospecies identifications of Simulium damnosum *complex.*

Sample	n	*dam	squa	yah	soubB
Agbokim Falls	10			10	
Kwa Falls	70		70		
Kudaru	25	25			
Bikili Dam	47	34	11	1	1
Site 1	60			60	

* *dam* = *S. damnosum* subcomplex (= *S. damnosum* s.s. and *S. sirbanum* together); *squa* = *S. squamosum*; *yah* = *S. yahense*; *soub*B = *S. soubrense* Beffa form.

The proportions of specimens from Bikili Dam that were identified as *S. damnosum* subcomplex and *S. squamosum* by larval cytotaxonomy and adult morphology do not differ significantly ($\chi^2 = 1.805$, P > 0.10). However, morphological identifications also revealed single specimens of *S. yahense* and *S. soubrense* Beffa form. It is possible that these species were not identified cytotaxonomically because of the small number of larvae examined. However, it is unlikely that *S. soubrense* Beffa form occurs there because it has never been

recorded anywhere in Cameroun. It is possible that it is morphologically similar to *S. mengense*, which is widespread in Cameroun, but the adults of *S. mengense* are morphologically unknown and their larvae have never been found breeding at Bikili Dam (Traoré-Lamizana & Lemasson, 1987 and personal communication). The most likely explanation is that it is *S. squamosum* because Wilson et al (1993) have shown that there is a small morphological overlap between these species. This explanation is supported by the values obtained by calculating the posterior probability of group membership for *S. soubrense* Beffa form (0.6203) and *S. squamosum* (0.3787). The fly identified as *S. yahense* was completely typical of that species, being dark for all colour characters, and the posterior probability of belonging to *S. yahense* was 1.0000. Larvae of *S. yahense* have been identified from this part of Cameroun, and Bikili Dam is a small heavily shaded stream not atypical of *S. yahense* breeding sites. It is therefore possible that this species was not identified from the larval sample simply because of the small number examined.

All adult females from Bioko were classified as *S. yahense*. These were very dark flies, and detailed analysis of the morphological characters showed that they all had dark fore coxae, dark antennae, dark wing tufts, black arculus and dark scutellar setae. The cytotaxonomic identifications revealed only "Bioko" form from this site. In fact only one cytospecies is known from the island (Post & Millest, 1991), and that is the endemic "Bioko" form. The taxonomic status of the "Bioko" form is not yet clear, because cytotaxonomic analysis (Post, unpublished data) has shown that 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. It is clear that whatever the taxonomic status of the "Bioko" form, it has a morphology very similiar to *S. yahense*.

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