

The Laboratory Maintenance and Rearing of *Simulium damnosum* Complex Species as a Research Tool for the Onchocerciasis Control Programme in the Volta River Basin

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Summary

The laboratory maintenance and rearing of *Simulium damnosum* complex species and the cytotoxic determination of reared larvae, has been useful to OCP in the following fields of investigation:

1. Species identification of reinventing females.
2. Distribution of biting populations of *S. damnosum* complex species.
3. Identification of eggs and small larvae found within the OCP area.
4. Host preferences of species within the complex.
5. Vector potential of *S. damnosum* complex species for *Onchocerca volvulus* and *O. ochengi*.
6. Provision of identified material for morphological investigation.

The technique of indirect adult identification using reared larvae, may eventually be largely superseded by progress in morphological taxonomy.

Introduction

Simulium damnosum s.l. comprises a sibling species complex. Eight members of this complex have so far been recognised in West Africa (Vajime and Dunbar 1975). Six of the species concerned are widespread and of major importance to the Onchocerciasis Control Programme (Vajime and Quillévéré 1978).

New information on *S. damnosum* s.l. is usually of limited value to the Programme unless it is known to which species within the complex it refers. Until recently the only known characters adequate for species determination have been the banding pattern sequences on the polytene chromosomes in the larval salivary glands. At present (March 1979) these chro-

Die Laboratoriumshaltung und Zucht von Arten des *Simulium damnosum*-Komplexes als Forschungsmittel für das Onchocercosebekämpfungsprogramm im Volta-Gebiet

Die Laboratoriumshaltung von Arten des *S. damnosum*-Komplexes und die zytotaxonomische Bestimmung der gezüchteten Larven ist für das Onchocercosebekämpfungsprogramm für folgende Untersuchungen wichtig: 1. Artbestimmung wiedereinfliegender Weibchen, 2. Verbreitung blutsaugender Populationen, 3. Bestimmung von Eiern und jungen Larven, 4. Wirtswahl der Arten des Komplexes, 5. Übertragungspotential der Arten für *Onchocerca volvulus* und *O. ochengi*, 6. Bereitstellung identifizierter Exemplare für taxonomische Untersuchungen. Die Technik der indirekten Bestimmung adulter Mücken anhand gezüchteter Larven könnte durch die Weiterentwicklung morphologischer Bestimmungsmethoden an Bedeutung verlieren.

mosomal differences in the larvae remain the most reliable taxonomic characters available. However, it is the adult female coming to bite man which the Programme needs to have identified. For this reason, the laboratory maintenance and rearing of *S. damnosum* complex species, combined with the cytotoxic determination of reared late instar larvae, has proved to be an important research tool for OCP.

Material and methods

For most investigations, adult female simuliids have been allowed to feed in the field on man or an animal host and then maintained until gravid in the laboratory. They have been induced to oviposit by immersion in water, or by decapitation, and larvae hatching from the eggs have been reared to a cytotoxi-

nominally identifiable stage. Adults of known species have been obtained by rearing single egg batches separately and identifying at least one larva from each batch.

Methods of rearing and maintaining *S. damnosum* s.l. in the laboratory have been reviewed by Raybould and Grunewald (1975) and Raybould (1979a). Rearing techniques initially employed in these investigations, used compressed air or electromagnetic stirrers to produce a water current. However, such methods provided inadequate numbers of late instar larvae and only a few small adults. A better technique was therefore developed for use in the OCP area (Raybould 1979b). This method has given better survival and more rapid development to full size larvae suitable for cytotoxic determination.

Larvae have been fixed and chromosomal preparations made, using the techniques described by Dunbar (1972) or those of Quillévére (1975).

Fields in which information of interest to OCP has been obtained

1) *The reinvasion problem*

In spite of the remarkable success of OCP, certain areas, especially in the Ivory Coast, have been subjected to a substantial reinvasion of *S. damnosum* s.l. from distant breeding sites (Walsh 1977, Le Berre et al. 1979, Garms et al. 1979, Walsh et al. 1979). The cytotoxic identification of larvae reared from eggs laid by reinventing females captured in 1976 (Table 1), showed them to be almost entirely of the two savanna cytospecies, *S. damnosum* s.s. and *S. sirbanum*. *S. squamosum* was identified on one occasion only. However, this simuliid, although essentially a forest species, is not limited to the forest zone.

These findings were further substantiated by additional evidence from several other sources (Le Berre et al. 1979). It was concluded that control in the forest zone of southern Ivory Coast where only the forest cytotypes *S. sanctipauli*, *S. soubrense* and *S. yahense* occur (see map by Vajime and Quillévére 1978) would not influence reinvasion of the OCP area.

In 1977, the situation was further complicated when new areas of reinvasion became apparent following an extension of the Control Programme area (Le Berre et al. 1979). The technique of indirect identification using reared larvae was not employed that year, but was

used again in the 1978 rainy season in the reinvasion zone in a south-western extension of the OCP area in western Ivory Coast.

The results obtained in 1978 are presented in Table 2. Although some of the collections listed in the tables were made soon after or even before, the onset of local control and therefore included many autochthonous flies, others were composed entirely, or almost entirely of reinventing individuals. With the exception of a single specimen of *S. sanctipauli*, all identified larvae belonged to the three species *S. damnosum* s.s., *S. soubrense* and *S. squamosum*, both *S. sirbanum* and *S. yahense* being completely absent. These findings suggest possible source areas from known breeding sites of the three reinventing species (Garms et al. 1979).

Although relatively few females were (indirectly) identified during these investigations, the importance of the results lies in their reliability which enables them to be used to check morphological determinations made in more comprehensive reinvasion studies. This is particularly necessary in the case of *S. squamosum* and of closely similar species pairs, namely *S. sirbanum/damnoscum* and *S. sanctipauli/soubrense* (Garms 1978, Garms et al. 1979).

2) *The distribution of biting populations of S. damnosum complex species*

During the past few years, the distribution of breeding sites of the six main *S. damnosum* complex species has been mapped for the OCP area and for substantial areas to the south (Vajime and Quillévére 1978). However, a fly biting near a breeding site may not have originated there. In places where the breeding sites of the different species are interspersed, as in the Volta region of Ghana and parts of Togo, the identity of biting flies cannot be safely deduced from the known distribution of breeding sites. In such locations, three or even more species may be collected biting together, and identification from reared larvae has been found to be of particular value.

3) *The identification of eggs and small larvae collected within the OCP area*

Resulting from the presence of reinventing flies

Table 1 The species composition of *S. damnosum complex larvae* reared from eggs laid by invading females collected within the OCP area in 1976

Country	Catching station Location	Dates Collected	No. of Egg Batches Obtained	No. of Larvae Reared	No. of Larvae Identified	Species Composition		
						<i>S. damnosum</i>	<i>S. siribanum</i>	<i>S. squamosum</i>
	Border of upper Volta and Ivory Coast	Leraba R. Road Bridge 7, 13, 21, 28 May; 12, 13 June	43	600	424	193	208	23
	Ivory Coast	"Gite 3" R. Bou 24 May; 1,8 June	22	47	26	26		
	Upper Volta	Nabere Bougouriba R. 9 July	2	18	15		15	
	Border of Ivory Coast and Ghana	Tagadi Black Volta R. 15 July	15	21	18	18		

*Most egg batches from "Gite 3" were reared separately and only a few larvae preserved for each batch

or incomplete control, eggs or small larvae may be found within the OCP area in the absence of larger larvae and pupae. These have sometimes been identified after rearing to late instar larvae. Such reared larvae could also be used for insecticide susceptibility tests.

4) Host preferences of species within the complex

Probably the majority of the twenty-seven known members of the *S. damnosum* complex do not feed on man. In West Africa, however, it has long been suspected, on the basis of strong circumstantial evidence, that

Table 2 The species composition of *S. damnosum complex larvae* from eggs laid by females captured in western Ivory Coast in 1978

Location	Date Collected	No. of egg Batches Obtained	No. of Larvae Reared	No. of Larvae Identified	Species Composition			
					<i>S. damnosum</i>	<i>s. soubrense</i>	<i>S. squamosum</i>	<i>S. sanctipauli</i>
Pont Seguela Yani R.	5,6,7 June	16	40	13	10		3	
Kato Upper Yani	10, 13 July	21	133	68	57		11	
Kongasso Marahoue R.	10 July	15	173	90	72		18	
Gite Mermis Upper Marahoue	17 May	7	100	71	71			
	15 May	15	89	77	77			
Bac Semien	18 May	18	95	44	3	40		1
Sassandra R.	10 June	8	291	199	16	183		
	12 July	11	197	120	120			
	29 July	14	256	104	94	10		
Biankouma	23 May	9	3	2	1	1		
Bafing R.	14 June	6	3	2		2		

the six common species are all anthropophilic. The fact that we have reared larvae of all of them from eggs laid by man-biting females has now proved this to be the case.

S. damnosum s.l. in West Africa bites a variety of animals and birds in addition to man (Crisp 1956, Disney and Boreham, 1969, Disney 1972, Quillévére et al. 1977b). Hitherto, however, the precise identity of zoophilic species has usually been uncertain although it has sometimes been inferred from the known distribution of breeding sites. The identification of reared larvae has provided some useful information in this respect, although investigations have so far been limited to a few areas in Togo and Ghana.

S. damnosum s.s., *S. sirbanum* and *S. soubrense* have been found to feed on cows at Akosombo in south-eastern Ghana (*S. damnosum* s.l. also bites goats, sheep, chickens and turkeys at Akosombo, but the species concerned have not yet been cytotaxonomically determined). Near Lama-Kara in Togo, cow-biting simuliids (kindly provided by Dr. A.K. Denke), were identified as *S. sirbanum*, while further south near Atakpame *S. damnosum* s.s. and *S. soubrense* were taken from the same host. Near Badu in western Togo *S. sanctipauli* feeds on cows and biting densities of up to a few hundred per hour have been recorded (Omar et al. 1979). Zoophily has not yet been demonstrated for either *S. squamosum* or *S. yahense*.

Further information on the degree of zoophily of *S. damnosum* complex species is required in connection with their role as vectors of animal filariae (see below).

5) *The vector potential of S. damnosum complex species*

In laboratory studies on the development of filarial larvae in members of the *S. damnosum* complex, problems of host identification are compounded by the fact that the infected flies must be kept alive for several days and then dissected. The living fly cannot be examined closely and tends to become badly rubbed during its confinement. Once dissected, the specimen is no longer morphologically

identifiable. The technique of indirect identification using reared larvae, has therefore proved to be very useful in this context. The most convenient procedure is to stimulate the infected fly to oviposit just prior to its being killed for dissection.

This technique was used to identify *S. sanctipauli* females from Asukawkaw Ferry in Ghana in which *O. volvulus* larvae were found to reach the infective stage (Zerbo, pers. comm.). This is of interest because *S. sanctipauli* is a suspected onchocerciasis vector in Liberia (Garms 1973) but is virtually never found naturally infected with *O. volvulus* in the Ivory Coast (Quillévére et al. 1977b).

Animal filariae are known to be common in *S. damnosum* s.l. in West Africa (Duke 1967; Garms and Voelker 1969; Quillévére et al., 1977b). Further information on these parasites is urgently required because some species are indistinguishable from *C. volvulus* and likely to confuse infection and infectivity rates obtained by dissection (Omar and Kuhlow 1978). Investigations are in progress, therefore, to determine the vectors of animal filariae (commonly found in members of the *S. damnosum* complex) by the cytotaxonomic identification of the larval progeny of the infected flies. So far this technique has been used to identify *S. sanctipauli* as a potential vector of *Onchocerca ochengi*, a parasite with larval stages both morphologically and histochemically indistinguishable (at present) from *O. volvulus* (Omar et al. 1979).

6) *The provision of identified material for morphological investigations*

If larvae, pupae and adults are reared from a single egg batch, and at least one larva is identified, the female parent and her progeny can then be used in the search for morphological differences between the species. Identified reared material of this kind has already contributed towards recent progress in morphological taxonomy (see below).

Possible future developments

Considerable strides have recently been made in the morphological taxonomy of *S. damno-*

sum complex females in West Africa (Quillévéré et al. 1977a, Garms 1978, Peterson and Dang 1979). Further progress in this field may one day obviate the need to identify biting females in the OCP area from their larval progeny. If this proves to be the case, rearing can then be confined to specific research investigations, and the work of OCP will be greatly facilitated. In other parts of Africa, however, where the possibility of implementing new control schemes is being studied, rearing will play an important part in enabling morphological taxonomists to check their results.

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