

SHORT COMMUNICATION

A new cytotype of *Simulium squamosum* from south-west Cameroon

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Abstract. A new cytotype of *Simulium squamosum* Enderlein (Diptera: Simuliidae) (here named *S. squamosum* 'D') is described from around Mount Cameroon in south-west Cameroon on the basis of sex-chromosome differences on the short arm of chromosome I. Two polymorphic inversions, IS-To (a new inversion) and IS-2, are partially Y linked. These inversions usually occur together, although IS-To has a higher frequency and is more strongly Y linked than IS-2. This sex-chromosome system has not been previously described and the occurrence and evolution of sex-chromosome systems in *S. squamosum* is discussed.

Key words. Simuliidae, *Simulium damnosum* complex, *S. squamosum*, onchocerciasis, sex chromosomes, sibling species, Cameroon.

In West Africa, anthropophilic members of the *Simulium* (*Edwardsellum*) *damnosum* Theobald complex transmit *Onchocerca volvulus* (Leuckart), the parasite responsible for human onchocerciasis. This vector complex consists of more than 40 cytospecies and cytoforms, which have all been described by micromorphological chromosome characteristics (Crosskey, 1987), but there are also differences in their biology, anthropophily and vector–parasite interactions, and as a consequence there are differences in their overall contribution to disease transmission (Duke *et al.*, 1966; Post & Boakye, 1992; Basáñez & Ricárdez-Esquina, 2001). Therefore, a full understanding of the taxonomy of the vector sibling species is important for understanding disease epidemiology and for the rational design of disease control programmes.

The distribution of the cytospecies of the *S. damnosum* complex was mapped in the Gulf of Guinea, including south-western Cameroon, during investigations into the potential for vector eradication on the island of Bioko (McCall *et al.*, 1998; Post *et al.*, 2003; Mustapha *et al.*, 2004). *Simulium damnosum* s.str., *S. mengense* Vajime and Dunbar and *S. squamosum* (Enderlein) were found breeding in south-west Cameroon and the 'Bioko' form of *Simulium yahense* Vajime and Dunbar was found breeding on Bioko.

Simulium squamosum and *S. yahense* are very closely related and together constitute the *S. squamosum* subcomplex within the *S. damnosum* complex (WHO, 1995). There are also two named cytotypes within this subcomplex, namely *S. squamosum kitetense* Elsen and Post, and the 'Bioko' form of *S. yahense*, both of which have restricted distributions, with the former known only from the around Kasongo in the Democratic Republic of Congo and the latter only from the island of Bioko in Equatorial Guinea (Elsen & Post, 1989; Post *et al.*, 2003). There are no published records of *S. yahense* from south-western Cameroon.

Simulium squamosum has a focal distribution throughout West and Central Africa from Sierra Leone through Cameroon to the eastern side of the Democratic Republic of Congo (Crosskey, 1987). It breeds in forest streams and small to medium-sized rivers in the forest and guinea savannah, especially in mountainous areas.

The *S. squamosum* subcomplex differs from the arbitrary designated standard chromosome sequence of *S. kilibanum* Gouteux by two inversions, IS-1 and IL-3 (Vajime & Dunbar, 1975). It also has numerous floating inversions whose presence or absence varies in different populations over its distribution range (Vajime & Dunbar, 1975; Boakye, 1993). Sex-chromosome differences have been used to describe members of the *S. squamosum* subcomplex. Vajime & Dunbar (1975) separated *S. yahense* from *S. squamosum* on the basis of sex linkage of the inversion IIL-18. There were no fixed inversion differences but their separate specific status was later confirmed by the discovery of species-specific

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isoenzyme variation (Meredith & Townson, 1981). The 'Bioko' form of *S. yahense* was also separated on the basis of sex linkage of the inversion IIL-18 (Post *et al.*, 2003). Vajime & Dunbar (1975) also recognized sex-chromosome variation within *S. squamosum s.str.* Males from Cameroon showed a long non-pairing segment at the centromere of chromosome I (IC), but elsewhere the non-pairing segment was not found. Traore-Lamizana *et al.* (2001) confirmed the existence of this cytotype and called it *S. squamosum 'A'*. A second cytotype, which had a much shorter and distinctively different non-pairing IC segment in males from river Sanaga in Cameroon, was named *S. squamosum 'B'*, and a third cytotype, *S. squamosum 'C'* had no sex-linked variation. West of the Dahomey Gap (in Togo and Benin where there is a break in the rain forest and the savannah reaches the coast) another cytotype has been recorded in which there is sex linkage of a variant centromere on chromosome III (Boakye, 1993; Post, unpublished observations). *Simulium squamosum kitetense* exhibited no sex-linked inversions and differed from *S. squamosum s.str.* by three fixed inversions (Elsen & Post, 1989).

During a survey of cytospecies in the *S. damnosum* complex in south-west Cameroon (Post *et al.*, 2003), *S. squamosum* exhibited two new sex-linked inversions. The purpose of this work is to describe these inversions and to define a new cytotype within *S. squamosum*.

Simuliid larvae and pupae were collected from 30 riverine breeding sites around Mount Cameroon in May 2001. *Simulium damnosum s.l.* larvae were fixed in Carnoy's solution (3:1 absolute alcohol: glacial acetic acid) and stored at 4°C for cytogenetic studies. Polytene chromosome preparations were made from dissected larval silk glands, according to standard methodologies (Boakye, 1993). Species were identified by comparison to reference chromosome maps published by Vajime & Dunbar (1975), Elsen & Post (1989), Boakye (1993) and Traore-Lamizana *et al.* (2001). Chromosome preparations (from the collections of the Natural History Museum, London, U.K.), which had already been identified as *S. squamosum* by Traore-Lamizana *et al.* (2001) from Cameroon and Nigeria, were also re-examined for comparison.

From the material collected in 2001, 59 specimens of *S. squamosum* were identified from 11 rivers around Mount Cameroon. Voucher specimens (larval bodies and chromosome preparations) have been deposited in the Natural History Museum, London, U.K. These specimens showed no fixed inversion differences from the *S. squamosum* karyotype described by Vajime & Dunbar (1975). The new samples (from 2001) from Cameroon exhibited two floating inversions on the short arm of chromosome I (Table 1), a new inversion IS-To (Fig. 1B) and inversion IS-2 (Fig. 1C). These inversions were sex linked, occurring together (Fig. 1A) in 87% of all males ($n=23$), and were both absent from 88% of all females ($n=25$). Table 2 shows the incidence and sex linkage of chromosome rearrangements in chromosome arm IS in the new samples (from 2001) and from re-examination of old specimens published by Traore-Lamizana *et al.* (2001).

Old samples from Nigeria and the river Menge conformed to *S. squamosum 'C'* with no sex linkage of any rearrangements. Inversion IS-To was absent and although IC was split in 38% of specimens and IS-2 heterozygous in a single specimen (Table 1), neither was sex linked. In the new samples and the old samples from Bikili dam, both inversions IS-To and IS-2 are strongly Y linked and although IS-To is at a higher frequency and more strongly Y linked than IS-2, the inversions usually occur together as shown by the high rate of double heterozygotes (Tables 1 and 2) (linkage disequilibrium $D/D_{\max}=86\%$, see Hartl, 2000).

The precise morphology of the split varied between those specimens, which showed an asynaptic centromere IC. The specimens from the river Menge, Bikili dam and the river Bolo (Table 1) had a long split similar to ICa as described by Traore-Lamizana *et al.* (2001). The other specimens had a short centromeric split (that extended into the short arm of chromosome I), which was different from split ICb (that extended into the long arm of chromosome I) and which Traore-Lamizana *et al.* (2001) used to define *S. squamosum 'B'*.

Many blackflies have microscopically indistinguishable sex chromosomes (such as *S. squamosum 'C'*, described by Traore-Lamizana *et al.*, 2001) and separate species of blackflies which share the same set of differentiated sex chromosomes are almost unknown (Post, 1982; Rothfels & Freeman, 1983). Furthermore, sex-chromosome differences are often the only cytogenetic differences between sibling species (for example *S. yahense* and *S. squamosum*). These general observations have led cytotaxonomists to place special emphasis on sex-chromosome differentiation (Vajime & Dunbar, 1975), and Rothfels (1980) and Procnier (1989) have argued that sex-chromosome differentiation can lead to speciation. The sex linkage of IS-To and IS-2 in *S. squamosum* populations around Mount Cameroon represents a new sex-chromosome system within *S. squamosum*, indicating the existence of a new cytotype that we have called *S. squamosum 'D'*. Re-examination of the old material from Traore-Lamizana *et al.* (2001) has shown that they misidentified some material from south-west Cameroon as *S. squamosum 'C'* because they did not notice the sex-linked inversions IS-To and IS-2 in the area. To date, *S. squamosum 'D'* has been found only in the Mount Cameroon region (both north and south-east of the mountain).

The four cytotypes in *S. squamosum* in Cameroon have been described on the basis of sex-chromosome variation. Such variation is common in blackflies (Post, 1982) and usually involves the sex linkage (to X or Y chromosomes) of centromere polymorphisms or inversions. The centromere polymorphisms (such as the non-pairing regions in *S. squamosum 'A'* and 'B' or the heterochromatin band dimorphism seen in *S. squamosum* west of the Dahomey Gap) are thought to be involved with the suppression of recombination between sex chromosomes (Post, 1985) and recombination-suppression is also considered the main function of inversions (Rees & Jones, 1977).

The pattern of sex-chromosome evolution in *S. squamosum* seems to involve a sex-determining locus somewhere

Table 1. Cytotaxonomic summary of *Simulium squamosum* from Nigeria and south-west Cameroon

Country/ locality*	Co-ordinates lat/long	Date	Sex†	IC split				IC not split				
				St/St	St/2	St/To	St/2.To	St/St	St/2	St/To	St/2.To	
Nigeria												
R. Oshun at Ede	07°44'/05°34'	14.08.82	m					2				
			f					6	1			
R. Assob at Assob	09°32'/08°38'	21.04.94	m					3				
			f									
R. Ogun at Eruwa	07°25'/04°29'	15.08.82	m									
			f	1								
Cameroon												
Bolo Moboka	04°52'/09°28'	07.05.01	m									1
			f	1				1				
R. Menge north of Ikiliwindi	04°45'/09°29'	07.05.01	m									
			f									
			u	3								
R. Menge north of Ikiliwindi	04°43'/09°21'	5.08.90	m	3				1				
			f	2				2				
R. Fiango at Bikili	04°37'/09°31'	5.04.89	m									3
			f					4				1
R. Fiango at Bikili	04°37'/09°31'	6.08.90	m				2					1
			f		3							
R. Bile at Marumba I	04°35'/09°21'	08.05.01	m									1
			f					3				
			u					1				
R. Kumba at Ediki	04°32'/09°28'	10.05.01	m				2					2
			f					2				
R. Yoke at Yoke	04°18'/09°26'	10.05.01	m									
			f	1				1				
R. Benoe south of Dibanda	04°07'/09°18'	06.05.01	m									3
			f					3				
R. Ndongo near Likomba	04°05'/09°21'	06.05.01	m									2
			f									
R. Essuke near Mutengene	04°05'/09°18'	13.05.01	m									1
			f									
R. Ombe west of Ombe	04°05'/09°17'	13.05.01	m			1					2	2
			f		1		3	2		2		
R. Moliwe at Moliwe	04°04'/09°15'	13.05.01	m									2
			f					2				
R. Limbe at Limbe	04°01'/09°12'	12.05.01	m									4
			f					5				
			u					4				2

*Samples are listed according to latitude N-S. Note that Mount Cameroon is 04°13'/09°10'.

†m, males; f, females; u, sex unknown.

near the centromere of chromosome I. In *S. squamosum* 'C' there is no visible recombination-suppression between the X and Y chromosomes, and west of the Dahomey Gap the sex locus has been translocated near the centromere of chromosome III. In *S. squamosum* 'A' and 'B', a sex-chromosome differential segment has developed (as expressed by the different asynaptic segments) near the centromere of chromosome I. This could be explained by selection for sex linkage of genes near the sex locus (Post, 1985). In *S. squamosum* 'D', the sex-differential segment seems to show progressive enlargement by sex linkage of IS-To and IS-2, bringing more genes into sex linkage (but in this case without a visible recombination suppression at the centromere).

Simulium squamosum is possibly the most variable of the cytospecies in the *S. damnosum* complex, comprising at least six cytotypes (see above) that have been described mostly on the basis of sex-chromosome differences, and it is not yet clear whether the cytotypes are correlated with known variation in isoenzymes (Thompson *et al.*, 1990) and repetitive DNA (Post & Flook, 1992; Mank *et al.*, 2004). The cause of this cytotoxic variation is unknown, but the Gulf of Guinea (including Cameroon) is a general biodiversity hot spot with a large number of endemic species of many taxonomic groups (Jones, 1994; Sosef, 1994; Mustapha *et al.* 2004). Four of the six cytotypes in *S. squamosum* have been described from Cameroon and it is likely that

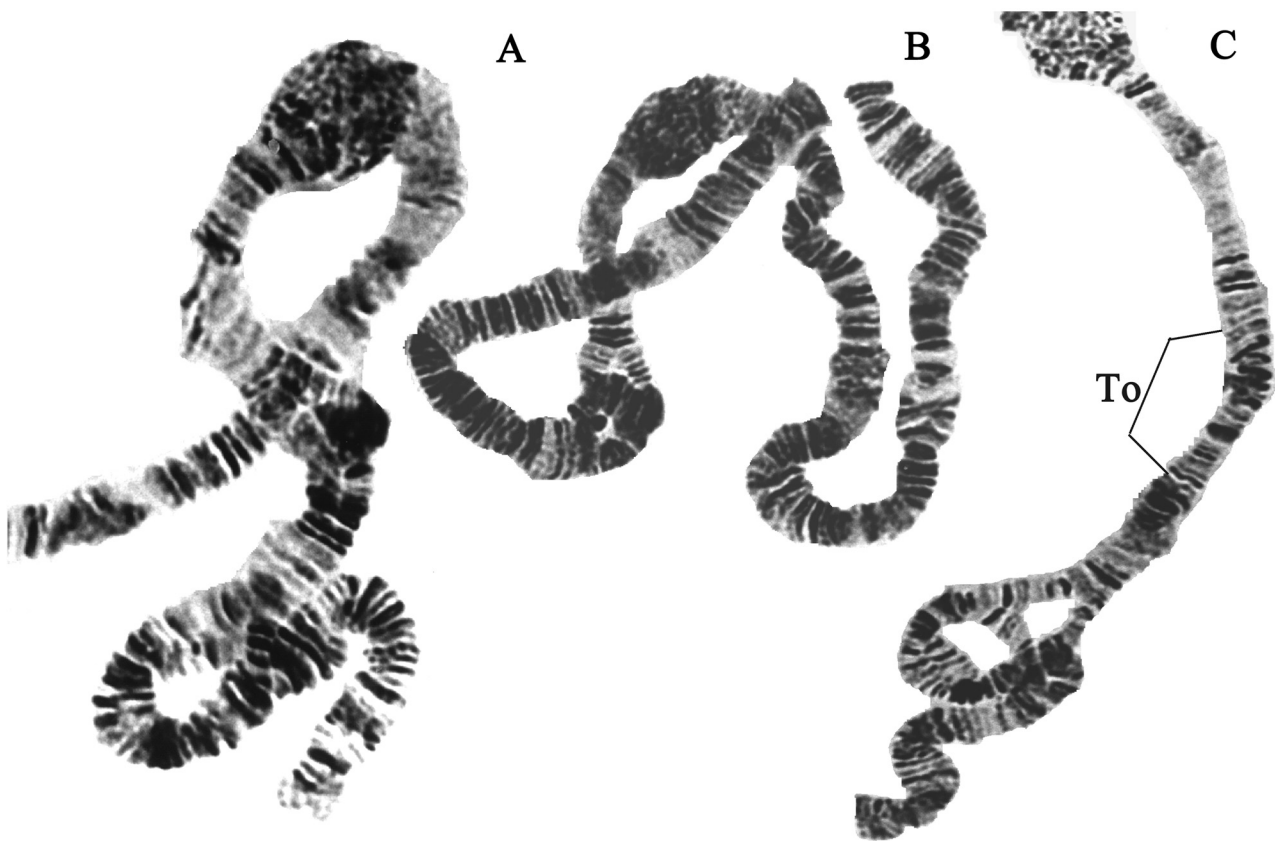


Fig. 1. Chromosome I of *Simulium squamosum* 'D': (A) Male from R. Ndonggo, heterozygous IS-St/To.2; (B) Male *S. squamosum* 'D' from R. Ombe, heterozygous IS-St/To; (C) Female from R. Ombe, with the breakpoints of inversion IS-To illustrated and heterozygous IS-St/2.

Table 2. Sex-linkage in *Simulium squamosum* 'D' from south-west Cameroon

Sex	IS Karyotype				
	St/St	St/2	St/To	St/To.2	Other
M	–	–	3	26	–
F	26	6	–	1	–

this taxonomic variation generally reflects the historical ecology of the Gulf of Guinea.

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