Pole 2

Medical and Veterinary Entomology (2001) **15**, 219–223

SHORT COMMUNICATION

Sex chromosome variation and cytotaxonomy of the onchocerciasis vector *Simulium squamosum* in Cameroon and Nigeria

M. oumouni TRAORE-LAMIZANA, S. SOMIARI*, H. B. MAFUYAI†, C. G. VAJIME‡ and R. J. POST§

Institute Pierre Richet, Bouaké, Côte d'Ivoire, *Department of Zoology, University of Calabar, Nigeria, †Department of Zoology, University of Jos, Nigeria, ‡Benue State University, Makurdi, Nigeria and §The Natural History Museum, London, U.K.

Abstract. On the basis of sex chromosome variation, three cytotypes of *Simulium squamosum* (Enderlein) (Diptera: Simuliidae) are described from Cameroon and Nigeria. *Simulium squamosum* A is the typical form as originally described by Vajime & Dunbar (1975) with chromosome I as the sex chromosome. It occurs throughout most of Cameroon and south-east Nigeria. A second cytotype, *S. squamosum* B, is described from the river Sanaga (Cameroon). It also has chromosome I as the sex chromosome, but the nature of the sex differential region is different. *Simulium squamosum* C has no sex-linked chromosomal rearrangements. It is widespread in Nigeria and occurs near Mount Cameroon, where it seems to hybridize with *S. squamosum* A.

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

The vectors of human onchocerciasis in Nigeria and Cameroon are members of the *Simulium* (*Edwardsellum*) *damnosum* complex (Diptera: Simuliidae). Nine sibling species, sometimes called 'cytospecies', were described by Vajime & Dunbar (1975, 1977) from West Africa on the basis of larval salivary gland polytene chromosome differences. Of these, *S. damnosum* Theobald *sensu stricto* and *S. sirbanum* V. & D. were recorded from both countries, with *S. squamosum* Enderlein and *S. mengense* V. & D. additional for Cameroon.

Traore-Lamizana & Lemasson (1987) surveyed the larval distribution of the *S. damnosum* complex in northern Cameroon and published distribution maps for five cytospecies. *Simulium damnosum s.s.* and *S. sirbanum* were common in the savanna zones; *S. squamosum* was associated with the forested zones and spread north into the savanna rivers during the rainy season. *Simulium yahense* V. & D. was reported locally in the west, whereas *S. mengense* had a scattered distribution throughout the area.

Correspondence: Dr R. J. Post, Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD, U.K. E-mail: R.Post@nhm.ac.uk

The Nigerian situation was reviewed by Wilson *et al.* (1994), Mafuyai *et al.* (1996a, b) and Somiari (1998). Both *S. damnosum s.s.* and *S. sirbanum* are widespread in savanna areas, with the latter tending towards a more northerly distribution. Conversely, *S. damnosum s.s.* spreads into the more forested SE and SW areas. The *S. sanctipauli* subcomplex (*S. sanctipauli* V. & D. and *S. soubrense* V. & D.) has been recorded from SW Nigeria and surprisingly also around the Jos Plateau. *Simulium yahense* is reliably known from only two sites in SE Nigeria, and *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 has a patchy distribution in forest, Guinea savanna and especially montane areas of both West and Central Africa, from Sierra Leone and Guinea in the west to Democratic Republic of Congo and Central African Republic in the east (Crosskey, 1987). This focal distribution might be expected to promote taxonomic variation and potential speciation. Geographical variation in genetic traits of *S. squamosum* involves isoenzymes (Thomson *et al.*, 1990), DNA sequences used for probes (Post & Flook, 1992) and chromosome polymorphism (Boakye, 1993). Frequencies of

© 2001 Blackwell Science Ltd

Fonds Documentaire IRD
010026001

Fonds Documentaire IRD
Cote : B* 26001 Ex : 1

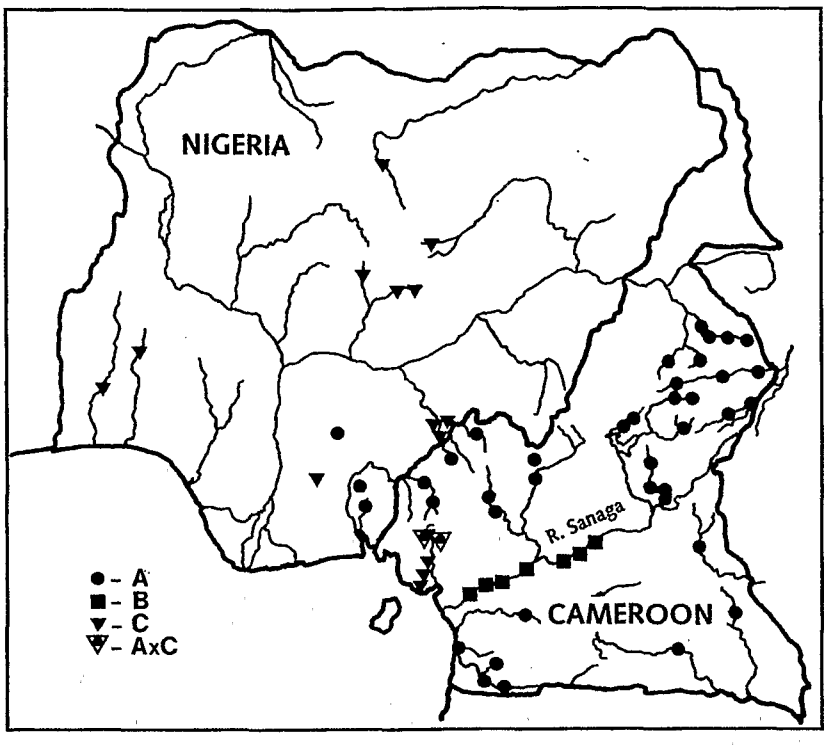

Fig. 1. Map showing the distribution of *Simulium squamosum* in Cameroon and Nigeria classified according to sex chromosome system as *S. squamosum* 'A', 'B' and 'C'.

several chromosomal floating inversions vary across the range of the species and one fixed (i.e. homozygous) inversion defines the south-eastern subspecies *S. squamosum kitetense* (Elsen & Post, 1989). Geographic variation in the sex chromosomes of *S. squamosum* has been reported by numerous workers (Vajime & Dunbar, 1975; WHO, 1988; Elsen & Post, 1989; Boakye, 1993).

Sex chromosomes are generally considered to be sensitive indicators of taxonomic divergence in the Simuliidae (White, 1978; Rothfels, 1979) and have been used to distinguish some of the sibling species and geographical races within the *S. damnosum* complex; for example, *S. yahense* (Vajime & 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 consider the taxonomic significance of sex chromosome variation among *S. squamosum* populations, which was discovered while surveying geographical distributions of the members of the *S. damnosum* complex in Cameroon and Nigeria.

Collection sites of *S. squamosum* were listed and mapped by Traore-Lamizana & Lemasson (1987) for northern Cameroon, and for the whole of Nigeria by Mafuyai *et al.* (1996a) and Somiari (1998). These sites, along with additional localities in southern Cameroon, are mapped on Fig. 1. Final and penultimate stage larvae of the *S. damnosum* complex 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 before 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 & Dunbar (1975), Quillévéré (1975), Elsen & Post (1989) and Boakye (1993).

Most populations of the *S. damnosum* complex sampled from Cameroon and Nigeria conformed with the standard cytotaxonomic descriptions by Vajime & Dunbar (1975, 1977). *Simulium yahense* showed typical sex linkage of inversion IIL-18 (homozygous in 97% of females and heterozygous in 65% of males). Chromosomal variation was observed in *S. damnosum s.s.* 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 & Dunbar, 1975). 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 SW Nigeria (36%).

Vajime & Dunbar (1975) described differences between the sexes of *S. squamosum* from Cameroon. Females ($n = 81$) were all homozygous for the chromosome I with complete

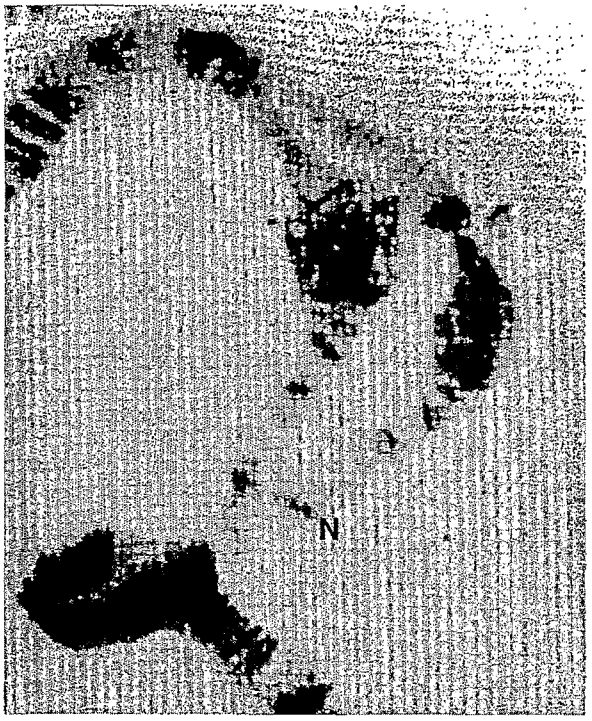

Fig. 2. Centromeric long asynaptic sex-differential region of chromosome I (ICa) from a male of the typical form of *Simulium squamosum*. N = Nucleolus Organiser.

synapsis (ICs), whereas 43% of males ($n = 69$) showed a centromeric long asynaptic (non-pairing) region on chromosome I, indicating a sex-differential region designated ICa (Fig. 2). Cameroon *S. squamosum* outside the Sanaga river and Mount Cameroon area (6587 larvae examined by us) conformed to this description. However, in the majority of males from the Sanaga river the asynaptic centromeric region of chromosome I was shorter, designated ICb (Fig. 3): all of 593 females and 39% (286) of males were ICs homozygous standard, whereas 61% (449) of males had the short ICb asynaptic sex-differential region. Sites around Mount Cameroon harboured a mixture of situations. *Simulium squamosum* sampled from rivers draining south-east from the mountain ($n = 91$ larvae) showed no sex-linked chromosome variation and were consistently synaptic standard ICs in both sexes. *Simulium squamosum* from two sites north of the mountain (river Fiango at Bikili dam and river Menge near Ikilivindi) carried the long asynaptic ICa region in both sexes: 18 larvae examined comprised three males and five females with long asynaptic ICa, plus four males and six females with synaptic ICs.

A total of 402 larvae of *S. squamosum* were identified from 21 sites in Nigeria. No sex-linked chromosome variation of any sort was observed at 18 sites, although three female larvae from the south-west had asynaptic ICa. From three sites (Kwa Falls, Koram river and Ibi river, in Cross River and Imo States). However, the 53 larvae examined were found to

Fig. 3. Centromeric short asynaptic sex-differential region of chromosome I (ICb) from a male of the Sanaga population of *Simulium squamosum*. N = Nucleolus Organiser.

possess the ICs-ICa sex chromosome system widespread in Cameroon (Table 1).

Our observations reveal at least three allopatric sex chromosome systems in *S. squamosum* from Cameroon and Nigeria. It is proposed to recognize these different populations as informal geographical races A, B and C of *S. squamosum*. The distribution of the three forms is clearly separate (Fig. 1), except north of Mount Cameroon in south-west Cameroon where a number of anomalies were found. The results of Vajime & Dunbar (1975) and the older samples collected and analysed for this study (1979–83) indicated the presence of *S. squamosum* A, but more recent samples (1989–94) indicate a mixture of *S. squamosum* A and C in this area. Further sampling is required to clarify the cytotaxonomic composition of *S. squamosum sensu lato* populations around Mount Cameroon.

Simulium squamosum A is the typical form, with sex-linked long asynaptic ICa (Fig. 2), originally described by Vajime & 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

Table 1. Cytotaxonomic summary of *Simulium squamosum* Vajime & Dunbar *sensu lato* from Nigeria and Cameroon

			IC condition			
				Asynaptic		
Type	Data	Sex	Synaptic ICs	Long ICa	Short ICb	Distribution
A	V & D 1975	♂	92 (57%)	69 (43%)	0	Cameroon general
		♀	81 (100%)	0	0	
	Ours	♂	1020 (29%)	2545 (71%)	0	Cameroon & SW Nigeria
		♀	3036 (99%)	9 (1%)	0	
B	Ours	♂	286 (39%)	0	449 (61%)	Cameroon Sanaga river
		♀	593 (100%)	0	0	
C	Ours	♂	102 (100%)	0	0	Nigeria and S.E. mt Cameroon
		♀	124 (98%)	3 (2%)	0	
		?	290 (100%)	0	0	
AxC	Ours	♂	4 (57%)	3 (43%)	0	N. mt Cameroon
		♀	6 (55%)	5 (45%)	0	

Note: The numbers of specimens here does not always correspond to the numbers mentioned in the text because it was not possible to determine the sex of all larvae, especially for Nigeria (marked ? above).

spreads across the border into south-east Nigeria, where it has been found at three localities.

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 characterized by the shorter asynaptic ICb region (Fig. 3) found in 61% of males. It is presumed to be responsible for the heavy man-biting in the area, requiring 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 IC asynapsis. It is the form distributed throughout most of Nigeria, occurring also around Mount Cameroon. *Simulium squamosum* C is a proven vector of onchocerciasis in Nigeria (Mafuyai *et al.*, 1997). To the north of Mount Cameroon, populations occur that appear to be a mixture of *S. squamosum* A and C, with the long asynaptic ICa in some larvae of both sexes, suggesting introgression between forms A and C in this area.

Acknowledgements

For intellectual, logistic and technical support we would like to thank D. Quillévéré, D. A. Boakye, S. E. O. Meredith, P. Enyong, I. Halid, N. R. Uzoigwe, F. X. Kenfack, T. Mvondo, B. Matur, J. Yohanna and A. L. Millest. This work was funded by several sources including the Fond d'Aide et de Coopération (FAC) for a feasibility study of onchocerciasis control in the region of the Logone river basin, Institut Francais de Recherche Scientifique pour le Developement en Cooperation (ORSTOM) and the World Health Organization Special Programme for Research and Training in Tropical Diseases (WHO/TDR research institute strengthening grant ID900314, research training grant ID880037, re-entry grant ID930332 and research project grant ID950612).

References

Boakye, D.A. (1993) A pictorial guide to the chromosomal identification of members of the *Simulium damnosum* Theobald complex in West Africa with particular reference to the Onchocerciasis Control Programme area. *Tropical Medicine and Parasitology*, **44**, 223–244.

Crosskey, R.W. (1987) A taxa summary for the *Simulium damnosum* complex, with special reference to distribution outside the control areas of West Africa. *Annals of Tropical Medicine and Parasitology*, **81**, 181–192.

Dunbar, R.W. (1972) *Polytene chromosome preparations from tropical Simuliidae*. Unpublished document WHO/ONCHO/72.95. World Health Organisation, Geneva.

Elsen, P. & Post, R.J. (1989) *Simulium (Edwardsellum) squamosum kitetense* ssp.n., a new chromosomal and morphological subspecies within the *Simulium damnosum* complex from East Zaire. *Annals of Tropical Medicine and Parasitology*, **83**, 85–92.

Mafuyai, H.B., Post, R.J., Molyneux, D.H. & Davies, D.H. (1997) First sibling-species identifications of Nigerian onchocerciasis vectors. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, **91**, 90–91.

Mafuyai, H.B., Post, R.J., Vajime, C.G. & Molyneux, D.H. (1996a) Cytotaxonomic identifications of the *Simulium damnosum* complex (Diptera: Simuliidae) from Nigeria. *Tropical Medicine and International Health*, **1**, 779–785.

Mafuyai, H.B., Wilson, M.D. & Post, R.J. (1996b) Morphological differentiation of adult females of the *Simulium damnosum* complex from Nigeria. *Medical and Veterinary Entomology*, **10**, 190–192.

Meredith, S.E.O., Cheke, R.A. & Garms, R. (1983) Variation and

distribution of forms of *Simulium soubrense* and *S. sanctipauli* in West Africa. *Annals of Tropical Medicine and Parasitology*, **77**, 627–640.

Post, R.J. (1986) The cytotaxonomy of *Simulium sanctipauli* and *Simulium soubrense* (Diptera: Simuliidae). *Genetica*, **69**, 191–207.

Post, R.J. & Flook, P. (1992) DNA probes for the identification of members of the *Simulium damnosum* complex (Diptera: Simuliidae). *Medical and Veterinary Entomology*, **6**, 379–384.

Quillévéré, D. (1975) Etude du complexe *Simulium damnosum* en Afrique de l'Ouest. I – Techniques d'étude. Identification des cytotypes. *Cahiers O.R.S.T.O.M., Série Entomologie Médicale et Parasitologie*, **13**, 87–100.

Quillévéré, D. (1979) Contribution a l'étude des caractéristiques taxonomiques, biologiques et vectrices des membres du complexe *Simulium damnosum* présents en Côte d'Ivoire. *Travaux et Documents de l'O.R.S.T.O.M.*, **109**, 1–304.

Rothfels, K.H. (1979) Cytotaxonomy of blackflies (Simuliidae). *Annual Review of Entomology*, **24**, 507–539.

Somiari, S.A.E. (1998) Cytological Studies and the Distribution of Simulium Damnosum Complex in Nigeria, Cameroon and Equatorial Guinea. PhD Thesis, Ahmadu Bello University, Zaria.

Surtees, D.P., Fiasorgbor, G., Post, R.J. & Weber, E.A. (1988) The cytotaxonomy of the Djodji form of *Simulium sanctipauli* (Diptera: Simuliidae). *Tropical Medicine and Parasitology*, **39**, 120–122.

Thomson, M.C., Renz, A. & Davies, J.B. (1990) A new electromorph diagnostic for *S. squamosum* from Sierra Leone and Togo but not found in *S. squamosum* from Cameroun. *Acta Leidensia*, **59**, 303–305.

Traore-Lamizana, M., Berl, D. & Chauvet, G. (1985) Mise en évidence d'une résistance au téméphos (Abate^{R}) dans le complexe *Simulium damnosum* sur le site du barrage de Song Loulou (Sanaga maritime, Cameroun). *Cahiers O.R.S.T.O.M., Série Entomologie Médicale et Parasitologie*, **23**, 143–148.

Traore-Lamizana, M. & Lemasson, J.J. (1987) Participation à une étude de faisabilité de campagne de lutte contre l'onchocercose dans la région du bassin du Logone. Répartition des espèces du complexe *Simulium damnosum* dans la zone camarounaise du projet. *Cahiers O.R.S.T.O.M., Série Entomologie Médicale et Parasitologie*, **25**, 171–186.

Vajime, C.G. & Dunbar, R.W. (1975) Chromosomal identification of eight species of the subgenus *Edwardsellum* near and including *Simulium (Edwardsellum) damnosum* Theobald (Diptera: Simuliidae). *Tropenmedizin und Parasitologie*, **26**, 111–138.

Vajime, C.G. & Dunbar, R.W. (1977) Chromosomal identification of *Simulium (Edwardsellum) mengense* new species (Diptera: Simuliidae). *Parassitologia*, **19**, 95–108.

White, M.J.D. (1978) *Modes of Speciation*. W.H. Freeman & Co, San Francisco.

WHO (1988) *Report of an informal consultative meeting on the Simulium damnosum complex*. Unpublished document WHO/VBC/88.954. World Health Organisation, Geneva.

Wilson, M.D., Mafuyai, H.B. & Post, R.J. (1994) Morphological identification of sibling species of *Simulium damnosum* (Diptera: Simuliidae) complex from Nigeria, Cameroon and Bioko. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (N.E.V.)*, **5**, 181–185.

Accepted 1 April 2001

Volume 15 • Number 2 • June 2001

ISSN 0269-283X

Medical and Veterinary Entomology

Editors: G. B. White (Medical) and R. Wall (Veterinary)

Published for the Royal Entomological Society

Blackwell Science

PM 278
25 JUIN 2001
Santé