

# The cytotaxonomy of *Simulium sanctipauli* sensu stricto (Diptera: Simuliidae) in Ghana and the Côte d'Ivoire

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## Abstract

The status of the different cytological variants of *Simulium sanctipauli* sensu stricto Vajime & Dunbar (*sensu* Post, 1986) found in Ghana and Côte d'Ivoire is cytotaxonomically evaluated in view of recent revisions of the *S. sanctipauli* subcomplex. Three geographical variants designated as 'Pra', 'Comoé' and 'Sassandra' forms of *S. sanctipauli* are described. The 'Pra' form is genetically differentiated from the other two forms by a distinct sex-determining system. The 'Comoé' and 'Sassandra' forms are considered as the two ends of an interspecific stepped cline with the River Bandama basin as the zone of contact. The possibility that the 'Comoé' form populations breeding in southwestern Ghana could serve as re-invading *S. sanctipauli* into the Onchocerciasis Control Programme (OCP) area in West Africa is discussed.

## Introduction

The cytotaxonomy of *Simulium sanctipauli* (Vajime & Dunbar) and *S. soubrense* (Vajime & Dunbar) (Diptera: Simuliidae) has been the subject of many reviews since these two cytotypes were described as distinct species. Quillévéré (1975) objected to their separation as distinct species because of the presence of a high proportion of heterozygotes for the diagnostic inversion IIL-7 in the Côte d'Ivoire. Later, he proposed the recognition of the two cytotypes (*S. sanctipauli* and *S. soubrense* Vajime & Dunbar) as subspecies of a single species *S. sanctipauli* (Quillévéré *et al.*, 1981). Meredith *et al.* (1983) also found high proportions of heterozygotes for IIL-7 in *S. soubrense* in Togo and Benin and described these populations as geographical variants of *S. soubrense* on the basis of their novel sex-determining system.

The correct identification of these species became more important when the populations resistant to insecticides in Côte d'Ivoire in 1980 and 1981 were identified as both *S. sanctipauli* and *S. soubrense* (Guillet *et al.*, 1980; Kurtak

*et al.*, 1982). Also, Meredith (1983) observed that on the River Tano a sample taken in July 1982, when a susceptibility test showed apparently a normal, sensitive population, was composed of IIL-4.6.7/4.6.7 individuals. Later Post (1986) recognized that IIL-7 was not diagnostic for members of the *S. sanctipauli* subcomplex but established instead that two previously unrecognized inversions, IL-A and IIL-A had diagnostic value. Using these inversions, he reclassified the members of the subcomplex into three species *S. sanctipauli*, *S. soubrense* and *S. soubrense* 'B'. Post & Kurtak (1987) identified the OP-insecticide resistant species within the *S. sanctipauli* subcomplex as *S. sanctipauli* on the basis of the inversion IIL-A.

Recently, Boakye *et al.* (1993) have reviewed the species status of populations of the subcomplex in Guinea and surrounding areas and also the inversions (fixed and floating) recorded in these populations. Four sibling species were described in this review; *S. sanctipauli*, *S. soubrense*, *S. leonense* Boakye, Post & Mosha (formerly *S. soubrense* B) and *S. konkourense* Boakye, Post, Mosha & Quillévéré. Within each of these species, chromosomal variants were observed in different areas of their distribution range and such variants were considered to be forms within the different

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species. Also, they reported that all the members of the *S. sanctipauli* subcomplex were distinguished from *S. squamosum* Enderlein (the standard chosen for the West African *S. damnosum* sensu lato by Vajime & Dunbar 1975) by the inversion IIL-4. Some inversions, such as, IIL-6 and IIL-2.17.4, recorded as fixed by Vajime & Dunbar (1975) and Post (1986) in all the members of the subcomplex, were found to be floating (intra-specific, being absent, present homozygously or heterozygously in different individuals of the same species) in some populations whilst other inversions such as IL-PQ, IIL-C<sub>1</sub>C<sub>2</sub> (Post 1986) were shown to be junior synonyms.

Members of the subcomplex east of the Côte d'Ivoire were, however, not included in this recent review and therefore continue to be classified according to the criteria of Post (1986). The observation that resistance to temephos and chlorphoxim occurred mostly in individuals with IIL-4.6/4.6 compared to those with IIL-4.6.7/4.6.7 on the River Pra in Ghana where no insecticide treatment had been undertaken (Meredith *et al.*, 1986), and the recent evidence that a stable

resistance to temephos exists on the River Comoé in the Côte d'Ivoire (Dr C. Back, personal communication), has prompted the re-evaluation of the cytotoxicity of the *S. sanctipauli* sensu lato in Ghana and Côte d'Ivoire. We report here a reclassification of the members of the *S. sanctipauli* subcomplex in Ghana and Côte d'Ivoire and provide evidence that untreated sites in Ghana could be the source of *S. sanctipauli* sensu lato resistant to organophosphates that repopulate the treated sites on the River Comoé. The Djodji form of *S. sanctipauli* described by Surtees *et al.* (1987), and the Beffa form of *S. soubrense* (Meredith *et al.*, 1983; Post, 1986) are not included in the present paper because the former is (apparently) extinct and the cytotoxicity of the latter remains unchanged.

### Material and methods

Samples of *S. damnosum* sensu lato larvae collected between 1986 and 1994 were identified from areas in Ghana and Côte d'Ivoire (fig. 1). Larval collections were undertaken

Table 1. Species composition of samples containing members of the *Simulium sanctipauli* subcomplex.

Country*	Site	Species composition				
		<i>S. sanctipauli</i> s.s.	<i>S. damnosum</i> s.s.	<i>S. sirbanum</i>	<i>S. squamosum</i>	<i>S. yahense</i>
<b>Ghana*</b>						
Anum	Esichu	2	8			
Anum	Muronem Bridge	21	9			
Anum	Njenasi	2	17			
Anum	Yankuma	62	67			1
Offin	Akuropon	144	2			
Offin	Asuang Village	51	1			
Pra	Abobo	24				
Pra	Bukuruso	34				
Pra	Downstr. Kumananta	162	1			
Pra	Hemang	48				
Pra	Kumananta	32	3			
Pra	Twifo Praso	10				
Tano	Acherensua	3	8		2	23
Tano	Boin Junction	198				
Tano	Domiabra Village	86				
Tano	Downstr. Dunkwa Br.	135	1		2	
Tano	Dunkwa Bridge	19				
Tano	Kpoikrom	1	9	1		
Tano	Bopa/Nsawora	121	8			
Tano	Ruru Village	51				
Tano	Sika Frebudja	49				
<b>Côte d'Ivoire*</b>						
Bandama	Chutes Gauthier	9	1			
Bandama	Marabadiassa	15	5			
Bandama	Ndenou	11	4			
Comoé	Ahinekro	11	3	1		
Comoé	Amiakouassikro	32	16			
Comoé	Amont Mbasso	40	2			
Comoé	Amouakoukro	95	33	9		
Comoé	Kabrankro	2	16	4		
Comoé	Koutoukro	227	36			
Comoé	Mbasso	21				
Diore	Kodiana	10	22	27		
Noun	Toyebli	1	8	3	1	
Nzi	Galegoua	18				
Nzo	Logouale Pont	1			44	
Sassandra	Amont Taobli	2	9	2		
Sassandra	Igesco	2	52	1		
Sassandra	Koperagui	102				
Sassandra	Tayaboui	232	6	1		

during routine surveillance of *S. damnosum* sensu lato breeding sites and sampling for insecticide susceptibility studies. Sampling bias, particularly, for insecticide test larvae were reduced by using only the control larvae. The larvae for cytotoxic determinations were fixed in modified Carnoy's solution (3:1 absolute ethanol:glacial acetic acid) and the chromosomes prepared as described by Dunbar (1972) and Boakye *et al.* (1993). Species recognition was based on the diagnostic chromosomal inversions of Vajime & Dunbar (1975), Meredith *et al.* (1983), Post (1986), Surtees *et al.* (1988) and Boakye *et al.* (1993). The various diagnostic criteria have been reviewed by Boakye (1993).

Inversion frequency data were obtained from samples that were fully karyotyped. The Hardy-Weinberg equation was used to test the data for genetic heterogeneity between different populations.

### Results

Two species of the *S. sanctipauli* subcomplex were recognized in Ghana, Côte d'Ivoire, Togo and Benin on the basis of the inversions IL-A and IIL-A (Post, 1986). All individuals with IIL-A but lacking IL-A were identified as *S. sanctipauli* and those without both IL-A and IIL-A as *S. soubrense*. Members of both species were fixed for IIL-4.6. Individuals with IL-A (*S. leonense*) were not observed.

A total of 2551 larvae were examined cytotoxicologically. Table 1 shows species compositions of the different samples which contained *S. sanctipauli* sensu lato. It was observed that *S. damnosum* sensu stricto and *S. sirbanum* Vajime & Dunbar were the commonest cytospecies, often found in sympatry with members of the *S. sanctipauli* subcomplex. *Simulium yahense* Vajime & Dunbar was rarely found breeding together with the *S. sanctipauli* sensu lato.

The frequencies of different inversions found in *S. sanctipauli* sensu stricto are shown in table 2. Analysis of these chromosomal polymorphisms by Chi square test indicated the presence of four different populations from the Côte d'Ivoire in the west to the Ghana-Togo border in the east. These populations inhabited the rivers Sassandra, Bandama, Comoé, Tano and Pra and their tributaries (fig. 1). They are described below.

#### Populations from Côte d'Ivoire

##### River Sassandra

In western Côte d'Ivoire, most populations found on the river Sassandra and tributaries had a high proportion of the inversions IIL-7 (fig. 2) and IIL-17+4 whilst IIL-B and IS-21 were absent. Figure 3 shows a typical chromosome III found in individuals of this cytotype. Inversion IS-A (fig. 4) was fixed. No sex linkage was observed among the different inversions. IL-B reported by Boakye *et al.* (1993) as fixed was found to be heterozygous at less than 1% frequency.

##### Rivers Comoé and Bandama

Inversion IIL-7 occurred rarely in samples from the two rivers. All specimens examined were homozygous for the inversions IL-B and IIL-4.6.A. (fig. 5). The inversions IS-A and IIL-B (fig. 6) occurred at a frequency of about 96%. A hitherto unrecognized chromosomal character (double banding of the centromere of chromosome II, fig. 5) was also observed at a very high frequency (about 98%). 3C-H (Post, 1986) was found to be polymorphic. IS-21 was absent and no sex linkage was observed (table 3).

Table 2. Proportion of inversions recorded in different populations of members of the *Simulium sanctipauli* subcomplex.

Country*	Site	Collection date	Proportion of inversions								
			IS-A	IS-21	IIS-6b	IIL-7	IIL-A	IIL-D	IIL-4+17	IIL-B	IIL-24
Ghana*											
Anum	Yankuma	30 Nov, 89	0.91	0.26	0.00	1.00	1.00	0.00	0.98	0.75	0.00
Offin	Akuropon	08 Jun, 89	0.55	0.10	0.00	0.95	1.00	0.00	1.00	0.60	0.00
Offin	Akuropon	29 Nov, 89	1.00	0.39	0.00	1.00	1.00	0.00	1.00	0.61	0.00
Pra	Abobo	29 Nov, 89	0.18	0.16	0.00	1.00	1.00	0.00	1.00	0.65	0.00
Pra	Bukuruso	14 Apr, 94	0.67	0.56	0.00	1.00	1.00	0.00	1.00	0.72	0.00
Pra	Bukuruso	26 May, 94	0.88	0.25	0.00	1.00	1.00	0.00	1.00	0.88	0.00
Pra	Bukuruso	20 Jun, 94	0.67	0.08	0.00	1.00	1.00	0.00	1.00	0.92	0.00
Pra	Downstr. Kumananta	29 Nov, 89	0.73	0.27	0.00	1.00	1.00	0.00	0.98	0.62	0.00
Pra	Twifo Praso	29 Nov, 89	0.44	0.00	0.00	1.00	1.00	0.00	1.00	0.69	0.00
Pra	Hemang	29 Nov, 89	0.09	0.26	0.00	1.00	1.00	0.00	1.00	0.68	0.00
Tano	Bopa/Nsawora	01 Dec, 89	1.00	0.11	0.00	0.77	1.00	0.00	0.97	0.73	0.00
Tano	Bopa/Nsawora	20 May, 94	1.00	0.33	0.00	1.00	1.00	0.00	1.00	0.83	0.00
Tano	Downstr. Dunkwa Br.	01 Dec, 89	0.95	0.00	0.00	0.09	1.00	0.00	1.00	0.98	0.00
Tano	Dunkwa Bridge	19 Apr, 94	1.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00	0.00
Tano	Ruru Village	01 Dec, 89	0.96	0.06	0.00	0.86	1.00	0.00	1.00	0.76	0.00
Tano	Sika Frebudja	01 Dec, 89	0.94	0.08	0.00	0.25	1.00	0.00	0.98	0.82	0.00
Côte d'Ivoire*											
Bandama	Chutes Gauthier	29 Jan, 87	1.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00	0.00
Bandama	Ndenou	05 Dec, 86	0.93	0.00	0.00	0.03	1.00	0.00	0.98	0.98	0.00
Comoe	Ahinekro	21 Jul, 94	0.50	0.00	0.00	0.00	1.00	0.00	1.00	1.00	0.00
Comoe	Koutoukro	28 Jul, 87	1.00	0.00	0.00	0.05	1.00	0.00	0.96	0.96	0.00
Comoe	Koutoukro	22 Dec, 93	1.00	0.00	0.00	0.01	1.00	0.00	1.00	1.00	0.00
Sassandra	Koperagui	01 Aug, 94	1.00	0.00	0.00	0.98	1.00	0.00	0.95	0.00	0.00

It was not always possible to score all inversions in every specimen, therefore sample sizes may be smaller than those listed in table 1.

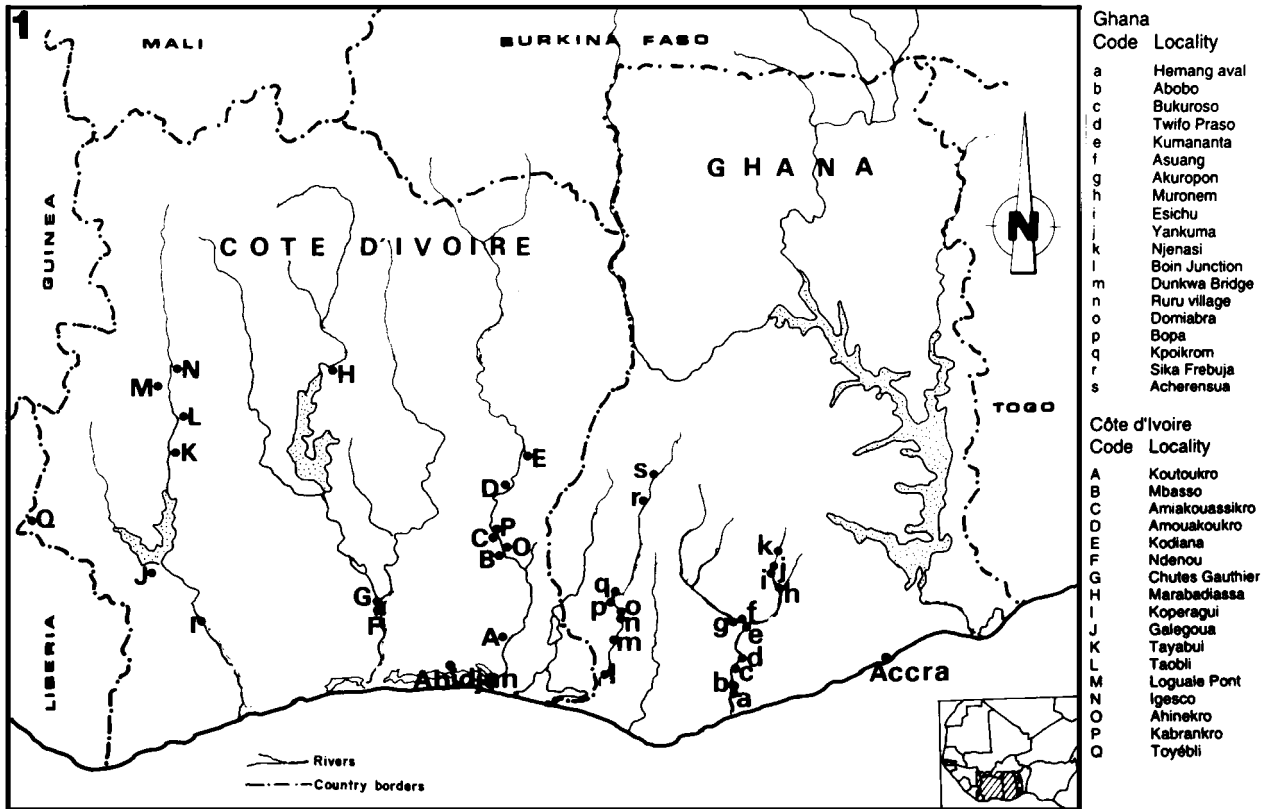
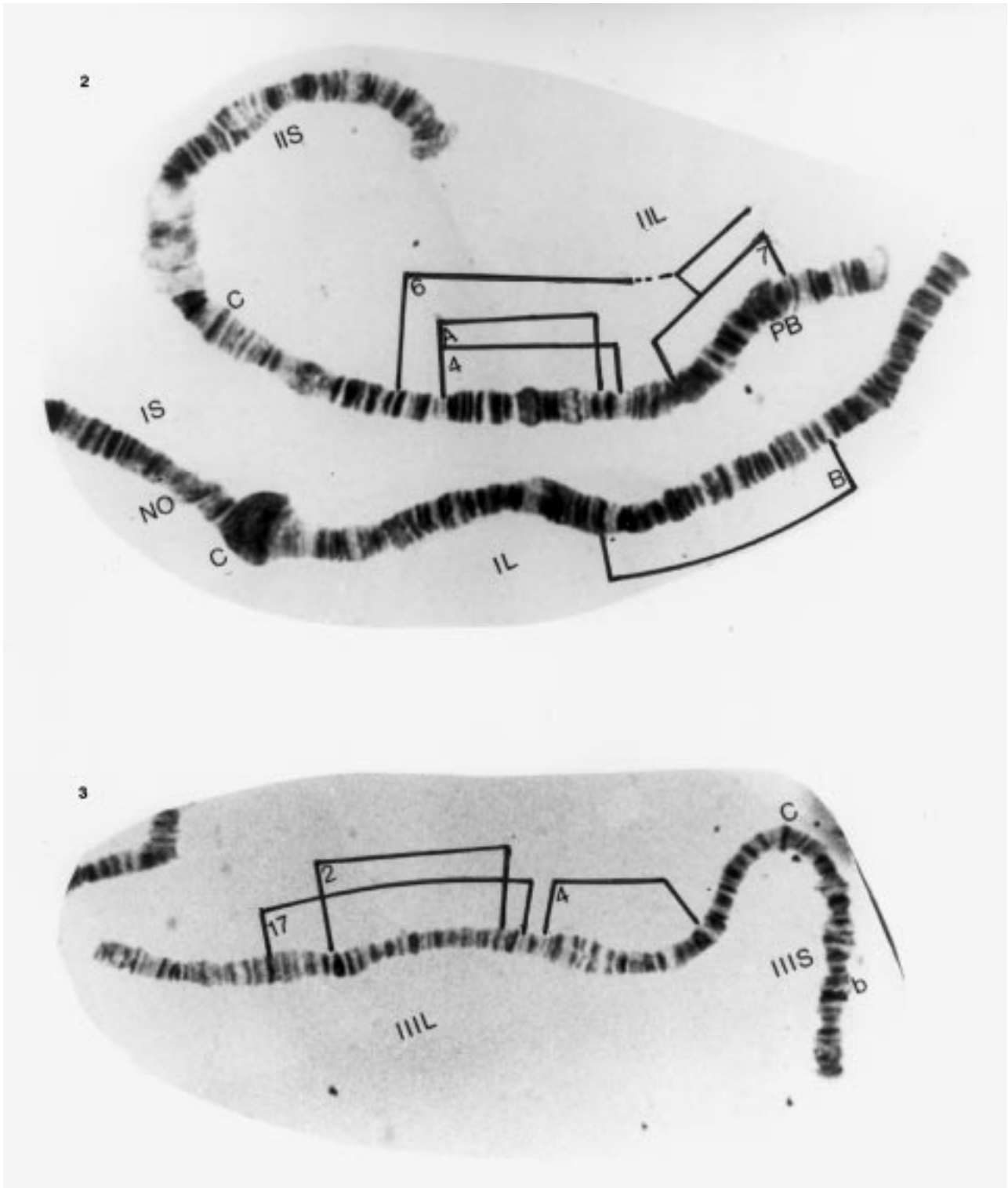


Fig. 1. Breeding sites of members of the *Simulium sanctipauli* subcomplex in Ghana and Côte d'Ivoire. (Inset is a map of west Africa showing the position of the two countries.)

Table 3. Relationship between sex and frequencies of the inversions IS-21 and IIIL-B in samples of *Simulium sanctipauli* from Pra, Tano Comoé and Sassandra river basins.

Rivers	Sex	IS-21			IIIL-B				
		Sample size	21/21	Ot/21	Ot/Ot	Sample size	B/B	Ot/B	Ot/Ot
Anum	M	37	0	14	23	42	3	34	5
	F	30	0	8	22	41	32	9	0
Offin	M	37	0	13	24	37	2	31	4
	F	33	0	15	18	32	25	7	0
Pra	M	129	0	46	83	134	12	114	8
	F	116	1	38	77	117	99	16	2
Tano	M	112	0	56	56	145	56	89	0
	F	103	0	61	42	128	112	12	4
Comoé	M	29	0	0	29	34	34	0	0
	F	32	0	0	32	39	39	0	0
Sassandra	M	8	0	0	8	9	0	1	8
	F	14	0	0	14	13	0	0	13

21/21; Ot/21; Ot/Ot =homozygous, heterozygous and absence for inversion IS-21. B/B; Ot/B; Ot/Ot = homozygous, heterozygous and absence for inversion IIIL-B



Figs 2-3. Chromosomes IL, II and III: 2, long arm of chromosome I and chromosome II of *Simulium sanctipauli* sensu stricto showing the inversions IL-B/B and IIL-4.6.A.7/4.6.A.7; 3, typical chromosome III sequence found in *S. sanctipauli* sensu stricto 'Sassandra' form.



Figs 4–6. Chromosomes I, II and III: 4, chromosome I of *Simulium sanctipauli* sensu stricto showing IS-A; 5, typical chromosome II of the 'Comoé form' showing inversions IIL-4.6.A/4.6.A and a double banding of the centromere (arrowed); 6, chromosome III of *Simulium sanctipauli* sensu stricto showing inversion IIIA-B which is autosomally fixed in the 'Comoé' form and sex-linked in the 'Pra' form.



### Populations from Ghana

#### River Pra

On the Pra river and its tributaries (River Anum and River Offin), populations were fixed for IL-B, IIL-4.6.A.7 and IIII-4.17.2. The inversion IS-A was highly polymorphic occurring at different frequencies in different samples. The frequency of this inversion varied between different collections of the same cytotype. For example, collections from different sites on the river Pra and its tributaries in November 1989 showed a south-to-north directional increase in frequency of the IS-A inversion possibly related to the rainy and dry seasons and/or vegetation zones.

Inversion IL-21 was present at a low frequency as an autosomal polymorphic inversion. In these populations, sex determination was associated with the inversion IIII-B (see fig. 6). About 80% of females were found to be homozygous for IIII-B whilst most males (about 90%) were heterozygous for the inversion (table 3).

#### River Tano

*Simulium sanctipauli* found on the river Tano and tributaries were a mixture of two populations. One population was similar to those on the Comoé river whilst the other was similar to populations on the Pra river. This is reflected in the proportion of the various inversions (table 2). In samples with fewer individuals having the inversion IIL-7, the inversion IIII-B was proportionately higher due to fixation of IIII-B in individuals lacking IIL-7.

The populations of the *S. sanctipauli* subcomplex found in south eastern Ghana (on the Dayi, Asukawkaw and tributaries) between 1986 and 1988 were all *S. sanctipauli* 'Djodji' form (Surtees *et al.*, 1988). This species has not been found in recent identifications because, as mentioned previously, it appears to be extinct.

*Simulium soubrense* was observed only in Togo and Benin. These populations were recognized as *S. soubrense* 'Beffa' form Meredith *et al.* (1983). Figures 7 and 8 show the chromosome II in *S. soubrense* (i.e. absence of IIL-A or IIL-D) whilst fig. 9 shows the typical chromosome III of *S. soubrense* 'Beffa' form.

### Discussion

Whilst most of the populations of the *S. sanctipauli* subcomplex identified from Ghana and Côte d'Ivoire were *S. sanctipauli* on the basis of Post (1986) and Boakye (1993), it was evident that on each of the major rivers (Pra, Tano, Comoé, Bandama and Sassandra) a distinct cytological population or a mixture of two such populations was present. No unique fixed inversions were observed for any of these distinct populations. However, populations on the river Pra (Pra form) showed a distinct sex-linkage to IIII-B. Sex chromosome differentiation is considered to be important in population sub-structuring leading to speciation (Procnier, 1982). Closely related members differ by only sex chromosomes but distant taxa differ by fixed inversions and sex chromosomes (Procnier, 1989).

Surtees *et al.* (1988) characterized the *S. sanctipauli* populations with sex-linkage to IS-21, which were found principally in the Asukawkaw and Dayi river systems as a geographical variant; they found no unique fixed inversions with other members of *S. sanctipauli*. Also, they were not found in sympatric situations with other populations of *S.*

*sanctipauli*. They diagnosed them as 'Djodji' form. It is believed to have been eradicated.

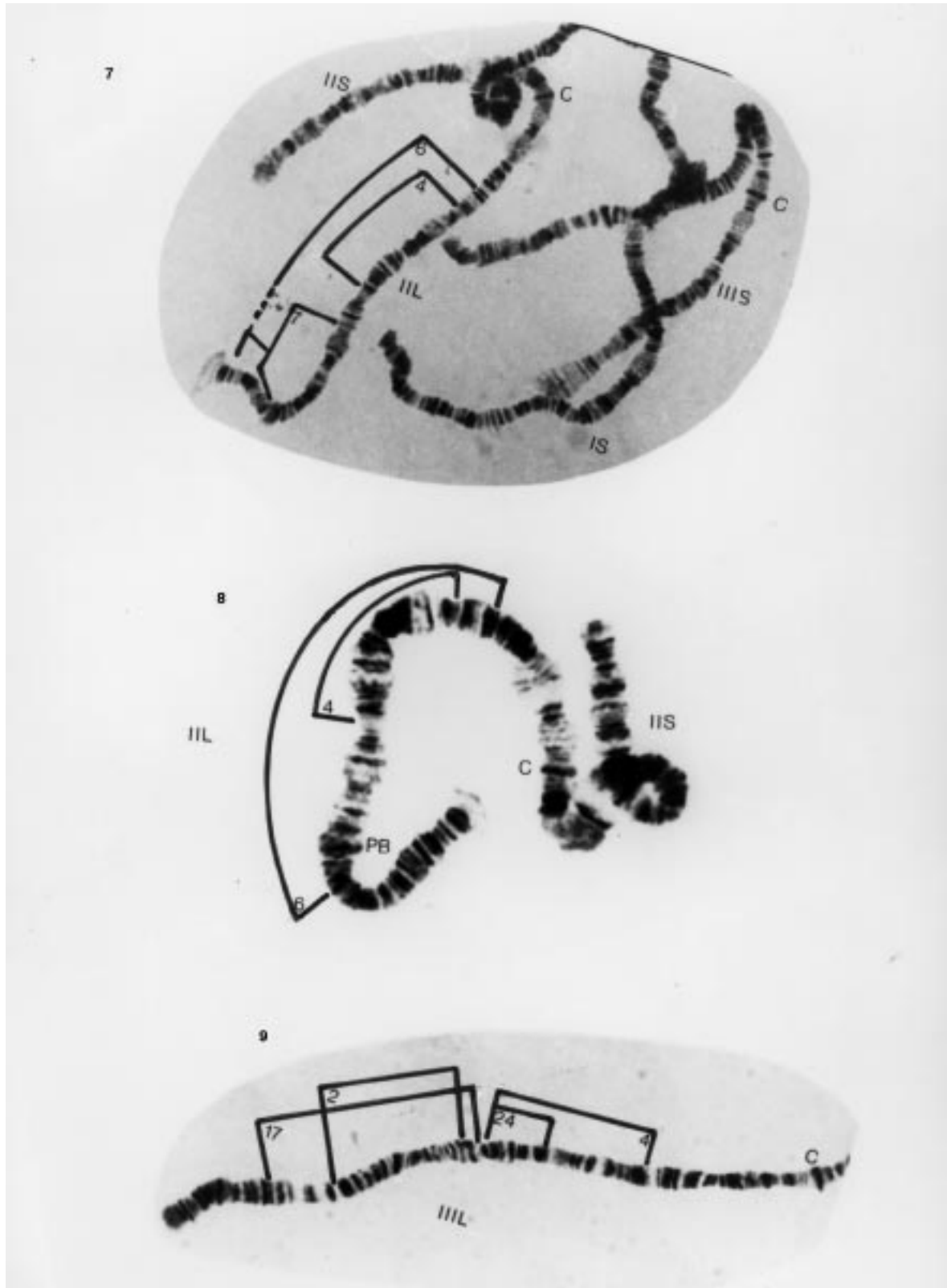
On the River Tano, the populations with sex-linkage to IIII-B were found in sympatry with the predominant cytotype found on the River Comoé (Comoé form). These two cytological forms showed a high degree of genetic isolation in that the inversion IIL-7 which is fixed in the population with sex-linkage to IIII-B was never found in a heterozygous combination. Furthermore, IS-21 was always absent in individuals lacking the inversion IIL-7. We consider the populations with sex-linkage to IIII-B as genetically differentiated from other *S. sanctipauli* sensu stricto populations. However, since they do not show any unique inversions from the other populations, they should be regarded as geographical variants within *S. sanctipauli* sensu stricto (probably in the process of speciation) designated as the 'Pra' form. The sex determining system in the 'Pra' form is similar to that reported for *S. soubrense* 'Chutes Milo' form (Boakye *et al.*, 1993) except that females of the former were homozygous for the inversion IIII-B.

Populations on the Comoé and Sassandra were also different with regard to the proportions of some floating inversions especially IIL-7. However, earlier identifications showed that heterozygotes for the inversion IIL-7 were observed in appreciable numbers on the River Bandama where both the 'Comoé' and 'Sassandra' forms occurred in sympatry (Quillévé, 1975; D. Boakye, unpublished observations). Unfortunately, most of these earlier samples were not fully karyotyped or identified on the basis of Post's (1986) criteria. Thus, it has not been possible to determine the complement of inversions found in these samples for comparison with those found on the Sassandra or Comoé rivers. The two apparently distinct populations could, however, represent the two ends of an intraspecific stepped cline (Boakye *et al.*, 1993) with the River Bandama basin area as the zone of contact. This would explain the earlier disagreement on the separation of these two populations as distinct species (Vajime & Dunbar, 1975; Quillévé, 1975). Recent identifications of samples collected from the River Bandama (which is under insecticidal control) indicate only the form predominant on the River Comoé (i.e. the 'Comoé' form) (unpublished data). It appears to be the resistant form within the *S. sanctipauli* sensu stricto.

In view of the above observations and the proximity of the untreated sites in southwestern Ghana to the River Comoé, populations of the 'Comoé' form found on the Tano and, probably, other areas in southwestern Ghana could be acting as the source breeding sites for the rapid repopulation of the treated breeding sites on the River Comoé with *S. sanctipauli* resistant to organophosphate insecticides. Due to the applied and operational importance of these repopulations to resistance management in the Onchocerciasis Control Programme in West Africa, the distribution of the 'Comoé' form should be mapped out for possible control.

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Figs 7–9. Chromosomes II and III: 7, chromosome II of *Simulium soubrense* showing the inversion III- 4.6.7/4.6.7; 8, chromosome II of *S. soubrense* showing the inversions III-4.6./4.6 (i.e. the absence of III-7); 9, typical chromosome III of *S. soubrense* 'Beffa' form with the inversions IIII-2.17.4.24/2.17.4.24.



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